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The Effects of Acute Ammonia Toxicity on Certain Blood Chemistries in Sheep

Gayleen Riedemann

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THE EFFECTS OF ACUTE AMMONIA TOXICITY ON CERTAIN BLOOD CHEMISTRIES IN SHEEP

by

GAYLEEN RIEDEMANN

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science, Major in Zoology, South Dakota State University 1980
THE EFFECTS OF ACUTE AMMONIA TOXICITY ON
CERTAIN BLOOD CHEMISTRIES IN SHEEP

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor

Head, Entomology-Zoology Dept.
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INTRODUCTION

Protein has always been in short supply in the world. This will be even more evident when the world's population reaches the projected 5.8 billion by the year 2000. Rosenfeld (1979) noted that not only will there be more mouths to feed, but there is also a decrease in cropland due to increased housing and industry. For these reasons, along with the monetary benefits, urea has been added to feeds for ruminants to prevent the waste of valuable protein.

The Board on Agriculture and Renewable Resources (1976) cited several factors which may cause urea toxicity to develop: (1) lack of an adequate adaptation period to urea-containing diets, suggesting that it was important to start feeding urea at low levels and increase gradually over a period of several days, especially if high levels of urea are fed; (2) fasting prior to urea consumption; (3) the feeding of urea in diets composed primarily of poor-quality roughages; (4) the feeding of diets that promote a high pH in ruminal fluid; and (5) low water intake. Errors in formulation and improper mixing of urea with other diet ingredients are probably the major factors causing urea toxicity in the feeding of ruminants.

Toxicity occurs when excess ammonia produced by hydrolysis of urea accumulates in body fluids and tissues. Symptoms of acute ammonia toxicity in the ruminant appear
to be progressive as follows: the animal becomes nervous and uneasy, salivates excessively, and demonstrates muscular tremors; these symptoms are followed by incoordination, respiratory difficulty, and frequent urination and defecation; the front legs begin to stiffen, and the animal becomes prostrate; violent struggling, bellowing, and terminal tetanic spasms occur in most animals; the jugular pulse is marked, and bloating is common; death occurs within 0.5 to 2.5 hours after the initial symptoms are observed. (The Board on Agriculture and Renewable Resources, 1976).

Treatment for urea toxicity is usually effective if applied before tetanic spasms occur. Therefore, it is important to be able to correctly diagnose toxicity. Blood ammonia levels can be used as a diagnostic tool, but are difficult to determine. The purpose of this study was to measure the changes occurring in certain blood chemistries during acute ammonia intoxication. It was hoped that this study would provide information which would provide a rapid diagnosis and a more effective treatment of urea toxicity. This information may also be used to develop more effective ways to use urea supplements, and therefore conserve the world's supply of protein.
Use of Urea in Feed

It was almost a century ago that it was first suggested that nitrogen from sources other than protein could be used beneficially in rations for ruminants. Briggs (1967) reported that until the mid-1930's it was widely disputed, and it was believed that urea was not converted to protein by the ruminant in any significant amount. Rather, the protein sparing action of urea was thought to be due to neutralization of organic acids formed in digestion.

Hart et al. (1939) published the results of the first intensive research in the U.S.A. on the use of urea and ammonium bicarbonate in ruminant rations. They concluded that ruminants could use simple nitrogen compounds through the action of the rumen microorganisms. By 1940 a considerable body of knowledge on the use of urea in ruminant rations had accumulated. Hart further concluded, that up to one-third of the nitrogen in a ration could be safely replaced by urea or ammonium salts, such as ammonium bicarbonate. However, the amount of urea which could be used in ruminant rations also depended upon the amount and kind of carbohydrates and true proteins in the diet.

Utilization of Urea

Nolan and Leng (1972) suggested that the digestive tract distal to the rumen was the major site of urea degradation.
in sheep, but the relative importance of the small intestine, caecum and large intestine was not known. The Board on Agriculture and Renewable Resources (1976) stated that when urea was fed, the following steps appeared to be involved in its complete utilization:

1. \[ \text{Urea} \xrightarrow{\text{urease}} \text{NH}_3 + \text{CO}_2 \]

2. Carbohydrates \( \xrightarrow{\text{enzymes}} \) Volatile Fatty Acids + Keto Acids

3. \( \text{NH}_3 + \text{Keto Acids} \xrightarrow{\text{enzymes}} \text{Amino Acids} \)

4. Amino Acids \( \xrightarrow{\text{enzymes}} \text{Microbial Protein} \)

5. Microbial Protein \( \xrightarrow{\text{animal enzymes in the abomasum and small intestine}} \) Free Amino Acids

6. Free amino acids are absorbed from the small intestine and used by the host animal.

**Physiology of Ammonia Toxicity**

**Transport of ammonia**

Davidovich et al. (1977a) observed that when urea hydrolysis occurred more rapidly than the resulting products could be utilized, excess ammonia diffused from the ruminal and omasal epithelium into the portal system where it traveled to the liver. The facility and rate of ammonia transfer across the epithelial wall was shown not only to depend on concentration gradient, but also on the pH of the rumen liquor, according to Briggs (1967). Ammonia was most toxic in conditions of high pH where the non-ionized \( \text{NH}_3 \) form was
formed; most membranes were more permeable to the NH$_3$ moiety than to the ammonium ion. Davidovich et al. (1977b) reported that the optimum pH for ureolytic activity was between 7.7 and 8.5, so a high ruminal pH would enhance the rate of ammonia production from urea. Blood ammonia began to rise when the liver could no longer detoxify the ammonia from portal blood.

Effects on electrolytes

Davidovich et al. (1977b) reported that arterial blood pH increased from 7.443 when cattle were dosed with urea solution to 7.500 when toxic symptoms occurred, while pCO$_2$ was essentially unchanged. Thus the pH increase indicated the manifestation of a metabolic alkalosis. Roller (1966) and Lloyd (1970) reported an initial increase in venous blood pH after urea dosing followed by a considerable decrease in pH at the time of death. Lloyd observed an increase in blood lactic acid to the time of death, which would increase the acidotic effect. Romkema (1978) also reported that acidosis occurred as intoxication progressed.

It was shown by Davidovich et al. (1977c) that serum calcium, phosphorus and magnesium decreased during ammonia toxicity. Chow and Pond (1971) reported that ammonia would react with magnesium and phosphorus ions in alkaline solutions to form magnesium ammonium hexahydrate, which made the magnesium biologically unavailable. Lloyd (1970) observed
that serum calcium, potassium and magnesium decreased immediately after urea was given to cattle or sheep, but then increased significantly until death. Potassium increased twofold, but the increases in calcium and magnesium were small. Roller (1966) reported a significant increase in potassium. Albano and Francavilla (1971) suggested that ammonia would replace potassium in the red blood cell while sodium remained unchanged. Thus, during ammonia toxicity, when blood ammonia was elevated, plasma potassium should increase while the potassium content of the red blood cell should decrease. Henry et al. (1977) demonstrated a decrease in magnesium and inorganic phosphorus, with no significant changes in calcium and potassium levels with high ruminal ammonia levels. Changes in the balance of monovalent (K⁺, NH₄⁺, Na⁺) and divalent (Ca⁺², Mg⁺², diamines) ions could alter membrane potentials and consequently, change nervous excitability which was noted during ammonia toxicity.

Effects on energy metabolism

Davidovich et al. (1977b) reported an increase in blood glucose at the time of toxicity. Singer (1969) attributed the hyperglycemia to reduced glucose utilization due to an imbalance in tricarboxylic-acid-cycle metabolism brought about primarily by overloading the urea cycle and to a lesser extent to hepatic glycogenolysis caused by
adrenalin release. However, Prior et al. (1971) stated that ammonia would cause refractiveness to insulin, resulting in decreased peripheral glucose uptake. Grunnet and Katz (1978) suggested that the stimulation of gluconeogenesis by ammonia was caused by an increase in the concentration of the amino acids involved in the transport of carbon across the mitochondrial membrane.

Romkema (1978) observed a significant increase in triglyceride levels during ammonia intoxication in sheep. He attributed this to the possible disruption of the tricarboxylic-acid-cycle. However, there was no significant changes in the levels of free fatty acids and volatile fatty acids between control and intoxicated animals.

It has also been demonstrated by Skaper et al. (1978) that excessive amounts of ammonia interfered with purine nucleotide biosynthesis by stimulating production of carbamyl phosphate through mitochondrial synthetase. The excess carbamyl phosphate in turn increased pyrimidine nucleotide synthesis de novo and diminished the phosphoribosyl pyrophosphate available for purine biosynthesis.

Effects on other enzyme systems

There are several enzymes of ammonia assimilation that may also be regulated by the concentration of ammonia in the rumen. Erfle et al. (1977) found that ammonia concentration had little effect on glutamate dehydrogenase
(GDH) activity. However, Salem et al. (1973) discovered that as diets approached 100% of the added nitrogen as urea, activity actually decreased. The formula follows:

$$\alpha$$-ketoglutarate + NH$_4^+$ + NAD(P)H $\xrightarrow{\text{GDH}}$ glutamate + NAD (P)$^+$

Decreased enzyme activity could be due to the fact that ammonia inhibited the conversion of isocitrate to $\alpha$-ketoglutarate, which was an important compound for the reaction of glutamate dehydrogenase. Therefore, the activity of glutamate dehydrogenase could be decreased due to a lack of substrate required for the following enzyme reaction:

$$\text{isocitrate} + \text{NAD} \rightleftharpoons \text{oxalosuccinate} + \text{NADH} + \text{H}^+ \rightleftharpoons \alpha$$-ketoglutarate + CO$_2$

Both studies above showed that glutamine synthetase (GS) activity was reversible (increased as urea concentration in the diet decreased and vice versa) as follows:

$$\text{glutamate} + \text{NH}_4^+ + \text{ATP} \xrightarrow{\text{GS}} \text{glutamine} + \text{ADP} + \text{phosphate}$$

This enzyme system was demonstrated to be a very important pathway for ammonia metabolism in urea-fed animals.

Salem et al (1973) noted that carbamyl phosphate synthetase was of minor importance in the ruminal assimilation of ammonia by the mixed rumen microbial population. However, Skaper et al. (1978) found that excess ammonia stimulated mitochondrial synthetase.

Erfle et al (1977) reported that when ammonia concentrations were high, alanine concentration rose rapidly.
Glutamic pyruvic transaminase (GPT) activity increased as ammonia concentrations increased. This suggested that alanine might serve as a storage depot for ammonia and also might provide an additional route for transport for glucose precursors, (i.e., pyruvate) from the rumen. The proposed formula is: glutamate + pyruvate $\xrightarrow{\text{GPT}}$ alanine + $\alpha$-ketoglutarate. The same study also demonstrated that ammonia concentration had no effect on glutamic-oxaloacetic transaminase (GOT) activity, which is shown as follows:

$\text{glutamate} + \text{oxaloacetate} \xrightarrow{\text{GOT}} \text{asparate} + \alpha$-ketoglutarate.

Therefore, it has been deduced that high concentrations of urea in the diet inhibited the activity of glutamate dehydrogenase and glutamine synthetase. Carbamyl phosphate synthetase and glutamic-oxaloacetic transaminase may or may not have been affected by urea concentrations in the diet. Glutamic pyruvic transaminase activity was increased by increased ammonia concentrations.
MATERIALS AND METHODS

Experimental Animals

This experiment was undertaken to determine the effects of ammonia toxicity on various blood chemistries. Ten young Southdown sheep were used as the experimental animals. The sheep were housed indoors and fed a mixture of alfalfa and brome grass hay ad libitum with water. Feed and water were withheld 24 hours prior to experimentation. Six sheep were randomly chosen for the principal group and four for the control group.

Experimental Procedures

The six principal sheep were weighed and ranged in weight from 18.2 kilograms to 31.4 kilograms. Prior to treatment, approximately 20 ml. of jugular vein blood was drawn. Each sheep was administered by drench 12.5 ml. of 3.3 M urea solution per kilogram of body weight. Following treatment, 20 ml. of blood was drawn by jugular venipuncture at 30-minute intervals until death occurred, at which time a final aliquot was drawn. The blood was collected in plain test tubes and stored in a refrigerator until the serum could be separated.

The four control sheep also had approximately 20 ml. of blood drawn prior to treatment. The sheep, ranging in weight from 17.7 kg. to 30 kg., were given 12.5 ml. of water per kilogram of body weight. Blood samples were subsequently drawn at 30-minute intervals. Final samples for the control
animals were collected at a time which approximated the average time of death of principal animals.

Analytical Procedures
Blood ammonia

Whole blood ammonia concentrations were determined to insure that the sheep were indeed intoxicated. The method used to determine blood ammonia nitrogen (BAN) was developed by Jaquette et al. (1974)

Blood chemistry determinations

The blood chemistries were performed on the Sequential Multiple Autoanalyzer Computer \(^a\) (SMAC). The explanations of each test method are as follows:

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is an enzyme that catalyzes the following reaction:

\[
\text{L-lactic acid} + \text{NAD}^+ \xrightleftharpoons{\text{LDH}} \text{pyruvic acid} + \text{NADH} + \text{H}^+
\]

Whereas NAD has no absorption at 340 nm., the reduced form of this coenzyme, NADH, has its absorption peak at this wavelength, and the enzymatic activity is proportional to the amount of NADH produced. In this method, LDH was measured by a two-point rate reaction.

\(^a\)Technicon Instruments Corporation, Tarrytown, New York 10591

(All reagents used in the test procedures are available through Technicon.)
A serum sample was added to the "LDH Substrate" (buffered lactic acid solution). Nicotinamide-adenine dinucleotide (NAD) was then added to initiate the enzyme reaction. After a brief incubation period (20 to 25 seconds) at 37°C, the absorbance was measured at 340 nm. in flowcell number 1. The reaction mixture was further incubated in a second 37°C incubation bath, and the final absorbance measurement made in flowcell number 2. The increase in absorbance with time was proportional to the LDH activity.

Alkaline Phosphatase

The determination of alkaline phosphatase was based on the enzymatic hydrolysis of p-nitrophenyl phosphate (PNPP). The serum sample was added to a stream of PNPP reagent. The reaction stream entered an incubator where the following color-producing reaction proceeded at a temperature of 37°C and at a pH of 10.25:

\[
p \text{-nitrophenyl phosphate (colorless)} \xrightarrow{\text{phosphatase}} p \text{-nitrophenol (bright yellow)} + \text{H}_3\text{PO}_4 + \text{H}_2\text{O}
\]

Following incubation, the free p-nitrophenol was dialyzed into a buffered Alkaline Phosphatase Recipient Diluent. The dialysis was performed to separate the p-nitrophenol from the bile pigment bilirubin. Since bilirubin absorbed light at approximately the same wavelength as p-nitrophenol, the added absorbance of bilirubin would cause a positive error in the determination of alkaline phosphatase activity.
The absorbance of the analytical stream was measured at 410 nm.

**Glutamic-Oxaloacetic Transaminase (GOT)**

Glutamic-oxaloacetic transaminase (GOT) activity was determined by a three-point rate reaction. The chemical reactions applicable to this method are as follows:

1. \[ \text{aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{GOT}} \text{oxaloacetate} + \text{glutamate} \]
2. \[ \text{oxaloacetate} + \text{NADH} \xrightarrow{\text{malate dehydrogenase}} \text{NAD} + \text{malate} \]

All reagents except the \(\alpha\)-ketoglutarate reagent were combined with the sample and incubated for 102 seconds at 37°C. Following the addition of the \(\alpha\)-ketoglutarate reagent, the transamination (1) and dehydrogenase (2) reactions proceed, with additional incubation of 66 seconds (also at 37°C) prior to measurement in flowcell number 1.

After removal of endogenous serum interferences in the first heating bath, the decrease in the absorbance of the analytical stream (determined at 340 nm.) was measured at three points: flowcells number 1, number 2, and number 3. The reaction slopes as determined from the absorbance measurements in flowcell number 1 and flowcell number 2, and flowcell number 3 were compared by the computer for linearity. If the slopes agreed, the absorbance difference between flowcell number 1 and flowcell number 3 was used to calculate the GOT activity.
Creatine Phosphokinase (CPK)

The creatine phosphokinase method required the use of two independent, but interrelated channels: a sample channel and a blank channel. The sample channel serum sample was added to the CP/ADP/Cysteine Reagent. In order to insure maximum CPK activity, an excess of sulfhydryl-groups (from cysteine) was incubated at 37°C for 2 minutes and 25 seconds, at which time the following reaction proceeded:

\[
\text{creatine phosphate (CP)} + \text{ADP} \underset{\text{CPK}}{\overset{\text{creatine} + \text{ATP}}{\rightleftharpoons}}
\]

The reaction stream was dialyzed into a recipient stream of NEM (N-ethylmaleimide) Reagent. The NEM Reagent terminated the enzymatic reaction and also prevented the sulfhydryl groups from interfering with the creatine coupling reaction.

The Diacetyl/Orcinal Reagent was added, and a condensation product was formed, which developed a strong color upon the addition of the Sodium Hydroxide Solution and incubation at 45°C. Ethylenediaminetetraacetic acid was added to prevent the precipitation of magnesium hydroxide.

The blank channel serum sample was added to a modified substrate which did not contain the creatine phosphate necessary for the enzymatic reaction. As a result, the absorbance measured in the blank channel was predominantly due to endogenous serum pigments.

The absorbance of each channel was determined at 520 nm. Blank subtraction was performed by differential colorimetry.
Total Bilirubin

The total bilirubin method required the use of two independent but interrelated test channels: a sample channel and a blank channel. In the sample channel, the serum sample was added to a stream of "Caffeine Diluent." This stream reacted with the "Diazo" reagent to form an azobilirubin complex. To the stream containing the azobilirubin complex (red color), a strongly alkaline sodium-potassium tartrate buffer was added, which solubilized protein and eliminated the effect of variation in sample pH. Upon the addition of the alkaline buffer reagent, a conversion in color occurred from a neutral pink to an alkaline blue azobilirubin. The final color appeared green since the blue alkaline azobilirubin complex was mixed with the yellow pigments derived from the reaction of caffeine with sulfanilic acid. Specificity was achieved by measuring the absorbance of the azobilirubin complex at 600 nm. In the blank channel, the sample was added to the "Caffeine Diluent." Subsequent additions of "Sulfanilic Acid" and "Sodium Potassium Tartrate" were made to provide a similar chemical environment to that present in the sample channel. However, missing from the blank channel and present in the sample channel was the "Diazo" reagent that combines with the bilirubin in the serum sample to form the azobilirubin complex. As a result, the absorbance determined in this channel was predominantly that of endogenous serum pigments.
The absorbance of each channel was measured at 600 nm. Blank subtraction was accomplished automatically by differential colorimetry.

**Direct Bilirubin**

The direct bilirubin method required the use of two independent but interrelated channels: a sample channel and a blank channel. In the sample channel, the serum sample was added to a stream of Direct Bilirubin Sample Diluent. This stream reacted with the "Diazo" reagent to form an azobilirubin complex. Since the direct reaction was time dependent, the reaction was stopped, after two minutes, by the addition of the ascorbic acid reagent which inactivated the "Diazo" reagent. After the reaction was quenched, a strongly alkaline sodium potassium tartrate buffer was added, which solubilized the protein and eliminated the effect of variation in sample pH. Upon the addition of the alkaline buffer to the serum sample, a conversion in color took place from the neutral pink to the alkaline blue azobilirubin.

In the blank channel, the sample was added to the Direct Bilirubin Sample Diluent. Subsequent additions of "Sulfanilic Acid", Ascorbic Acid Reagent, and "Sodium Potassium Tartrate" were made to provide a similar chemical environment to that present in the sample channel. The "Diazo" reagent that combined with the bilirubin in the serum sample to form the azobilirubin complex was used in the sample channel, but
was not used in the blank channel. As a result, the absorbance determined in the blank channel was produced predominantly by endogenous serum pigments.

As the sample and blank analytical streams reached the colorimeter flowcells, the absorbance of each stream was measured at 550 nm. Blank subtraction was accomplished automatically.

Total Protein

The total protein method required the use of two independent but interrelated channels: a sample channel and a blank channel. In the sample channel, the serum sample was added to an air-segmented stream of biuret reagent. During the ensuing reaction, the protein in the sample combined with copper in the biuret reagent to form a purple complex. Sodium potassium tartrate acted as a complexing agent, and potassium iodine prevented autoreduction.

In the blank channel, the sample was added to an air-segmented stream of total protein blank solution. The blank solution contained all of the constituents of the biuret reagent except copper sulfate and sodium potassium tartrate. These two reagents normally react with the protein in the sample to produce the final reaction color. Their absence from the blank channel prevented any color development in the channel. As a result, the absorbance determined in the blank channel was predominantly due to endogenous serum pigments.
The absorbance of each channel was determined at 550 nm. Blank subtraction was performed by differential colorimetry.

Albumin

The serum sample was added to the bromcresol green (BCG) reagent to form the albumin-BCG complex. The pH of the reaction was regulated by a buffer in the BCG reagent. Optical interferences from turbid or lipemic samples were minimized by the use of a surfactant within the BCG reagent.

The absorbance of the analytical stream was measured at 630 nm.

Albumin-Globulin Ratio

This ratio was computed by using the following equation:

\[
A/G = \frac{\text{albumin (gm/dl)}}{\text{total protein (gm/dl) - albumin (gm/dl)}}
\]

Glucose

The principle of the glucose method was that it combined the specificity of a glucose oxidase reaction with a new peroxidase indicator reaction.

A direct sample was diluted with Glucose Sample Diluent, mixed, and dialyzed into a buffered Glucose Oxidase Reagent. The solution was incubated in a 37° C heating bath to allow the enzymatic reaction to take place.

A solution of 3-methyl-2-benzothiazolinone hydrazone and dimethylaniline (MBTH/DMA) was added and mixed with the
recipient stream that contained the glucose oxidase reagent. Peroxidase Reagent was added and mixed with the indicator hydrogen peroxide (H$_2$O$_2$) solution and oxidative coupling of MBTH and DMA formed the soluble indamine dye. The amount of dye formed was proportional to the amount of glucose present in the original sample. The color reaction took place at room temperature, and the stable blue color of the reaction product was measured at 600 nm.

**Blood Urea Nitrogen (BUN)**

The serum sample was diluted in an air-segmented stream of BUN Sample Diluent. The urea which was highly soluble in water, was dialyzed across a Type C membrane into a stream of "BUN Color Reagent" containing diacetyl-monoxine and thiosemicarbazide. "BUN Acid" reagent, containing ferric chloride and sulfuric acid, was added to the recipient stream. After the addition of the "BUN Acid," the recipient stream was heated to 90°C, at which point the thiosemicarbazide in the "BUN Color Reagent" intensified the color. The absorbance of the analytical stream was measured at 520 nm.

**Creatinine**

The serum sample was diluted with Creatinine Sample Diluent. This diluted sample was dialyzed against the Creatinine Recipient Solution to remove the creatinine in the sample from protein and other endogenous serum interferences. Sodium Hydroxide Solution (creatine) and
"Creatinine Color Reagent" were added to the recipient stream to form the red-colored chromogen in an alkaline medium. This reaction mixture was heated to 37° C to accelerate development of the chromogen. The absorbance of the analytical stream was measured at 505 nm.

BUN-Creatinine Ratio

This ratio was derived by dividing the BUN value by the creatinine value.

Cholesterol

The serum sample was combined with an air-segmented stream of "Cholesterol Color Reagent." During the ensuing reaction, the color of the mixture changed from red, to violet, and finally to green. The following mechanism has been proposed for the reaction of cholesterol with Liebermann-Burchard reagent:

\[
\begin{align*}
(1) \text{Cholesterol} + H_2SO_4 & \longrightarrow \text{bis-cholestadienyl monosulfonic acid} \\
(2) \text{Bis-cholestadienyl} + H_2SO_4 & \longrightarrow \text{bis-cholestadienyl disulfonic acid}
\end{align*}
\]

Since these reactions were highly exothermic, a heat exchanger was used to maintain the optimum reaction temperature. The absorbance of the analytic stream was measured at 630 nm.

Triglyceride

The serum triglycerides method required the use of two independent but interrelated channels: a sample channel
and a blank channel. In the sample channel the pre-diluted serum sample was added to the lipase reagent. This solution was incubated at 37°C at which time the following enzymatic reaction occurred:

$$\text{triglycerides} + \frac{\text{lipase}}{\text{protease}} \rightarrow \text{glycerol} + \text{free fatty acids}$$

The glycerol product of this enzymatic reaction was dialyzed into a recipient solution of glycerol kinase reagent and glycerol substrate reagent. This solution was incubated at 37°C to allow the following reactions to take place:

1. $$\text{glycerol} + \text{ATP} \rightarrow \text{glycerol phosphate} + \text{ADP}$$
2. $$\text{ADP} + \text{phosphoenolpyruvate} \rightarrow \text{ATP} + \text{pyruvate}$$
3. $$\text{pyruvate} + \text{NADH} \rightarrow \text{lactate} + \text{NAD}$$

The absorbance of the reaction solution was measured at 340 nm. The absorbance decrease determined in this channel was predominantly due to endogenous serum interferences. Blank subtraction was performed automatically by the SMAC system, and the concentration of the triglycerides was inversely proportional to the absorbance measured.

Low Density Lipoprotein (LDL)

This parameter was based on the following calculation:

$$\text{LDL} = \text{Cholesterol} - \left( \frac{\text{Triglyceride}}{5} + 45 \right)$$
Calcium

Prediluted serum was added to a diluted solution of hydrochloric acid (HCl) containing 8-hydroxyquinoline. The HCl released the protein bound calcium, and the 8-hydroxyquinoline bound the free magnesium ions present in the serum. The free ionized calcium ions were dialyzed across a semipermeable, type H membrane into the analytical stream of "Cresolphthalein Complexone", containing additional 8-hydroxyquinoline. Upon the addition of Diethylamine to the analytical stream, a colored complex was formed between the calcium and the dye. The reaction stream was heated at 37°C to fully develop the colored complex. The absorbance of the reaction product was measured at 570 nm.

Iron

The serum iron method was based on the interaction of the chromogen Ferrozine with protein-free iron. The pre-diluted serum sample was further diluted with the Ascorbic Acid Reagent which liberated the iron from transferrin and reduced it to the ferrous state. The Ascorbic Acid Reagent Diluent contained sodium chloride to prevent Donan equilibrium effects and neocuproine hydrochloride to prevent interference by copper.

Free ferrous ions were dialyzed into a recipient stream of "Iron Color Reagent" by means of a 12-inch dialyzer equipped

--
bHach Chemical Company, Ames, Iowa
with a type H membrane. A FerroZine complex was formed in
the presence of sodium acetate and the absorbance of the
reaction stream was measured at 560 nm.

Inorganic Phosphorus
The serum sample was mixed with Inorganic Phosphorus
Sample Diluent and dialyzed into an Inorganic Phosphorus
Recipient Diluent. The method utilized a dialyzer with a
12-inch path length. Following dialysis, ammonium molybdate
was added, and the phosphomolybdate complex formed passed to
the colorimeter where the absorbance was measured at 340 nm.

Sodium
The serum sample was first added to a buffer solution.
The buffered serum sample was heated to 55°C to eliminate
potassium in transient effects. As the buffered serum sample
flowed along the glass membrane of the sodium selective elec-
trode, it caused a change in electrical potential between
the ionically constant outer surface and the ionically
variable inner surface. This change in electrical potential
was measured against a reference electrode by an operational
amplifier, and the resulting difference signal was sent to
the computer.

Potassium
The serum sample was added to an air-segmented stream
of buffer solution. As the buffered serum sample flowed
past the valinomycin membrane of the ion-selective electrode, it caused a change in electrical potential between the ionically constant outer surface and the ionically variable inner surface which was in contact with the analytical stream. This change in electrical potential was measured against a reference electrode by an operational amplifier, and the resulting difference signal was sent to the computer.

Chloride

The chloride sample was added to an air-segmented stream of Chloride Sample Diluent. The stream then was dialyzed against an air-segmented stream of Chloride Recipient Solution. Dialysis was performed to remove the chloride sample from protein and serum pigment inferences. The "Chloride Color" reagent was added to the recipient stream where the following reactions occurred:

\[
(1) \quad \text{Hg(SCN)}_2 + 2\text{Cl}^- \rightarrow \text{HgCl}_2 + 2\text{(SCN)}^- \\
(2) \quad 3\text{(SCN)}^- + \text{Fe}^{3+} \rightarrow \text{Fe} \quad \text{(SCN)}_3 \quad \text{red complex}
\]

The absorbance of the analytical stream was measured at 480 nm.
RESULTS

Two of the six sheep in the principal group died 90 minutes following the administration of the 3.3 M urea solution. Therefore, only four values were used in the averages of blood chemistries for the terminal time period. Average values for blood chemistries at each of the sampling times are recorded in tables 1, 2 and 3 for the principal and control sheep. When blood chemistry values exceeded the upper limits of linearity, this upper limit was used for that value when the averages for each time period were calculated. Those parameters showing significant changes (P < 0.05) during intoxication were included in these tables. The values for all of the chemistries measured are in the appendix.

Table 4 lists the blood chemistries that were significant at the 0.05 level. This table includes the significant step and the variance explained, which reveals the amounts of variability within the treatment or principal group.

The enzymes alkaline phosphatase, glutamic-oxaloacetic transaminase, and creatine phosphokinase increased during the course of increased ammonia toxicity. Lactate dehydrogenase did not change to any significant degree.

There were varied changes in the organic blood chemistries as time progressed during intoxication. Total bilirubin, direct bilirubin, total protein A/G ratio and cholesterol remained near pretreatment values. Albumin, glucose,
blood urea nitrogen, creatinine, BUN/creatinine ratio and low density lipoprotein levels rose throughout the experimental period in the principal group sheep. Triglycerides were the only organic parameter to decrease with time.

All of the inorganic blood chemistries demonstrated some degree of change during the experimental period. Calcium, iron, inorganic phosphorus, sodium and potassium increased, while chloride levels decreased as toxicity progressed. No such trend occurred in the control group.
Table 1-Average enzyme values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Alkaline Phosphatase (U/L)</th>
<th>Glutamic-Oxaloacetic Transaminase (U/L)</th>
<th>Creatine Phosphokinase (U/L)+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>129</td>
<td>91</td>
<td>119</td>
</tr>
<tr>
<td>30 minutes</td>
<td>148</td>
<td>98</td>
<td>120</td>
</tr>
<tr>
<td>60 minutes</td>
<td>153</td>
<td>98</td>
<td>153</td>
</tr>
<tr>
<td>90 minutes</td>
<td>154</td>
<td>100</td>
<td>209</td>
</tr>
<tr>
<td>terminal*</td>
<td>153</td>
<td>125</td>
<td>757</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>132</td>
<td>80</td>
<td>63</td>
</tr>
<tr>
<td>30 minutes</td>
<td>137</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>60 minutes</td>
<td>129</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>90 minutes</td>
<td>137</td>
<td>78</td>
<td>96</td>
</tr>
<tr>
<td>terminal**</td>
<td>131</td>
<td>78</td>
<td>108</td>
</tr>
</tbody>
</table>

+Creatine phosphokinase averages include some values using the upper limit of 2000 U/L, which was the upper limit of linearity.

*Six sheep were drenched with urea solution, but only four survived past the 90 minute interval. Average enzyme values in the terminal interval were calculated, using data collected for these four principal animals only.

**Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 2-Average organic blood chemistries for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Albumin (gm/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Blood Urea Nitrogen (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Blood Urea Nitrogen/Creatinine</th>
<th>Triglyceride (mg/dl)</th>
<th>Lipoprotein Density (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>4.6</td>
<td>70</td>
<td>25</td>
<td>0.9</td>
<td>29.47</td>
<td>12</td>
<td>13.2</td>
</tr>
<tr>
<td>30 minutes</td>
<td>4.7</td>
<td>87</td>
<td>47</td>
<td>0.9</td>
<td>50.81</td>
<td>6</td>
<td>16.9</td>
</tr>
<tr>
<td>60 minutes</td>
<td>4.7</td>
<td>149</td>
<td>58</td>
<td>1.0</td>
<td>59.17</td>
<td>3</td>
<td>18.7</td>
</tr>
<tr>
<td>90 minutes</td>
<td>4.8</td>
<td>171</td>
<td>65</td>
<td>1.3</td>
<td>62.26</td>
<td>3</td>
<td>18.6</td>
</tr>
<tr>
<td>terminal*</td>
<td>5.1</td>
<td>228</td>
<td>67</td>
<td>1.2</td>
<td>56.38</td>
<td>0</td>
<td>19.5</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>4.4</td>
<td>74</td>
<td>29</td>
<td>0.8</td>
<td>37.91</td>
<td>14</td>
<td>9.8</td>
</tr>
<tr>
<td>30 minutes</td>
<td>4.3</td>
<td>76</td>
<td>28</td>
<td>0.8</td>
<td>34.54</td>
<td>16</td>
<td>8.6</td>
</tr>
<tr>
<td>60 minutes</td>
<td>4.4</td>
<td>74</td>
<td>28</td>
<td>0.8</td>
<td>34.85</td>
<td>12</td>
<td>9.7</td>
</tr>
<tr>
<td>90 minutes</td>
<td>4.5</td>
<td>71</td>
<td>28</td>
<td>0.8</td>
<td>35.65</td>
<td>13</td>
<td>11.2</td>
</tr>
<tr>
<td>terminal**</td>
<td>4.4</td>
<td>72</td>
<td>29</td>
<td>0.9</td>
<td>33.35</td>
<td>12</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Six sheep were drenched with urea solution, but only four survived past the 90 minute interval. Average organic blood chemistries in the terminal interval were calculated, using data collected for these four principal animals only.

**Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 3-Average inorganic blood chemistries for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calcium (mg/dl)</th>
<th>Iron (mcg/dl)</th>
<th>Inorganic Phosphorus (mg/dl)</th>
<th>Sodium(^+) (mEq/L)</th>
<th>Potassium(^++) (mEq/L)</th>
<th>Chloride (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>10.1</td>
<td>136</td>
<td>6.0</td>
<td>149</td>
<td>5.5</td>
<td>106</td>
</tr>
<tr>
<td>30 minutes</td>
<td>10.2</td>
<td>138</td>
<td>6.2</td>
<td>151</td>
<td>6.3</td>
<td>107</td>
</tr>
<tr>
<td>60 minutes</td>
<td>9.7</td>
<td>139</td>
<td>6.3</td>
<td>152</td>
<td>6.5</td>
<td>106</td>
</tr>
<tr>
<td>90 minutes</td>
<td>9.9</td>
<td>146</td>
<td>7.0</td>
<td>154</td>
<td>7.2</td>
<td>105</td>
</tr>
<tr>
<td>terminal(*)</td>
<td>10.3</td>
<td>153</td>
<td>8.5</td>
<td>157</td>
<td>9.3</td>
<td>102</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>10.1</td>
<td>153</td>
<td>6.3</td>
<td>143</td>
<td>5.1</td>
<td>98</td>
</tr>
<tr>
<td>30 minutes</td>
<td>10.0</td>
<td>150</td>
<td>6.5</td>
<td>143</td>
<td>5.0</td>
<td>98</td>
</tr>
<tr>
<td>60 minutes</td>
<td>10.0</td>
<td>148</td>
<td>6.2</td>
<td>144</td>
<td>5.0</td>
<td>98</td>
</tr>
<tr>
<td>90 minutes</td>
<td>10.1</td>
<td>152</td>
<td>5.9</td>
<td>143</td>
<td>4.6</td>
<td>98</td>
</tr>
<tr>
<td>terminal(**)</td>
<td>10.1</td>
<td>152</td>
<td>6.1</td>
<td>143</td>
<td>5.0</td>
<td>101</td>
</tr>
</tbody>
</table>

\(^+\)Sodium averages include some values using the upper limit of 160 mEq/L, which was the upper limit of linearity.

\(^++\)Potassium averages include some values using the upper limit of 10 mEq/L, which was the upper limit of linearity.

\(*\)Six sheep were drenched with urea solution, but only four survived past the 90 minute interval. Average inorganic blood chemistries in the terminal interval were calculated, using data collected for these four principal animals only.

\(**\)Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 4—Blood chemistries that are significant at the 0.05 level when compared to time previous to death in ammonia intoxicated sheep.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significant Step</th>
<th>Variance Explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>Linear &amp; Quadratic</td>
<td>17.91%</td>
</tr>
<tr>
<td>Glutamic-Oxaloacetic Transaminase</td>
<td>Linear, Quadratic, &amp; Cubic</td>
<td>40.97%</td>
</tr>
<tr>
<td>Creatine Phosphokinase</td>
<td>Linear, Quadratic, &amp; Cubic</td>
<td>47.25%</td>
</tr>
<tr>
<td>Albumin</td>
<td>Linear &amp; Quadratic</td>
<td>18.98%</td>
</tr>
<tr>
<td>Glucose</td>
<td>Linear &amp; Quadratic</td>
<td>69.02%</td>
</tr>
<tr>
<td>Blood Urea Nitrogen</td>
<td>Linear</td>
<td>49.61%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Linear &amp; Quadratic</td>
<td>76.18%</td>
</tr>
<tr>
<td>Blood Urea Nitrogen/Creatinine</td>
<td>Linear</td>
<td>31.28%</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>Linear</td>
<td>58.46%</td>
</tr>
<tr>
<td>Low Density Lipoprotein</td>
<td>Linear</td>
<td>55.66%</td>
</tr>
<tr>
<td>Calcium</td>
<td>Linear &amp; Quadratic</td>
<td>25.49%</td>
</tr>
<tr>
<td>Iron</td>
<td>Linear &amp; Quadratic</td>
<td>22.90%</td>
</tr>
<tr>
<td>Inorganic Phosphorus</td>
<td>Linear, Quadratic, &amp; Cubic</td>
<td>59.39%</td>
</tr>
<tr>
<td>Sodium</td>
<td>Linear &amp; Quadratic</td>
<td>40.15%</td>
</tr>
<tr>
<td>Potassium</td>
<td>Linear, Quadratic, &amp; Cubic</td>
<td>85.57%</td>
</tr>
<tr>
<td>Chloride</td>
<td>Linear</td>
<td>41.39%</td>
</tr>
</tbody>
</table>
DISCUSSION

In this investigation, the BAN levels of the principal sheep were compared with those of the control sheep to insure that they were indeed intoxicated. Once ammonia intoxication was established, the data was subjected to analysis of variance to detect differences in treatment of principal and control animals. I have chosen to discuss in detail only those parameters in which the regression accounts for more than 50% of variance, as seen in Table 4. These parameters are glucose, creatinine, triglycerides, low density lipoprotein, inorganic phosphorus and potassium. Even though the remaining parameters were significant, the variance explained was considered too low to justify further discussion.

The blood chemistry levels of the control animals agreed favorably with those measured by Smith et al. (1978). The only exception was lactate dehydrogenase which was higher when compared to the study reported by Smith. A difference in the breed of sheep used may account for the higher lactate dehydrogenase values in this investigation.

The activity of lactate dehydrogenase or alkaline phosphatase did not change to any significant degree during intoxication. Serum glutamic-oxaloacetic transaminase did increase slightly, especially prior to death of the principal sheep. Erfle et al. (1977) concluded that there was no change in activity of this enzyme, however, the experimental animals were not given lethal doses of urea. As intoxication
progressed, the damage to the liver may have increased causing increased levels of glutamic-oxaloacetic transaminase. The enzyme revealing the most change was creatine phosphokinase. The increased levels of this enzyme are most likely due to the muscular tremors experienced by the principal sheep during intoxication.

This study, along with several previous studies, demonstrated an increase in glucose levels in the principal sheep with time. An increased glucose could be the result of increased production, decreased utilization or a combination of both. Hormonal response to the stress of ammonia intoxication may play an important role in the increased production of glucose. Singer (1969) suggested that the release of adrenalin may result in hepatic glycogenolysis. Adrenalin also enhances lipolysis for fatty acid mobilization and protein catabolism to increase amino acid availability for gluconeogenesis. Glucagon release is also stimulated by adrenalin which in turn results in glycogenolysis. The glucocorticoids released during a stressful situation may also elevate glucose levels via gluconeogenesis, which is accomplished by increasing the synthesis of hepatic enzymes that catalyze the metabolic degradation of amino acids and enzymes needed for gluconeogenesis. Grunnet and Katz (1978) suggested that ammonia stimulated gluconeogenesis due to the increased concentration of amino acids involved in the transport of carbon across the mitochondrial
membrane. All of the above factors lead to hyperglycemia. Failure to utilize the glucose available may also cause hyperglycemia. Singer (1969) stated that overloading the urea cycle during ammonia toxicity could cause an imbalance in the tricarboxylic-acid cycle. Prior et al. (1971) reported that ammonia may cause the cell to become refractive to insulin, therefore, resulting in decreased peripheral glucose uptake. Anything affecting the permeability of the cell membrane may reduce its ability to utilize glucose. Glucose is also dependent upon a carrier before it can diffuse into a cell. Increased ammonia levels may therefore decrease membrane permeability or hinder the carrier in its transport of glucose into the cell, resulting in hyperglycemia.

Serum triglyceride concentrations were decreased in this study. Because glucose was not being utilized, triglycerides could possibly have been broken down as a source of energy. The acidosis reported by Roller (1966), Lloyd (1970) and Romkema (1978) could in part be the result of lipid breakdown releasing ketones which would lower the pH.

Eisenberg (1976) states that low density lipoprotein is derived mainly from very low density lipoproteins which are acted upon by lipases. They are also derived from chylomicrons or directly synthesized in the liver and intestines. Low density lipoproteins increased as ammonia toxicity progressed in this experiment. The presence of high concentra-
tions of urea in the rumen could stimulate the intestinal mucosal cells to produce elevated amounts of low density lipoprotein.

The ornithine cycle, which Gantarow and Schepartz (1967) report is primarily concentrated in the liver, removed ammonia from the body to form urea. When the concentration of ammonia in the blood increases, the liver is stimulated to produce more urea. A rise in blood urea nitrogen occurred in this experiment. An increase may also reflect failure of the kidneys to excrete the blood urea nitrogen. If the animal becomes dehydrated, the rate of urea removal from the blood decreases, due to the fact that the urea concentration gradient is larger for the glomerular filtrate than the renal tubular intracellular fluid as well as interstitial fluid. The differential concentration gradient enhances back-diffusion of urea. The time for passive reabsorption is also longer when the urine volume is minimal.

Creatinine became significantly elevated during ammonia toxicity as time progressed. Since glucose was not effectively being utilized for reasons mentioned earlier, the animal's body may have been using creatine phosphate as a source of energy. Creatine combines with ATP to form ADP and creatine phosphate. The reaction is enhanced by the increased activity of creatine phosphokinase, which was also elevated. The creatine phosphate will then spontaneously
break down into phosphoric acid and creatinine, releasing energy in the process. Failure by the renal tubules to actively excrete creatinine could also lead to accumulation in the blood.

The level of calcium did not change to any significant degree as ammonia toxicity progressed. Other researchers have also reported varying results of calcium during ammonia toxicity. Davidovich et al. (1977c) reported a decrease in serum calcium, while Lloyd (1970) observed an initial decrease followed by an increase in calcium until death. It is doubtful that the decrease measured at the 60 and 90 minute intervals was solely responsible for the tetany exhibited by the animals as they approached death. Smith et al. (1978) reported a normal range of 8.4 - 10.8 mg./dl. for calcium. The lowest calcium measured in this experiment was 8.9 mg./dl. which is well within the normal range reported.

Inorganic phosphorus changes were also significant relative to time in principal animals. A steady increase in phosphorus occurred as the experimental animals approached death. Previous studies performed by Davidovich et al. (1977c) and Henry et al. (1977) report a decrease in phosphorus levels. However, neither of the above studies involved administration of lethal doses of urea. The accumulation of phosphorus in the blood could be caused by the release of phosphoric acid during the metabolism of ATP.
in the muscles. Because the kidneys govern the excretion of phosphorus, renal failure could also lead to an accumulation of phosphorus as the animal approached death.

Several studies, including this one, reported a significant increase in potassium during ammonia toxicity. Lloyd (1970) demonstrated an initial decrease in potassium levels followed by a two-fold increase at death. Roller (1966) also reported an increase in serum potassium levels. Albano and Francavilla (1971) suggested that the elevated potassium levels during ammonia toxicity were due to the influx of ammonium ions into the erythrocytes. An influx of ammonium ions forced potassium ions to efflux, thus increasing the serum potassium levels. Serum potassium concentrations are also affected by blood pH. In human subjects, potassium rises about 0.6 mEq. per liter for each 0.1 unit fall in blood pH. Previous studies have revealed that acidosis occurred when animals were administered lethal doses of urea. The renal cells respond to acidosis by retaining potassium ions and excreting hydrogen ions, thus causing hyperkalemia.

Serum sodium levels rose slightly in the principal animals as ammonia toxicity progressed. The renal cells also retain sodium in response to acidosis. The level of potassium may be the greatest factor in sodium retention. An increase of 1 mEq. per liter of potassium will result in a three-fold increase in aldosterone secretion.
Aldosterone acts on the distal renal tubules to enhance reabsorption of sodium.

Serum chloride levels remained constant until death at which time there was a slight drop. Chloride ions are excreted with hydrogen ions to maintain acid-base balance during acidosis. The loss of chloride allows for the reabsorption of bicarbonate ions. The bicarbonate would combine with the excess hydrogen ions to produce carbonic acid. Carbonic acid dissociates into water and carbon dioxide, which can be removed by the lungs. Romkema (1978) reported a significant increase in pCO₂ as the principal animals approached death. Without analysis of urine samples, the role played by the kidneys in ammonia toxicity is only supposition.
The effects of acute ammonia toxicity on certain blood chemistries in sheep were investigated. Ten young Southdown sheep were divided into two groups. Six animals in the principal group were given by drench 12.5 ml. of 3.3 M urea solution per kilogram of body weight. Four control sheep received by drench 12.5 ml. of water per kilogram of body weight. Five aliquots of blood were drawn from each animal, with the exception of two principal animals having only four aliquots drawn, by jugular venipuncture. The first aliquot was drawn prior to drenching, subsequent aliquots were drawn at 30, 60 and 90 minutes after drenching, and a terminal aliquot was collected at death. For two of the principal animals, the 90-minute aliquot and the terminal aliquot were one and the same. Blood was analyzed for blood ammonium nitrogen and twenty serum chemistries which were performed on the SMAC analyzer by Technicon.

Several serum chemistries increased slightly in the principal animals as toxicity progressed. They are as follows: alkaline phosphatase, glutamic-oxaloacetic transaminase, creatine phosphokinase, albumin, blood urea nitrogen, iron, sodium and chloride. Calcium decreased slightly, followed by a slight increase as death approached. Lactate dehydrogenase, total bilirubin, direct bilirubin,
total protein and cholesterol did not change from pretreatment values during ammonia toxicity.

Analysis of lines of regression for principal and control groups demonstrated significant differences for blood ammonium nitrogen levels. It also revealed a significant increase in glucose, creatinine, low density lipoprotein, inorganic phosphorus and potassium. Triglyceride levels were significantly decreased with time.
LITERATURE CITED


Table 3: Analysis of variance for lines of regression of tested parameters with time.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
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<th>M.S. 2</th>
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**APPENDIX**
Table 5.-Analysis of variance for lines of regression of tested parameters with time.

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<th>Time Treat</th>
<th>Remainder</th>
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<th>Time</th>
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<th>Time Treat</th>
<th>Remainder&lt;sup&gt;d&lt;/sup&gt;</th>
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*Significant at 0.05 level.
**Significant at 0.01 level.
<table>
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<th>Parameter</th>
<th>Treat&lt;sup&gt;c&lt;/sup&gt;</th>
<th>A/TRT</th>
<th>A/TRC</th>
<th>Time Rgrsn</th>
<th>Time Treat</th>
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<sup>c</sup> Error term for treatment (treat) is the pooled mean squares for animals within principal animals (A/TRT) and control animals (A/TRC).

<sup>d</sup> Remainder is used for testing time regression (time rgrsn) and time X treatment (time treat).

* Significant at 0.05 level.

** Significant at 0.01 level.
Table 6. - Blood ammonium nitrogen (BAN) values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

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</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 7.—Serum lactate dehydrogenase values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Lactate Dehydrogenase (U/L)</th>
<th>Principal Sheep</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>pretreatment</td>
<td>600</td>
<td>575</td>
<td>600</td>
<td>577</td>
<td>533</td>
<td>600</td>
</tr>
<tr>
<td>30 minutes</td>
<td>600</td>
<td>581</td>
<td>600</td>
<td>600</td>
<td>551</td>
<td>600</td>
</tr>
<tr>
<td>60 minutes</td>
<td>600</td>
<td>554</td>
<td>600</td>
<td>600</td>
<td>580</td>
<td>600</td>
</tr>
<tr>
<td>90 minutes</td>
<td>600</td>
<td>564</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
<td>600</td>
<td>600</td>
<td>-</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>521</td>
<td>557</td>
<td>600</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>527</td>
<td>551</td>
<td>600</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>523</td>
<td>556</td>
<td>600</td>
<td>600</td>
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</tr>
<tr>
<td>90 minutes</td>
<td>539</td>
<td>588</td>
<td>600</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>terminal**</td>
<td>541</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.

+ LDH values in excess of 600 U/L were not recorded because of lack of linearity in the measurement.
Table 8.—Serum alkaline phosphatase values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Alkaline Phosphatase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal Sheep</strong></td>
</tr>
<tr>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>88 111 101 218 248 110</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>96 109 88 233 249 114</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>101 96 102 235 267 118</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>116 99 89 246 257 114</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
<tr>
<td>- 116 90 - 272 133</td>
</tr>
<tr>
<td><strong>Control Sheep</strong></td>
</tr>
<tr>
<td>7 8 9 10</td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>182 118 103 126</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>193 122 110 124</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>186 113 97 119</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>189 126 103 130</td>
</tr>
<tr>
<td>terminal**</td>
</tr>
<tr>
<td>181 121 101 119</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 9.-Serum glutamic-oxaloacetic transaminase values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Glutamic-oxaloacetic Transaminase (U/L)</th>
<th>Principal Sheep</th>
<th>Control Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>pretreatment</td>
<td>84</td>
<td>96</td>
</tr>
<tr>
<td>30 minutes</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>60 minutes</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>90 minutes</td>
<td>114</td>
<td>98</td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
<td>122</td>
</tr>
<tr>
<td>pretreatment</td>
<td>85</td>
<td>64</td>
</tr>
<tr>
<td>30 minutes</td>
<td>86</td>
<td>64</td>
</tr>
<tr>
<td>60 minutes</td>
<td>81</td>
<td>64</td>
</tr>
<tr>
<td>90 minutes</td>
<td>86</td>
<td>64</td>
</tr>
<tr>
<td>terminal**</td>
<td>85</td>
<td>68</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.
** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 10.-Serum creatine phosphokinase values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Creatine Phosphokinase (U/L)⁺</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>146</td>
<td>165</td>
<td>67</td>
<td>81</td>
<td>134</td>
<td>121</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>143</td>
<td>155</td>
<td>59</td>
<td>116</td>
<td>142</td>
<td>108</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>149</td>
<td>75</td>
<td>95</td>
<td>201</td>
<td>225</td>
<td>173</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>359</td>
<td>89</td>
<td>90</td>
<td>278</td>
<td>183</td>
<td>254</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>141</td>
<td>2000</td>
<td>-</td>
<td>348</td>
<td>539</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>51</td>
<td>61</td>
<td>54</td>
<td>86</td>
</tr>
<tr>
<td>30 minutes</td>
<td>69</td>
<td>98</td>
<td>73</td>
<td>67</td>
</tr>
<tr>
<td>60 minutes</td>
<td>68</td>
<td>112</td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td>90 minutes</td>
<td>80</td>
<td>132</td>
<td>96</td>
<td>76</td>
</tr>
<tr>
<td>terminal**</td>
<td>88</td>
<td>172</td>
<td>96</td>
<td>77</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.

+ CPK values in excess of 2000 U/L were not recorded because of lack of linearity in the measurement.
Table 11.-Total serum bilirubin values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>60 minutes</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>90 minutes</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>60 minutes</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>90 minutes</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>terminal**</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 12.-Serum direct bilirubin values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Direct Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal Sheep</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
<tr>
<td><strong>Control Sheep</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal**</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 14.-Serum albumin values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Albumin (gm/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
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<td>4.4</td>
<td>4.5</td>
<td>4.8</td>
<td>4.8</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>4.5</td>
<td>4.5</td>
<td>4.4</td>
<td>5.0</td>
<td>4.8</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>4.5</td>
<td>4.3</td>
<td>4.3</td>
<td>5.1</td>
<td>4.9</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>90 minutes</td>
<td>4.8</td>
<td>4.4</td>
<td>4.3</td>
<td>5.2</td>
<td>5.1</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
<td>5.0</td>
<td>4.5</td>
<td>-</td>
<td>5.5</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>4.4</td>
<td>4.8</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>30 minutes</td>
<td>4.4</td>
<td>4.6</td>
<td>3.8</td>
<td>4.5</td>
</tr>
<tr>
<td>60 minutes</td>
<td>4.4</td>
<td>4.7</td>
<td>3.9</td>
<td>4.4</td>
</tr>
<tr>
<td>90 minutes</td>
<td>4.5</td>
<td>4.8</td>
<td>3.9</td>
<td>4.6</td>
</tr>
<tr>
<td>terminal**</td>
<td>4.4</td>
<td>4.9</td>
<td>3.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 13.-Serum total protein values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Total Protein (gm/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>Principal Sheep</td>
<td>6.9</td>
<td>6.7</td>
<td>7.0</td>
<td>7.3</td>
<td>7.1</td>
<td>7.7</td>
</tr>
<tr>
<td>30 minutes</td>
<td>Principal Sheep</td>
<td>7.2</td>
<td>6.7</td>
<td>6.8</td>
<td>7.6</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td>60 minutes</td>
<td>Principal Sheep</td>
<td>7.3</td>
<td>6.8</td>
<td>6.6</td>
<td>7.6</td>
<td>7.3</td>
<td>7.8</td>
</tr>
<tr>
<td>90 minutes</td>
<td>Principal Sheep</td>
<td>7.8</td>
<td>6.9</td>
<td>6.6</td>
<td>8.0</td>
<td>7.4</td>
<td>7.7</td>
</tr>
<tr>
<td>terminal*</td>
<td>Principal Sheep</td>
<td>-</td>
<td>7.8</td>
<td>6.9</td>
<td>-</td>
<td>7.9</td>
<td>8.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>Control Sheep</td>
<td>6.8</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td>30 minutes</td>
<td>Control Sheep</td>
<td>6.8</td>
<td>6.7</td>
<td>7.1</td>
</tr>
<tr>
<td>60 minutes</td>
<td>Control Sheep</td>
<td>6.7</td>
<td>6.7</td>
<td>7.1</td>
</tr>
<tr>
<td>90 minutes</td>
<td>Control Sheep</td>
<td>6.9</td>
<td>6.9</td>
<td>7.2</td>
</tr>
<tr>
<td>terminal**</td>
<td>Control Sheep</td>
<td>6.7</td>
<td>7.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 15.-Serum albumin-globulin ratios for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>A/G Ratio</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>1.56</td>
<td>1.91</td>
<td>1.80</td>
<td>1.92</td>
<td>2.09</td>
<td>1.57</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>1.67</td>
<td>2.05</td>
<td>1.83</td>
<td>1.92</td>
<td>2.18</td>
<td>1.67</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>1.61</td>
<td>1.72</td>
<td>1.87</td>
<td>2.04</td>
<td>2.04</td>
<td>1.69</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>1.60</td>
<td>1.76</td>
<td>1.87</td>
<td>1.86</td>
<td>2.22</td>
<td>1.75</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>1.79</td>
<td>1.88</td>
<td>-</td>
<td>2.29</td>
<td>1.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>1.83</td>
<td>2.29</td>
<td>1.22</td>
<td>1.73</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>1.83</td>
<td>2.19</td>
<td>1.15</td>
<td>1.88</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>1.91</td>
<td>2.35</td>
<td>1.22</td>
<td>1.76</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>1.88</td>
<td>2.29</td>
<td>1.18</td>
<td>1.70</td>
</tr>
<tr>
<td>terminal**</td>
<td></td>
<td>1.91</td>
<td>2.23</td>
<td>1.19</td>
<td>1.76</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 16.-Serum glucose values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>64</td>
<td>78</td>
<td>72</td>
<td>70</td>
<td>72</td>
<td>62</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>82</td>
<td>107</td>
<td>72</td>
<td>99</td>
<td>85</td>
<td>74</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>221</td>
<td>146</td>
<td>116</td>
<td>227</td>
<td>76</td>
<td>105</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>172</td>
<td>197</td>
<td>172</td>
<td>191</td>
<td>87</td>
<td>205</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>219</td>
<td>195</td>
<td>-</td>
<td>268</td>
<td>229</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>73</td>
<td>76</td>
<td>74</td>
<td>72</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>82</td>
<td>80</td>
<td>70</td>
<td>71</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>80</td>
<td>81</td>
<td>72</td>
<td>64</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>77</td>
<td>72</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>terminal**</td>
<td></td>
<td>71</td>
<td>80</td>
<td>68</td>
<td>69</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 17.—Blood urea nitrogen values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Blood Urea Nitrogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal Sheep</strong></td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
</tbody>
</table>

| **Control Sheep**           | 7  | 8  | 9  | 10 |
| pretreatment                | 27 | 26 | 35 | 26 |
| 30 minutes                  | 26 | 26 | 34 | 26 |
| 60 minutes                  | 26 | 26 | 35 | 26 |
| 90 minutes                  | 26 | 25 | 35 | 26 |
| terminal**                  | 27 | 26 | 35 | 27 |

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 18.-Serum creatinine values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Creatinine (mg/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>1.3</td>
<td>1.0</td>
<td>0.9</td>
<td>1.3</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>1.2</td>
<td>1.2</td>
<td>-</td>
<td>1.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>60 minutes</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>90 minutes</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>terminal**</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 19.-Blood Urea Nitrogen-Creatinine ratios for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Blood Urea Nitrogen-Creatinine Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Sheep</td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
</tbody>
</table>

| Control Sheep    | 7 | 8 | 9 | 10 |
| pretreatment     | 38.57 | 43.33 | 43.75 | 26.00 |
| 30 minutes       | 37.14 | 32.50 | 42.50 | 26.00 |
| 60 minutes       | 37.14 | 32.50 | 43.75 | 26.00 |
| 90 minutes       | 37.14 | 35.71 | 43.75 | 26.00 |
| terminal**       | 33.75 | 28.89 | 43.75 | 27.00 |

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 20.-Serum cholesterol values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/dl)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Principal Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>58</td>
<td>57</td>
<td>66</td>
<td>62</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>30 minutes</td>
<td>65</td>
<td>61</td>
<td>64</td>
<td>68</td>
<td>56</td>
<td>65</td>
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<td>60 minutes</td>
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<td>62</td>
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<td>67</td>
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<td>64</td>
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<tr>
<td>90 minutes</td>
<td>65</td>
<td>64</td>
<td>62</td>
<td>70</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
<td>68</td>
<td>66</td>
<td>-</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>58</td>
<td>56</td>
<td>61</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>58</td>
<td>54</td>
<td>60</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>59</td>
<td>54</td>
<td>61</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 minutes</td>
<td>59</td>
<td>56</td>
<td>62</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>terminal**</td>
<td>58</td>
<td>55</td>
<td>61</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 21.- Serum triglyceride values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Triglyceride (mg/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>12</td>
<td>16</td>
<td>11</td>
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<tr>
<td>30 minutes</td>
<td></td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>6</td>
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<tr>
<td>90 minutes</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th></th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>30 minutes</td>
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<td>15</td>
<td>17</td>
<td>14</td>
<td>17</td>
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<tr>
<td>60 minutes</td>
<td></td>
<td>10</td>
<td>9</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>7</td>
<td>8</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>terminal**</td>
<td></td>
<td>9</td>
<td>9</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
**Table 22.-Serum low density lipoprotein values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.**

<table>
<thead>
<tr>
<th>Low Density Lipoprotein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
</tr>
<tr>
<td>Principal Sheep</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>Terminal*</td>
</tr>
</tbody>
</table>

| Pretreatment                  |
| Control Sheep                | 7   | 8   | 9   | 10  |
| 30 minutes                   | 10.0 | 5.6  | 12.2 | 6.6  |
| 60 minutes                   | 12.0 | 7.2  | 13.0 | 6.4  |
| 90 minutes                   | 12.6 | 9.4  | 14.2 | 8.4  |
| Terminal**                   | 11.2 | 8.2  | 13.0 | 7.4  |

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 23.-Serum calcium values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Principal Sheep</td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>10.0</td>
</tr>
<tr>
<td>30 minutes</td>
<td>10.0</td>
</tr>
<tr>
<td>60 minutes</td>
<td>9.9</td>
</tr>
<tr>
<td>90 minutes</td>
<td>11.3</td>
</tr>
<tr>
<td>terminal*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Sheep</td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>9.8</td>
</tr>
<tr>
<td>30 minutes</td>
<td>9.9</td>
</tr>
<tr>
<td>60 minutes</td>
<td>9.9</td>
</tr>
<tr>
<td>90 minutes</td>
<td>9.8</td>
</tr>
<tr>
<td>terminal**</td>
<td>9.7</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 24.-Serum iron values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Iron (mcg/dl)</th>
<th>Principal Sheep</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td></td>
<td>154</td>
<td>115</td>
<td>97</td>
<td>142</td>
<td>179</td>
<td>126</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>160</td>
<td>113</td>
<td>94</td>
<td>150</td>
<td>178</td>
<td>135</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>161</td>
<td>115</td>
<td>94</td>
<td>155</td>
<td>178</td>
<td>130</td>
</tr>
<tr>
<td>90 minutes</td>
<td></td>
<td>184</td>
<td>120</td>
<td>98</td>
<td>166</td>
<td>178</td>
<td>132</td>
</tr>
<tr>
<td>terminal*</td>
<td></td>
<td>-</td>
<td>142</td>
<td>108</td>
<td>-</td>
<td>201</td>
<td>159</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Sheep</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>156</td>
<td>186</td>
<td>129</td>
<td>141</td>
</tr>
<tr>
<td>30 minutes</td>
<td>153</td>
<td>182</td>
<td>125</td>
<td>140</td>
</tr>
<tr>
<td>60 minutes</td>
<td>152</td>
<td>181</td>
<td>121</td>
<td>139</td>
</tr>
<tr>
<td>90 minutes</td>
<td>155</td>
<td>186</td>
<td>124</td>
<td>143</td>
</tr>
<tr>
<td>terminal**</td>
<td>160</td>
<td>190</td>
<td>121</td>
<td>135</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 25. Serum inorganic phosphorus values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Inorganic Phosphorus (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal Sheep</strong></td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Control Sheep</strong></th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretreatment</td>
<td>7.2</td>
<td>6.2</td>
<td>5.4</td>
<td>6.5</td>
</tr>
<tr>
<td>30 minutes</td>
<td>7.2</td>
<td>6.6</td>
<td>5.7</td>
<td>6.5</td>
</tr>
<tr>
<td>60 minutes</td>
<td>7.0</td>
<td>5.5</td>
<td>5.6</td>
<td>6.8</td>
</tr>
<tr>
<td>90 minutes</td>
<td>6.2</td>
<td>5.5</td>
<td>5.5</td>
<td>6.3</td>
</tr>
<tr>
<td>terminal**</td>
<td>6.8</td>
<td>4.9</td>
<td>5.8</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.
Table 26.-Serum sodium values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th>Sodium (mEq/L)⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Sheep</td>
</tr>
<tr>
<td>pretreatment</td>
</tr>
<tr>
<td>30 minutes</td>
</tr>
<tr>
<td>60 minutes</td>
</tr>
<tr>
<td>90 minutes</td>
</tr>
<tr>
<td>terminal*</td>
</tr>
</tbody>
</table>

| Control Sheep   | 7   | 8   | 9   | 10  |
| pretreatment    | 144 | 144 | 141 | 143 |
| 30 minutes      | 143 | 144 | 140 | 143 |
| 60 minutes      | 144 | 145 | 141 | 144 |
| 90 minutes      | 142 | 143 | 141 | 144 |
| terminal**      | 144 | 143 | 140 | 144 |

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.

⁺ Sodium values in excess of 160 mEq/L were not recorded because of lack of linearity in the measurement.
Table 27.-Serum potassium values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Principal Sheep</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>pretreatment</td>
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<td>6.0</td>
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</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.

Potassium values in excess of 10 mEq/L were not recorded because of lack of linearity in the measurement.
Table 28.-Serum chloride values for principal and control sheep drenched with 12.5 ml. of 3.3 M urea solution and 12.5 ml. of water/kg. of body weight, respectively.

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<th>Chloride (mEq/L)</th>
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<td>terminal**</td>
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<td>99</td>
<td>97</td>
<td>96</td>
</tr>
</tbody>
</table>

* Animals 1 and 4 died at the 90 minute interval.

** Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals.