ACUTE AMMONIUM SALT POISONING IN SHEEP

by

Robert Henry Singer

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Pathology

Approved:

Iowa State University
Of Science and Technology
Ames, Iowa

1969

TABLE OF CONTENTS

INTRODUCTION

Considerable interest has developed in the use of ammonia and ammonium salts as a source of nitrogen for crops and in the use of ammonium salts to supplement the protein nitrogen of ruminant feeds. Urea is employed for the same purposes and is more widely used in feeds than are the ammonium salts. Because urea is hydrolyzed to ammonia and carbon dioxide by the enzyme urease that occurs in the gastrointestinal tract of animals, it is of interest as a source of ammonia.

Although ammonium salts and urea are used extensively in feeds for ruminants and numerous studies dealing with their utilization and metabolism by ruminants under different feeding conditions have been reported, only a few reports are available that pertain to the clinical and pathologic effects that may be produced in sheep and other ruminants by toxic amounts of them.

The present study was designed to demonstrate the clinical and pathologic alterations produced in sheep by ammonium salts administered intraruminally. Different ammonium salts were used to prove that the alterations were due to the ammonium ion and not to the various anions. In one phase of the study, blood samples were collected at ten-minute intervals after dosing to determine the sequence and significance of chemical changes as they may be related to clinical signs. The methods, results, and conclusions of this study follow.

LITERATURE REVIEW

The use of ammonium salts and urea to supplement the protein nitrogen requirement of ruminants was based on observations made before 1900. According to Maynard (44), Zuntz made observations in 1891 that led him to conclude that rumen bacteria preferentially use amides, amino acids, and ammonium salts instead of protein for the synthesis of nitrogenous compounds. This led to numerous investigations of the possibility of utilizing various nitrogen compounds to supply a part of the protein nitrogen requirement of ruminants. In 1937, Fingerling et al. (14) produced clear evidence that urea could be utilized to supply a part of the protein needs for growth of cattle, and Hart et al. (23) found that ammonium bicarbonate served this purpose in growing calves. Very little attention has been given, however, to the toxic effects of excessive quantities of these compounds. According to Maynard (44), it was assumed that urea was hydrolyzed in the rumen and that the toxicity experienced when excessive quantities were consumed was due to the excessive ammonia produced.

The Cause of Ammonium Salt and Urea Poisoning

Various writers are in disagreement concerning the role ammonium salts play as the cause of the syndrome observed in ruminants that have consumed excessive quantities of urea or of ammonium salts. Dinning et al. (11) reported that while their study of urea poisoning in sheep and cattle revealed that signs of poisoning could be correlated with the peripheral blood ammonia level, severe alkalosis was also produced. They concluded that the clinical signs were caused by alkalosis rather than the high blood ammonia concentrations.

Severe alkalosis was also observed during urea poisoning in sheep by Clark et al. (7). They were unable to produce similar signs in sheep in which severe alkalosis was brought about with non-nitrogenous compounds. They concluded that urea poisoning was due to some unidentified products formed by the reaction of the ammonia released from the urea with metabolic products of the rumen flora.

Carbamate has also been suggested as a factor in urea and ammonium salt poisoning in ruminants. Kaishio et al. (31a) reported that ammonium carbamate administered intravenously to goats produced clinical signs similar to those they had observed in cattle and goats given intraruminal doses of urea. They suggested that ammonium carbamate formed by the hydrolysis of urea in the rumen or abomasum was the cause of poisoning. The toxicity of oral doses of ammonium carbamate to sheep was investigated by Hale and King (22). They observed the same clinical signs in sheep given ammonium carbamate as those given urea or ammonium salts and agreed with Kaishio et al. (31a) that ammonium carbamate formed from urea in the rumen was the real cause of poisoning.

The reports of Kaishio et al. (31a) and Hale and King (22) do not provide analytical evidence that ammonium carbamate was present in the gastrointestinal contents or tissues of any of the animals, but it is inferred in both reports that the carbamate portion of the ammonium carbamate was responsible for the poisoning. These researchers failed to consider the ammonium portion of the molecule; it cannot be disregarded so easily.

The possible formation of ammonium carbamate during the enzymatic hydrolysis of urea was first suggested by Fenton (13) in 1885. Subsequent studies of the hydrolysis of urea in aqueous unbuffered solutions, as

reported by Mack and Villars (40), Sumner et al. (60), and Wang and Tarr (67), established that ammonium carbamate was formed although it rapidly decomposed to ammonia and carbon dioxide. Sumner et al. (60) demonstrated that ammonium salts and carbonates were formed immediately in buffered solutions and that ammonium carbamate was not formed as an end product. This was confirmed by the studies of Soejima et al. (59). It is questionable that ammonium carbamate could exist in toxic quantities in the gastrointestinal contents of ruminants.

On the other hand, a number of investigators have concluded that ammonia is directly responsible for the toxicity of ammonium salts and of urea for ruminants. Dinning et al. (11) reported that the clinical signs observed during poisoning with urea in sheep and cattle could be correlated with the peripheral blood ammonia levels. Similar observations were made by Lewis (37), Repp et al. (55), Davis and Roberts (10), and Oltjen et al. (51). The latter four groups related the clinical signs of poisoning by ammonium salts and urea to the direct effect of the ammonia in the peripheral blood. In each instance, signs of poisoning began to appear when the peripheral blood ammonia nitrogen increased to approximately 1.0 mg. per 100 ml. of blood.

Repp et al. (55) observed that the toxicities of ammonium formate, acetate, and propionate to sheep would equate with the toxicity of urea when the dosage of each was expressed in terms of ammonia nitrogen.

A more thorough report of the importance of blood ammonia in ammonium salt poisoning in sheep was presented by Lewis et al. (38). They found that changes in the rumen-ammonia concentration were paralleled by changes in portal blood-ammonia concentrations. When the rumen-ammonia level reached

60 millimoles per liter of rumen contents, the portal blood-ammonia was high (over 1.4 mg. per 100 ml.), while the peripheral blood-ammonia level remained normal. When the rumen-ammonia was increased above 60 millimoles per liter of rumen contents by administering ammonium acetate, the peripheral blood-ammonia concentration increased, and when it exceeded 0.84 to 1.26 mg. per 100 ml., signs of poisoning appeared.

Factors Influencing Ammonia Poisoning

The oral dosage of ammonium salts required to cause poisoning varies considerably and appears to depend on a number of factors. The effect of diet, the ability of the gastrointestinal microflora to utilize ammonia, the pH of the gastrointestinal contents, the concentration of ammonium salts, and the ability of the liver to detoxify absorbed ammonia are a few of the factors that have received some attention.

It has been demonstrated by Coombe and Tribe (9), Head (25), Reis and Reid (54), Annison (1), and Clark et al. (7) that when the available carbohydrate of the rumen is increased, ammonia utilization within the rumen is greatly increased, and the free ammonia concentration is greatly decreased. The adequacy of carbohydrate in the ration is considered to be an important factor in the prevention of poisoning while feeding urea.

Lewis (37), Davis and Roberts (10), and Kita et al. (32) found that ruminants could adapt to urea and to ammonium salts so that a few days after an initial dose was given, several times the dose normally found to be toxic could be tolerated. The adaption was explained on the basis that the

balance of the microflora had changed in response to the increased quantities of ammonia and the new combination was able to utilize it at a greater rate.

The absorption of ammonia from the rumen has been found by Hogan (26), Bloomfield et al. (5), Gaertner (16), and Gaertner and von Engelhardt (17) to be dependent on the pH of the rumen. It was demonstrated by Hogan (26) that the transport of ammonia across the rumen epithelium at pH 4.5 was negligible and by Gaertner (16) that at pH 5.0 to 6.0 the transport was only 66 percent of that at pH 6.9 to 7.8. It was suggested by Gaertner and von Engelhardt (17) that only nonionized ammonia would penetrate the rumen epithelium.

There is another factor related to pH that has been found when urea is the source of ammonia. The optimum pH for urease activity was found by Howell and Sumner (28) to range from 6.4 to 6.9 in various buffer systems and that at lower pH values, urease activity was considerably reduced. Clark et al. (7), Davis and Roberts (10), Szwabowicz (61), Repp et al. (55), and Koval and Vas'ko (36) reported that they were successful in treating urea poisoning with oral doses of acetic acid. The reduction of the pH of the rumen contents and the consequent inhibition of urease activity were expressed as the reasons for this success.

The concentration of ammonia in the rumen contents is a factor governing the toxicity of ammonium salts and urea. Hogan (26) reported that the transport of ammonia across the rumen epithelium increased with concentration gradient at pH 6.5. Gaertner (16) demonstrated that at levels over 10 mg. of ammonia nitrogen per 100 ml. of rumen contents, there was a linear relationship between its concentration and passage through the rumen mucosa.

Lewis et al. (38) also observed that the portal blood ammonia concentration paralleled the ammonia concentration in the rumen.

According to Snyder (58), in his review on liver function, ammonia absorbed from the gastrointestinal tract of all mammals enters the liver from the portal blood and is converted to urea. Mann and Bollman (41) demonstrated the liver as the main site of ammonia metabolism. They were able to recover ammonia quantitatively from the blood, tissues, and urine of dehepatized animals several hours after parenteral treatment with ammonium salts. Lewis et al. (38) suggested that when the quantity of ammonia absorbed is greater than the liver's capacity to convert it to urea, the peripheral blood ammonia concentration increases. Signs of poisoning were observed by Repp et al. (55), Lewis et al. (38), and Thamm et al. (62) when the ammonia nitrogen reached levels of 0.84 mg. to 1.50 mg. per 100 ml. Considering that the normal portal blood ammonia nitrogen of ruminants may at times be in excess of 0.84 mg. to 1.50 mg. per 100 ml., according to McDonald (39) and Lewis et al. (38), the speed with which the liver can convert it to urea can be a critical factor in ammonium salt poisoning. White et al. (71), Bessman and Bessman (4), and Phillips et al. (52b) have reported that signs of ammonia poisoning are observed in people suffering from impaired liver function, such as cirrhosis of the liver, and that the poisoning can result from a high protein diet or from therapy with ammonium salts, because the absorbed protein-derived ammonia or the ammonium salts can pass unchanged through the damaged liver to the peripheral blood.

Toxicity of Ammonium Salts and Urea

In view of the number of factors involved in the metabolism of ammonium salts and urea, it is difficult to establish a definite toxic dose, especially when the dose is by the oral route. Therefore, the quantities reported as toxic doses should be accepted with reservations because there may be considerable difference in the degree of response from animal to animal depending upon the various factors involved.

Without referring to the weight of the animals, Nunn (50) lists the oral toxic doses of ammonium chloride for dogs, sheep, and horses as 8 g., 25 to 80 g., and 500 g., respectively.

The toxic doses of ammonium salts and urea for several animal species that have been reported on a weight basis are listed in Table 1.

Clinical and Pathologic Observations of Acute Ammonium Salt and Urea Poisoning

Ruminants

Clinical signs The clinical signs of acute ammonium salt and urea poisoning in ruminants have been described by a number of investigators. The clinical signs during urea poisoning in cattle have been reported by Davis and Roberts (10), Dinning et al. (11), Koval and Vas'ko (36), Szwabowicz (61), and Thamm et al. (62); those in sheep by Clark et al. (7) and Repp et al. (55); and those in goats by Fujimoto and Tajima (15) and Kita et al. (32). The clinical signs during ammonium salt poisoning in cattle have been described by Priouzeau (53) and those in sheep by Lewis (37), Oltjen et al. (51), and Repp et al. (55). The syndrome was very similar in each instance, as indicated in the following description.

Table 1. A summary of the toxicity of ammonium salts and urea as reported by various investigators

Animal	Ammonium compound	Route of administration	Dose range g./kg. body wt.	Reference
Cattle	Ammonium sulfate	oral	0.29-0.47	Priouzeau (53)
	Urea	oral	0.5-1.0	Davis & Roberts (10)
Sheep	Ammonium acetate	oral	2.6	Repp et al. (55)
	Ammonium formate	oral	2.1	Repp et al. (55)
	Ammonium nitrate	oral	2.0	Topchyan & Averyanova (64)
	Ammonium propionate	oral	3.0	Repp et al. (55)
	Diammonium phosphate	oral	2.2	Oltjen et al. (51)
	Urea	oral	0.5-1.0	Gallup et al. (18)
				Nix & Anthony (49)
				Oltjen et al. (51)
Dog	Ammonium chloride	parenteral	0.15	Bassini & Ferrante (3)
Rabbit	Ammonium chloride	oral	0.5-1.0	Govan & Parkes (21)
	Ammonium nitrate	oral	2.0	Topchyan & Averyanova (64)
	Ammonium sulfate	oral	0.5	Priouzeau (53)
Guinea pig	Ammonium chloride	oral	0.9-1.5	Koenig & Koenig (33,34,35)
Rat	Ammonium acetate	parenteral	0.75	Underhill & Kapsinow (65)
	Ammonium benzoate	parenteral	1.25	Underhill & Kapsinow (65)
	Ammonium bicarbonate	parenteral	1.25	Underhill & Kapsinow (65)
	Ammonium bromide	parenteral	1.25	Underhill & Kapsinow (65)
	Ammonium carbonate	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium chloride	parenteral	1.25	Underhill & Kapsinow (65)
	Ammonium chromate	parenteral	1.00	Underhill & Kapsinow (65)
	Ammonium citrate	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium formate	parenteral	1.00	Underhill & Kapsinow (65)

Table 1. (Continued)

Animal	Ammonium compound	Route of administration	Dose range g./kg. body wt.	Reference
Rat (Cont.)	Ammonium iodide	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium lactate	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium nitrate	patenteral	1.25	Underhill & Kapsinow (65)
	Ammonium phosphate dibasic	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium phosphate tribasic	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium sulfate	parenteral	1.25	Underhill & Kapsinow (65)
	Ammonium tartrate	parenteral	1.50	Underhill & Kapsinow (65)
	Ammonium valarianate	parenteral	1.50	Underhill & Kapsinow (65)
Mice	Ammonium acetate	intravenous	0.32	Warren (69)
	Ammonium bicarbonate	intravenous	0.13	Warren (69)
	Ammonium carbonate	intravenous	0.14	Warren (69)
	Ammonium chloride	intravenous	0.24	Warren (69)
	Ammonium hydroxide	intravenous	0.06	Warren (69)

Within 30 to 60 minutes after the intraruminal administration of ammonium salts or urea, dullness was noted. This was followed by marked hyperesthesia, severe muscular twitching over the entire body, atony of the rumen, and ataxia. Severe tetanic spasms involving all of the skeletal muscles then followed. The animals went down with legs stiffly extended and tonic convulsions occurred. Breathing became extremely labored and regurgitation of ruminal contents often occurred just before death. Frothing at the mouth, pulmonary edema, and cyanosis were seen in the advanced states of poisoning. A pulse rate of 100 to 200 and a subnormal body temperature were reported. Death usually came 1.5 to 2.5 hours after the appearance of clinical signs. Kita et al. (32) reported that the terminal stage was characterized by respiratory paralysis.

Blood chemistry Very few measurements of the blood components of ruminants have been made during ammonium salt or urea poisoning. Other than the blood ammonia nitrogen discussed earlier, blood urea nitrogen, carbon dioxide, and the pH are the only measurements that have received attention.

Blood ammonia nitrogen levels were observed to increase considerably in both cattle and sheep during urea and ammonium salt poisoning as reported by Dinning et al. (11), Repp et al. (55), Lewis et al. (38), Lewis (37), Davis and Roberts (10), Thamm et al. (62), and Oltjen et al. (51). In surviving animals, the blood ammonia nitrogen concentration did not rise above 4.0 mg. per 100 ml. of blood. Those that died exceeded this concentration. Juhász (30) observed that ruminal motility was completely inhibited when the blood ammonia nitrogen reached 0.6 mg. per 100 ml.

Urea nitrogen measurements were made of the blood of cattle during urea poisoning by Dinning et al. (11), of sheep by Lewis (37) during ammonium salt poisoning, and by Repp et al. (55) during both urea and ammonium salt poisoning. In all the animals, the urea nitrogen of the blood increased considerably. Lewis (37) considered that the increase in blood urea was a result of its formation from the high concentration of ammonia that entered the liver by way of the portal blood.

Carbon dioxide and pH measurements of the blood of sheep during ammonium salt and urea poisoning were conducted by Lewis (37). He found that an acidosis developed during ammonium chloride poisoning and an alkalosis during ammonium acetate and urea poisoning.

Pathology The lesions in sheep that died as a result of urea poisoning have been briefly described by Clark et al. (7). They observed an acute circulatory collapse with severe venous stasis, severe pulmonary congestion and edema, hydrothorax, epicardial and endocardial hemorrhages, hemorrhagic diathesis in the small intestines, and severe degeneration of the liver and kidneys. The rumen contents were strongly alkaline and gave off an odor of ammonia. Microscopic examination of the liver and kidney tissues revealed severe diffuse fatty degeneration of the liver and degeneration and necrosis of the tubules of the renal cortex. The lesions in cattle that died as a result of acute ammonium sulfate poisoning were reported by Priouzeau (53) as consisting of numerous petechial hemorrhages in the skin and throughout the carcass, large hemorrhagic patches in the mucosa of the rumen, reticulum, abomasum and intestines, edema and ulcerations in the intestines, and an enlarged, friable liver.

A pathologic study of acute urea poisoning in goats has been reported by Fujimoto and Tajima (15). They observed an acute catarrhal gastroenteritis, nephrosis, acute catarrhal bronchitis accompanied by peribronchial hemorrhages, intra-alveolar hemorrhages of the lung tissues, intensive congestion and hemorrhages of the central nervous system, and degeneration of ganglion cells.

Non-ruminants

Clinical signs Acute ammonium salt poisoning has been described in dogs by Marfori (42), in rabbits by Koenig and Koenig (34), Marfori (42), and Priouzeau (53), and in cats, rats, and guinea pigs by Koenig and Koenig (34). The clinical signs included dyspnea, muscle fasciculations, and convulsions in each instance. Death was due to early acute pulmonary edema in rats, guinea pigs, and cats. Although some pulmonary edema was observed in rabbits, it was not deemed sufficient to have caused death.

Blood chemistry Studies of the chemical changes in blood occurring in non-ruminants during ammonium salt poisoning have revealed alterations of blood sugar levels. Morita (48) demonstrated that a pronounced hyperglycemia, persisting for several hours, occurred in cats administered ammonium salts. This observation was further investigated in rabbits by Gigon (20), Hazard and Vaille (24), Horvath (27), Masamune (43), Miyaura (46), Miyoshi (47), and Wantoch (68) and in dogs by Tolone (63). They considered hyperglycemia to be a constant finding in ammonium salt poisoning. Satake (57) demonstrated the hyperglycemic effect in adrenalectomized rabbits poisoned with ammonium salts and concluded that it was not due to increased

adrenalin release. The authors did not offer an alternate reason for the hyperglycemia.

Pathology A study of the lesions caused in rats, guinea pigs, and cats by acute ammonium chloride poisoning was made by Koenig and Koenig (35). Windle et al. (72) made a similar study in guinea pigs. The gross pathology was confined to the lungs and upper respiratory tract. The bronchi, trachea, and nasal passages were filled with a frothy fluid, blood tinged in the rat and guinea pig but serous in the cat. Pleural fluid was not seen. The lungs were enlarged and filled with fluid. Severe congestion and small to diffuse hemorrhagic patches occurred in the lungs. On section, the lungs were deep red and contained a frothy serosanguineous fluid.

Windle et al. (72) reported that histological alterations were limited to the lung and brain. The most important change was massive pulmonary edema accompanied by variable degrees of hemorrhage. The alveolar spaces were filled with a precipitated pale eosinophilic material and often contained erythrocytes. Interstitial edema was not a prominent finding except in the rat where periarterial edema was always present. Congestion of the larger vessels and alveolar capillaries was severe. The brain lesions were described as similar to those observed in deaths due to acute anoxia.

Lesions in rabbits caused by poisoning with ammonium chloride were described in the studies by Koenig and Koenig (34) and Govan and Parkes (21). In contrast to lesions that occurred in rats, guinea pigs, and cats, pulmonary edema was not found in rabbits. Fat emboli deposited in the lung tissues and necrosis of the renal tubular epithelium were the outstanding lesions.

Fazekas (12b) studied the microscopic lesions of the central nervous system of cats and rabbits that died of acute ammonium chloride poisoning. Lesions in the brain consisted of watery swellings in the vascular walls, fatty degeneration of the endothelial cells, and increase of migratory cells around the vessels, swelling of the glial fibers, and degeneration of the ganglion cells.

METHOD OF PROCEDURE

Materials

Animals

Sheep originating from ranches in the Kerrville, Texas, vicinity were used in this study. Forty-five adult ewes 3 to 10 years of age of mixed breeding were selected. Each animal was identified by a numbered ear tag. They were conditioned for one month prior to study on a grain and hay concentrate at the rate of 1½ lbs. per day per ewe and on coastal Bermuda hay ad libidum. Ten of the ewes were pregnant, the rest barren. All appeared in satisfactory health.

Ammonium salts

The ammonium chloride[1], dibasic ammonium phosphate[1], ammonium sulfate[2], and ammonium carbonate[2] used to produce poisoning were of analytical reagent grade, meeting American Chemical Society specifications, obtained commercially.

Preparation of animals and individual controls

To determine if any of the clinical signs, blood chemical, or urine changes observed during poisoning were due to handling, the collection of blood and urine samples, or the dosing methods, the following procedures were conducted prior to the administration of ammonium salts. The sheep were placed individually into portable pens four feet square to facilitate

---

[1]Mallinkrodt Chemical Works, St. Louis, Missouri.

[2]Matheson, Coleman and Bell, Norwood, Ohio.

handling and sample collection. Each ewe was fitted with a retention catheter connected by plastic tubing to a liter plastic bottle for the continuous collection of urine. Blood and urine samples were taken 1½ hours after catheterization. Each ewe was then given 500 ml. of water intraruminally via stomach tube. The second blood and urine samples were taken 1½ hours after the administration of the water. These control measures were followed immediately by the administration of ammonium salts. Clinical observations were made and recorded throughout the period of these trials.

Administration of Ammonium Salts

Administration of ammonium chloride

Ammonium chloride dissolved in distilled water was administered intraruminally to two groups of sheep. One group was used to study the clinical and pathologic alterations caused by ammonium chloride and to compare these with those found in the groups receiving other ammonium salts. The second group was used to study the blood chemical alterations at 10-minute intervals during the course of poisoning. Sufficient ammonium chloride was given in each case to cause poisoning and subsequent death.

Study of clinical and pathologic alterations Doses of ammonium chloride, each dissolved in 500 ml. of distilled water, were administered to eleven animals intraruminally via stomach tube. The study was made on not more than two sheep per day and included all eleven by the end of two weeks.

It was desired to cause acute poisoning and subsequent death within one to five hours. To determine the quantity of ammonium chloride necessary to obtain such effects, 4 ewes were given different dosages. Two ewes

(1 and 2) were given 1.0 g. per kg. of body weight. Severe clinical signs of poisoning appeared within a few minutes; both recovered and were eating hay within two hours. Twenty-four hours after the initial dose, each was given a second dose of 1.5 g. per kg. of body weight which produced clinical signs of poisoning and the death of the animals within 2 hours. A third ewe (3) was administered a dose of 2.5 g. per kg. of body weight. Clinical signs of poisoning appeared immediately, and the animal died 26 minutes after the administration. A fourth ewe (4) was given 1.0 g. per kg. of body weight. Within a few minutes after administration, mild signs of poisoning appeared and then began to subside within 20 minutes. The animal appeared to be normal at the end of an hour. A second dose of 1.0 g. per kg. of body weight was administered 1 hour and 55 minutes after the initial dose. Progressive signs of poisoning developed, and death occurred 1 hour and 35 minutes after dosing.

Because a dose of 2.5 g. of ammonium chloride per kg. of body weight was more than that required to produce optimum effects and a dose of 1.0 g. per kg. of body weight was less, the remaining seven ewes were administered doses of 1.5 g. per kg. of body weight. This quantity was sufficient to cause signs of poisoning and subsequent death in five of the seven. Additional doses of ammonium chloride were required in two of the seven.

The weight of each animal, the doses and quantity of ammonium chloride each received, and the interval of time that transpired from the initial dose to the time of death of each animal are recorded in Table 2.

In order to provide additional control information on the influence that handling, sample collection, and dosing via stomach tube may have on clinical signs, blood chemical, and urine changes, two of the animals (12

Table 2. Ammonium chloride poisoning: dosage and survival time

Animal number	Kg. body wt.	NH_4Cl given g. per kg. body wt.	Survival time after initial dose hours:minutes
1	30.7	1.0 g. initial dose 1.5 g. 24 hrs. later	25:20
2	46.4	1.0 g. initial dose 1.5 g. 24 hrs. later	25:36
3	33.2	2.5 g. single dose	:26
4	38.9	1.0 g. initial dose 1.0 g. 1 hr. 55 min. later	3:30
5	40.7	1.5 g. single dose	1:10
6	34.2	1.5 g. single dose	:25
7	35.9	1.5 g. initial dose 0.5 g. 50 min. later	3:40
8	39.1	1.5 g. single dose	2:55
9	41.6	1.5 g. single dose	:56
10	34.8	1.5 g. initial dose 0.5 g. 1 hr. 20 min. later 0.5 g. 35 min. later 0.5 g. 1 hr. 5 min. later 1.0 g. 1 hr. 45 min. later	5:52
11	39.3	1.5 g. single dose	1:12

and 13) were used as controls. They were handled in the same manner and at the same time as another two (5 and 8) that were administered ammonium chloride. However, instead of ammonium chloride, they were administered 500 ml. of water. Clinical observations and collections of blood and urine

samples were made in the same manner and at the same time as those administered ammonium salts.

Study of progressive blood chemical alterations Six ewes were used to study the sequency of blood chemical changes and to determine, if possible, how these changes relate to the poisoning syndrome. A dose of 2.5 g. per kg. of body weight of ammonium chloride was administered to each ewe. The ammonium chloride was dissolved in 300 ml. of distilled water and administered through the left paralumbar fossa into the rumen by syringe and needle. A single dose was sufficient to cause progressive clinical signs of poisoning and subsequent death in 5 of the 6 ewes. In the exception, ewe 15, clinical signs of poisoning appeared within 20 minutes but subsided within an hour. The ewe appeared to be normal after two hours. A second dose of 2.5 g. per kg. of body weight was administered 24 hours after the initial dose to this ewe, and signs of poisoning appeared within the first 10 minutes. The ewe died 1 hour later.

The weight of each animal, the doses and quantity of ammonium chloride each received, and the interval of time that transpired from the initial dose to the time of death for each animal are recorded in Table 3.

In order to determine the effects of handling, intraruminal inoculations, and the drawing of blood samples may have on blood chemical levels, two ewes (20 and 21) were used as controls. Each ewe was administered 300 ml. of water instead of ammonium chloride. Otherwise, they were dosed, bled, and handled in the same manner and for a similar period of time as were the ewes that received the ammonium chloride.

Table 3. Ammonium chloride poisoning: dosage and survival time

Animal number	Kg. body wt.	NH_4Cl given g. per kg. body wt.	Survival time after initial dose hours:minutes
14	38.4	2.5 g. single dose	2:22
15	33.5	2.5 g. initial dose 2.5 g. 24 hrs. later	25:25
16	36.2	2.5 g. single dose	:50
17	41.0	2.5 g. single dose	2:50
18	42.3	2.5 g. single dose	1:27
19	40.0	2.5 g. single dose	2:21

Administration of ammonium sulfate

Twelve ewes were used to study clinical, blood chemical, and histologic changes caused by the intraruminal administration of ammonium sulfate.

Doses of ammonium sulfate, each dissolved in 500 ml. of distilled water, were administered intraruminally via stomach tube to eight ewes. To cause acute poisoning and death, it was necessary to administer multiple doses (see Table 4) to all but one of the ewes. In each case, succeeding doses were not administered until the ewe had recovered from the signs produced by the preceding dose.

The weight of each animal, the doses and quantity of ammonium sulfate each received, and the interval of time that transpired from the initial dose to the time of death of each animal are recorded in Table 4.

Table 4. Ammonium sulfate poisoning: dosage and survival time

Animal number	Kg. body wt.	$NH_4(SO_4)_2$ given g. per kg. body wt.	Survival time after initial dose hours:minutes
22	40.5	1.5 g. initial dose 1.0 g. 25 min. later 1.5 g. 30 min. later 1.0 g. 30 min. later 2.0 g. 45 min. later	5:07
23	36.7	1.5 g. initial dose 1.0 g. 25 min. later 1.0 g. 30 min. later 25 g. 1 hr. 20 min. later	4:12
24	38.9	2.5 g. initial dose 2.5 g. 30 min. later	1:58
25	31.1	2.5 g. initial dose 2.5 g. 30 min. later	1:25
26	34.6	3.0 g. initial dose 3.0 g. 30 min. later	1:40
27	35.7	3.0 g. initial dose 3.0 g. 45 min. later 3.0 g. 45 min. later 3.0 g. 45 min. later	3:03
28	32.3	3.5 g. single dose	1:57
29	39.1	3.5 g. initial dose 3.0 g. 40 min. later 2.0 g. 48 min. later	3:41

The other four animals (30, 31, 32, and 33) were used as controls to determine the influence that handling, sample collection, and dosing via stomach tube may have on clinical signs, blood chemical, urine, and tissue changes. They were handled in the same manner and at the same time as four

ewes (22, 24, 26, and 29) that were administered ammonium sulfate with the exception that they were administered 500 ml. of water. Clinical observations and collections of blood and urine samples were made in the same manner and at the same time as with the ewes administered ammonium sulfate, and then they were electrocuted.

Administration of ammonium salt mixture

Twelve ewes were used in this study to determine clinical, blood chemical, and histologic changes caused by the intraruminal administration of a mixture of inorganic ammonium salts.

Eight ewes were administered a mixture of four ammonium salts in sufficient quantities to cause clinical signs of poisoning and subsequent death. The mixture consisted of equal quantities by weight of ammonium chloride, ammonium sulfate, ammonium carbonate, and ammonium phosphate. Each dose of the mixture was dissolved in 500 ml. of distilled water and administered intraruminally via stomach tube.

An initial dose of 2.0 g. of mixture per kg. of body weight was given to each of the eight ewes. This was sufficient to cause clinical signs and subsequent death in four of the eight. The other four required additional doses of 1.0 to 2.0 g. per kg. of body weight to cause death.

The weight of each animal, the doses and quantity of the ammonium salt mixture, and the interval of time that transpired from the initial dose to the time of death of each animal are recorded in Table 5.

Four animals (42, 43, 44, and 45) were used as controls. They were handled in the same manner and at the same time as four ewes (34, 38, 39, and 40) that were administered the ammonium salt mixture with the exception

Table 5. Ammonium salt mixture[a] poisoning: dosage and survival time

Animal number	Kg. body wt.	Ammonium salts given in g. per kg. body wt.	Survival time after initial dose hours:minutes
34	30.5	2.0 g. single dose	:25
35	35.2	2.0 g. single dose	:39
36	34.9	2.0 g. initial dose 1.0 g. 1 hr. 36 min. later	2:31
37	34.3	2.0 g. initial dose 1.0 g. 48 min. later	1:42
38	33.4	2.0 g. single dose	:48
39	43.2	2.0 g. initial dose 1.0 g. 1 hr. 53 min. later 1.0 g. 30 min. later	3:27
40	39.3	2.0 g. initial dose 2.0 g. 2 hrs. 51 min. later	3:39
41	39.3	2.0 g. single dose	:43

[a]Ammonium salt mixture: a mixture of equal quantities by weight of ammonium chloride, carbonate, phosphate, and sulfate.

that they were administered 500 ml. of water instead of ammonium salts. Collections of blood and urine samples and clinical observations were made in the same manner and time as with the ewes given ammonium salts. They were then electrocuted.

Clinical Procedure

Observations

Observations of the general health and behavior of all animals were made during the a.m. and p.m. daily prior to the administration of ammonium salts. On the day that each animal was poisoned, it was under constant observation during the control measures taken prior to the administration of ammonium salts and thereafter until the animal died. Control animals were observed in the same manner. Individual changes in respiration, excreta, and other clinical signs were recorded. Ruman motility, respiration and pulse rate, and body temperature were observed and recorded for each animal before and after each step of the pretreatment period and several times after the administration of ammonium salts.

Blood chemistry

With the exception of the group of animals used in the study of progressive blood chemical alterations, three jugular blood samples were collected from each ewe. One was collected before the intraruminal administration of 500 ml. of distilled water, the second prior to the administration of the ammonium salts, and the third just preceding the death of the sheep. Heparin sodium was used as the anticoagulant. These samples were used for determinations of the non-protein nitrogen, urea nitrogen, amino acid nitrogen, glucose, chlorides, and pH.

Multiple blood samples were collected from each animal in the group used in the study of progressive blood chemical alterations. Each animal was bled before and every 10 minutes after the administration of ammonium chloride until subsequent death of the poisoned animal.

For control purposes to determine if blood chemical changes were influenced by handling procedures or the frequent blood collection, two animals were bled before the intraruminal administration of 500 ml. of water and every 10 minutes thereafter. A total of eleven collections were made from each control.

At each bleeding, two samples were prepared. One was collected for the measurement of non-protein nitrogen, urea nitrogen, amino acid nitrogen, glucose, chlorides, and pH. Heparin sodium was used as the anticoagulant. The other was collected under special conditions for the measurement of ammonia nitrogen. Blood collected for this purpose must be preserved immediately in order to prevent new ammonia formation. (Ammonia forms in unpreserved shed blood and blood filtrates due to deamination reactions.) The method of Gangolli and Nicholson (19) for the preparation of blood samples and spectrophotometric estimation of ammonia nitrogen was employed using diffusion chambers described by Conway and Byrne (8) for the distillation of ammonia.

The procedures used on all blood samples for the measurement of pH, glucose, amino acid nitrogen, urea nitrogen, non-protein nitrogen, and chlorides were the same. The pH was measured on the heparinized blood samples immediately after collection, employing a Beckman pH meter. Shortly thereafter, protein-free blood filtrates were prepared by the tungstic acid method of Folin and Wu (Oser 52a, p. 1027). The filtrates were used for the other measurements made in the following order: glucose, amino acid, nitrogen, urea nitrogen, non-protein nitrogen, and chlorides. The glucose, amino acid nitrogen, and urea measurements were conducted on the freshly

prepared filtrates. The remainder of each filtrate was placed in a test tube, sealed, and frozen until time permitted the measurement of the non-protein nitrogen and chlorides.

The procedures used for these measurements were as follows: glucose was estimated by the method of Folin and Wu (Oser 52a, pp. 1052-1053); amino acid nitrogen by the method of Davidson (Oser 52a, pp. 1048-1050); urea by the method of Karr (Oser 52a, pp. 1037-1038); non-protein nitrogen by the method of Folin and Wu (Oser 52a, pp. 1033-1034); and chlorides by the method of Schales and Schales (Oser 52a, pp. 1109-1110). All colorimetric measurements were made with a Bausch and Lomb Spectronic 20 spectrophotometer.

Urology

Urine samples were collected into 1 liter plastic bottles on a continuous basis by means of a retention catheter. Three samples were collected from each animal. The first sample was urine collected for 1½ hours following the placement of the catheter. The second sample was the urine collected for 1½ hours following the intraruminal administration of water. The third sample was the total urine eliminated from the time of administration of ammonium salts until death of the animal. Urine samples collected from control animals corresponded in sequence and time of collection to those that received ammonium salts.

The color of each urine sample was noted, and each was examined for the presence of erythrocytes, albumin, and glucose. The examination for the presence of erythrocytes was made on sediment from centrifuged urine

samples with the aid of a microscope. The presence of albumin was determined by the Osgood-Haskins (Oser 52a, p. 1187) method and of glucose by the method of Benedict (Oser 52a, p. 1175).

Necropsy Procedure

Complete necropsies were performed immediately after death. All gross lesions were recorded, and the following tissues were taken from each animal for microscopic examination.

Cardiovascular system

- Left and right ventricle of the heart
- Anterior aorta

Digestive system

Esophagus	Abomasum	Colon
Recticulum	Duodenum	Rectum
Dorsal rumen wall	Jejunum	Liver
Ventral rumen wall	Ileum	Pancreas
Omasum	Cecum	

Endocrine system

Adrenal gland	Pituitary gland	Thyroid gland

Lymphatic system

Bronchial lymph node	Mesenteric lymph node
Cervical lymph node	Parotid lymph node
Hepatic lymph node	Spleen
Mediastinal lymph node	Thymus

Muscular system

Skeletal muscle from the pectoral region

Nervous system

Frontal lobe of the cerebral cortex

Occipital lobe of the cerebral cortex

Cerebellar cortex

Corpus striatum

Thalamus

Hippocampus

Midbrain

Anterior medulla oblongata

Posterior medulla oblongata

Cervical spinal cord

Thoracic spinal cord

Lumbar spinal cord

Respiratory system

Larynx

Mid-trachea

Dorsal and ventral regions of right and left apical lobes of the lungs

Dorsal and ventral regions of the right and left cardiac lobes of the lungs

Anterior and posterior regions of the right and left diaphragmatic lobes of the lungs

Midlateral and median regions of the right and left diaphragmatic lobes of the lungs

Urogenital system

Kidney

Urinary bladder

Ovary

Uterus

Uterine caruncles

Tissues, 6 to 10 mm thick, collected for the microscopic examination were submerged in 10% formalin for a minimum of 48 hours. The tissues were trimmed, dehydrated in graduated concentrations of ethyl alcohol, cleared

in Clearing Agent[1] and infiltrated in paraffin in the routine manner in an Autotechnicon[1]. Tissues were embedded in paraffin, sectioned at eight microns, and mounted on glass slides for staining.

All sections were stained in a routine manner with Mayer's hemotoxylin and eosin Y as described in the Armed Forces Institute of Pathology, Manual of Histologic and Special Staining Technics (2).

---

[1]The Technicon Company, Chauncey, New York.

FINDINGS

Clinical Findings

Clinical effects of poisonous doses of ammonium chloride

Clinical signs Clinical signs of poisoning were apparent within 5 to 20 minutes after the sheep had received the ammonium chloride. Approximately the same signs were seen in all but one of the sheep. Those observed in ewe number 7 differed from those observed in the others and will be described separately.

The animals first became abnormally apprehensive and restless. This was followed by mild trembling of the musculature extending over the entire body accompanied by extreme hyperesthesia. They responded violently to noise and touch but not to moving objects. At about the same time, mydriasis, an increased pulse rate, a marked increase in respiratory volume, and atony of the rumen were observed.

As the muscle tremors became more severe, tonic muscle spasms began to occur that increased in frequency and severity within a few minutes. Spastic movements of the facial and cervical musculature were first noticed. After a few moments, the muscle spasms were also seen in the musculature of the body and limbs. At the time that the muscle spasms began, rotatory nystagmus and weakness of the posterior limbs were also observed. A short time later, the animals dropped to their sternums in a normal recumbent position and usually could not regain their feet.

Within a few minutes after the animals were down, severe tonic convulsions occurred (Figure 1). In appearance, the convulsions were characterized by opisthotonus, violent trembling with legs fully extended,

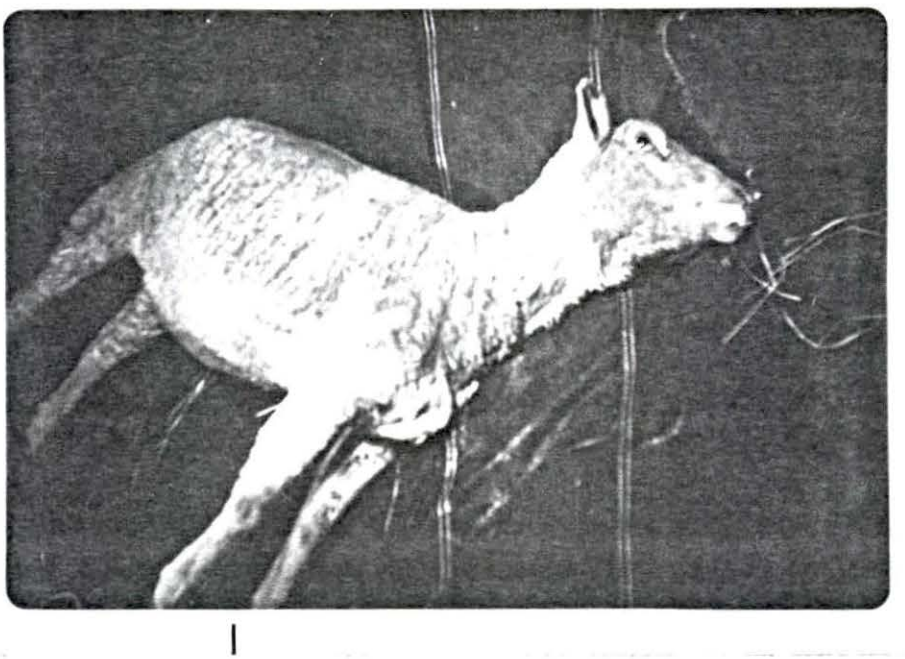


Figure 1. Sheep poisoned with ammonium chloride undergoing a tonic convulsion

rigidity of all observable body musculature, and the arrest of respiration. The heart rate was accelerated from 120 to 216 beats per minute. The mucous membranes of the body openings were cyanotic during each convulsion. The convulsions recurred at varying time intervals. During the intervals between convulsions, there was some relaxation of the muscles, although the body remained rigid and the head, neck, and legs remained extended. The respiration was labored, and after the second or third convulsion, there was some frothing at the mouth. The body temperature remained normal or increased by only 1.0 to 1.5 degrees Fahrenheit during the first and succeeding convulsions but increased as much as 5.0 degrees 10 to 15 minutes prior to death.

Respiration became more difficult as the course of poisoning progressed due to partial paralysis of the costal and abdominal muscles. During the interval between the last two agonal convulsions, vomition with violent retching was observed in sheep 4 and 7. Death occurred after a final violent convulsion. When complete relaxation of the musculature occurred and respiration ceased, the heart continued to beat for another 1 to 5 minutes. Heart function terminated in fibrillation in one of the animals. At the time of death, the venous blood was bright red in some of the ewes and dark red in others. The urine of all of the ewes was pink during the last stages of poisoning.

The poisoning syndrome in sheep number 7 was different from that observed in the others. Clinical signs appeared about ten minutes after dosing, characterized by mild muscular trembling accompanied by depression. After thirty minutes, the animal no longer trembled and appeared to recover. After the second dose of ammonium chloride, the muscular trembling recurred

and again was accompanied by depression. The animal was lethargic and not responsive to noise, touch, or moving objects. Muscular weakness was apparent, and the animal could no longer stand 1 hour and 40 minutes after the initial dose. The animal lost consciousness soon after going down and remained in a comatose state. Thirty minutes after the animal went down, tonic muscle spasms of the body musculature were observed that increased in tempo until clonic-tonic convulsions occurred. Opisthotonus and paddling of all four limbs occurred with each seizure. Severe convulsions of this type continued intermittently until death an hour and twenty-five minutes later. Respiration, heart rate, and body temperature followed a course similar to that observed in the other ewes.

The respiration rate, pulse rate, body temperature, and rumen motility rate of each of the sheep in this group are listed in Table 6.

Blood chemistry findings The chemical findings made on the blood samples collected from this group of sheep are listed in Table 7. They include measurements of the pH, glucose, amino acid nitrogen (AAN), blood urea nitrogen (BUN), non-protein (NPN), and chlorides made of the blood of the sheep administered ammonium chloride and on the controls. The blood pH decreased considerably in all of the poisoned sheep, indicating that an acidotic condition had developed. The blood glucose levels of 4 of the 11 animals were slightly above the normal range prior to the administration of ammonium chloride. Severe hyperglycemia was found in each after dosing. The glucose level had increased from 3 to 5 times the predosing level.

The non-protein nitrogen was substantially increased in all of the sheep following ammonium chloride administration. The two non-protein nitrogen fractions, amino acids, and urea accounted for most of the

Table 6. Respiration, pulse, and rumen motility rates and body temperatures of sheep poisoned with ammonium chloride and of controls

Animal number	Respiration rate per min.		Pulse rate per min.		°F. body temperature		Rumen motility rate per min.	
	Before[a]	After[b]	Before[a]	After[b]	Before[a]	After[b]	Before[a]	After[c]
1	44	48	80	170	102.0	106.2	2	0
2	32	44	92	180	102.8	108.0	1	0
3	28	48	76	160	102.6	105.8	2	0
4	36	48	96	176	102.0	105.8	3	0
5	48	34	128	180	103.6	106.8	1	0
6	28	44	80	160	103.4	104.4	3	0
7	40	36	80	120	103.6	106.0	2	0
8	46	52	80	160	103.2	105.8	2	0
9	44	44	72	200	102.8	107.8	2	0
10	24	48	84	160	103.6	105.2	2	0
11	64	44	96	216	101.8	107.4	1	0
				c o n t r o l s				
12[d]	30	32	84	76	103.2	103.0	2	2
13[d]	54	48	92	92	102.0	102.0	2	2

[a]Observed just before the administration of ammonium chloride.

[b]Observed after administration of ammonium chloride 5 to 15 minutes before death.

[c]Observed after administration of ammonium chloride before convulsive seizures occurred.

[d]Control animals observed before and after receiving 500 ml. of water instead of ammonium chloride.

Table 7. Blood chemical findings: sheep poisoned with ammonium chloride and controls

Animal number	Sample[a]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
1	C	7.77	43	6.8	12.1	34.9	505
	P1	7.57	54	8.5	15.8	37.5	550
	P2	6.87	184	16.2	30.2	58.2	590
2	C	7.81	30	7.2	11.9	34.0	515
	P1	7.49	76	6.0	14.9	37.2	560
	P2	7.49	154	13.4	16.9	40.5	565
3	C	7.89	49	6.5	10.7	34.9	451
	P	7.21	161	15.6	17.5	40.9	600
4	C1	7.72	39	5.2	8.2	27.2	512
	C2	7.76	60	5.5	8.1	23.5	480
	P	7.15	200	16.6	15.5	37.9	550
5	C1	7.77	41	6.0	12.5	28.9	458
	C2	7.70	44	5.0	12.3	27.0	425
	P	7.27	160	10.6	14.5	38.9	496
6	C1	7.62	60	8.0	8.4	24.5	590
	C2	7.76	73	7.9	9.2	27.0	595
	P	6.92	234	13.2	12.7	38.4	676

[a]Samples: C1-Control sample collected before mock-treatment with water.
C and C2-Control sample collected before second mock-treatment or before dosing with ammonium chloride.
C3-Control sample collected from control animals after second mock-treatment with water.
P1-Sample collected during severe convulsion from poisoned animals that recovered.
P2-Sample collected 5 to 15 minutes before death from animals poisoned a second time.
P -Sample collected from animals administered ammonium chloride 5 to 15 minutes before death.

Table 7. (Continued)

Animal number	Sample[a]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
7	C1	7.65	50	6.6	9.6	23.1	536
	C2	7.81	73	5.6	9.4	24.0	599
	P	7.38	234	10.3	14.3	33.8	588
8	C1	7.60	35	9.9	12.2	29.7	485
	C2	7.72	38	5.9	11.6	28.1	521
	P	7.19	140	10.2	16.6	41.1	545
9	C1	7.70	45	6.4	10.0	30.0	560
	C2	7.73	45	7.3	8.7	25.1	530
	P	7.23	154	16.2	13.0	34.1	550
10	C1	7.88	45	4.3	15.1	34.1	478
	C2	7.71	45	6.1	15.4	32.1	485
	P	7.18	130	16.9	28.4	64.1	625
11	C1	7.70	45	6.2	9.0	24.8	545
	C2	7.73	65	5.9	10.7	26.9	520
	P	7.26	154	12.5	13.5	37.1	575
			c o n t r o l s				
12	C1	7.62	41	6.7	11.2	26.8	510
	C2	7.70	57	6.1	12.5	28.4	515
	C3	7.69	40	6.1	11.4	27.8	500
13	C1	7.75	40	4.9	9.9	24.9	485
	C2	7.62	40	6.0	12.1	24.0	485
	C3	7.76	68	6.1	11.2	26.3	482

increase with the greatest increase occurring in the amino acid fraction in all but sheep number 8.

The blood chloride levels after dosing were found to be elevated in all but one (sheep 9) of the animals.

With the exception of a slight increase of the blood glucose, blood chemical levels of the control animals remained within normal bounds.

Urine findings The findings made on the urine samples collected from this group of sheep consisting of the color and the presence of erythrocytes, albumin, and glucose are listed in Table 8. Hematuria, characterized by the pink discolored urine and the presence of erythrocytes, was observed in all of the animals during the latter stages of poisoning. Albuminuria was found in all but three of the poisoned animals and glycosuria in one. Abnormal findings were not disclosed in the urine of the two control sheep.

Clinical effects of poisonous doses of ammonium sulfate

Clinical signs Clinical signs of poisoning observed in the sheep administered ammonium sulfate, with minor exceptions, were not visibly different from those observed in the sheep poisoned with ammonium chloride. None of them exhibited the central nervous system depression observed in sheep number 7 of the ammonium chloride group, and while severe tonic convulsions were seen in all of the animals, clonic-tonic convulsions were also observed in sheep number 28. Vomition was observed in only one of the sheep (number 29), and it occurred during the interval between the last two convulsions.

Table 8. Urine findings: ammonium chloride poisoned sheep and controls

Animal number	Sample[a]	Color	Erythrocytes[b]	Albumin[c]	Glucose[d]
1	C	yellow	negative	negative	negative
	P1	yellow	positive	negative	negative
	P2	pink	positive	positive	negative
2	C	yellow	negative	negative	negative
	P1	pink	positive	negative	negative
	P2	amber	positive	positive	negative
3	C	amber	negative	negative	negative
	P	pink	positive	positive	negative
4	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
5	C1	amber	positive	negative	negative
	C2	amber	negative	negative	negative
	P	pink	positive	positive	negative
6	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	amber	positive	negative	negative

[a]Samples: C1-Total urine collected during 1½ hours before mock-treatment with water.
C and C2-Total urine collected during 1½ hours before second mock-treatment or dosing with ammonium chloride.
C3-Total urine collected from control animals after second mock-treatment with water.
P1-Total urine collected during poisoning from animals that later recovered.
P2-Total urine collected during second poisoning period.
P -Total urine collected during poisoning.

[b]Erythrocytes reported negative for none observed and positive for present.

[c]Albumin reported negative for none detected and positive for positive test.

[d]Glucose reported negative for none detected and positive for positive test.

Table 8. (Continued)

Animal number	Sample[a]	Color	Erythrocytes[b]	Albumin[c]	Glucose[d]
7	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	P	pink	positive	negative	positive
8	C1	amber	negative	negative	negative
	C2	amber	negative	negative	negative
	P	pink	positive	positive	negative
9	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	negative	negative
10	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
11	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
12	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	pale yellow	negative	negative	negative
13	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	C3	yellow	negative	negative	negative

The respiration rate, pulse rate, body temperature, and rumen motility of each of the sheep in this group are listed in Table 9.

Blood chemistry findings The chemical findings made on the blood samples collected from this group of sheep are listed in Table 10. The changes are consistent from animal to animal and do not differ from those found in the sheep poisoned with ammonium chloride with the exception of the chloride levels. The blood chloride levels of these sheep did not

Table 9. Respiration, pulse, and rumen motility rates and body temperature of sheep poisoned with ammonium sulfate and of controls

Animal number	Respiration rate per min.		Pulse rate per min.		°F. body temperature		Rumen motility rate per min.	
	Before[a]	After[b]	Before[a]	After[b]	Before[a]	After[b]	Before[a]	After[c]
22	40	44	80	160	103.4	105.4	3	0
23	36	48	96	220	103.8	107.2	3	0
24	40	48	80	160	103.6	107.0	1	0
25	28	32	80	172	104.4	106.2	2	0
26	28	72	84	230	103.2	105.6	2	0
27	32	52	120	168	103.4	107.4	2	0
28	36	56	80	216	103.4	106.8	1	0
29	36	60	88	192	103.6	107.6	1	0
				controls				
30[d]	36	36	80	84	103.0	103.2	2	1
31[d]	40	36	84	80	103.2	102.8	2	2
32[d]	28	32	80	80	103.0	103.0	1	1
33[d]	36	32	96	884	102.4	102.6	3	2

[a]Observed just before the administration of ammonium sulfate.

[b]Observed after administration of ammonium sulfate 5 to 15 minutes before death.

[c]Observed after administration of ammonium sulfate before convulsive seizures occurred.

[d]Control animals observed before and after receiving 500 ml. of water instead of ammonium sulfate.

Table 10. Blood chemical findings: sheep poisoned with ammonium sulfate and controls

Animal number	Sample[a]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
22	C1	7.66	55	6.4	10.7	27.0	483
	C2	7.68	69	5.9	10.6	26.6	477
	P	6.94	200	17.3	26.0	61.5	483
23	C1	7.50	52	5.9	8.5	23.7	529
	C2	7.60	44	5.5	8.6	22.4	475
	P	6.93	173	14.4	19.4	47.9	485
24	C1	7.62	76	5.0	9.1	22.7	408
	C2	7.65	92	4.8	8.7	22.4	427
	P	7.26	158	10.0	13.6	32.6	420
25	C1	7.61	92	6.0	10.4	28.8	435
	C2	7.63	114	5.0	11.5	25.4	445
	P	7.32	191	10.4	14.3	35.0	435
26	C1	7.69	50	5.9	11.6	28.1	478
	C2	7.67	66	5.2	11.3	23.0	462
	P	7.04	126	11.9	23.5	44.9	NS

[a]Samples: C1-Control sample collected before mock-treatment with water.
C2-Control sample collected before second mock-treatment or before dosing with ammonium sulfate.
C3-Control sample collected from control animals after second mock-treatment with water.
P -Sample collected from animals administered ammonium sulfate 5 to 15 minutes before death.

Table 10. (Continued)

Animal number	Sample[a]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
27	C1	7.66	53	5.2	10.5	26.4	NS
	C2	7.72	74	4.9	10.3	26.3	470
	P	6.90	186	18.9	27.6	53.6	500
28	C1	7.70	44	5.0	10.1	24.0	447
	C2	7.71	58	5.9	9.8	24.2	433
	P	7.30	166	12.1	21.1	52.1	NS
29	C1	7.71	53	6.4	11.7	29.9	450
	C2	7.68	71	6.5	11.8	30.0	450
	P	7.21	198	10.8	23.3	47.0	480
			c o n t r o l s				
30	C1	7.73	62	5.3	7.0	17.9	462
	C2	7.73	92	4.8	6.9	17.7	485
	C3	7.78	59	3.4	5.9	14.1	457
31	C1	7.64	25	7.0	15.7	35.0	498
	C2	7.60	20	6.1	16.7	37.2	536
	C3	7.64	13	7.0	17.2	30.1	487
32	C1	7.67	67	4.7	13.3	24.8	503
	C2	7.67	92	5.0	13.9	29.0	493
	C3	7.66	77	4.9	12.1	26.4	468
33	C1	7.61	54	5.9	9.0	23.9	485
	C2	7.62	54	4.8	9.0	21.2	492
	C3	7.60	90	5.3	10.0	25.0	527

increase. An acidosis and hyperglycemia were constant features of ammonium sulfate poisoning. The non-protein nitrogen increased in these sheep in a manner similar to that found in the ammonium chloride group. As with the ammonium chloride group, the urea and amino acid fractions accounted for most of the increase, and the greatest increase was in the amino acid fraction.

Urine findings The findings made on the urine samples collected from this group of sheep are listed in Table 11. Hematuria and albuminuria were found in all of the poisoned sheep. Glycosuria was found in only two of the animals. None of the findings of the urine from the control sheep were abnormal.

Clinical effects of poisonous doses of ammonium salt mixture

Clinical signs The clinical signs observed in the sheep poisoned with the ammonium salt mixture were very similar to those observed in the group poisoned with ammonium chloride and those poisoned with ammonium sulfate. The central nervous system depression observed in sheep number 7 of the ammonium chloride group was not observed in any of the sheep of this group. Severe tonic convulsions occurred in each of the poisoned sheep of this group. In animal number 40, clonic-tonic convulsions were also observed. Vomition occurred in three of the sheep (37, 38, and 41).

The respiration rate, pulse rate, body temperature, and rumen motility rate of each of the sheep of this group are listed in Table 12.

Blood chemistry findings The blood chemical findings for this group of sheep, listed in Table 13, are similar to those found in the other two groups, those poisoned with ammonium chloride and those poisoned with

Table 11. Urine findings: ammonium sulfate poisoned sheep and controls

Animal number	Sample[a]	Color	Erythrocytes[b]	Albumin[c]	Glucose[d]
22	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
23	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	positive
24	C1	amber	negative	negative	negative
	C2	amber	negative	negative	negative
	P	dark amber	positive	positive	negative
25	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	positive
	P	pink	positive	positive	positive
26	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	P	amber	positive	positive	negative
27	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative

[a]Samples: C1-Total urine collected during 1½ hours before mock-treatment with water.
C2-Total urine collected during 1½ hours before second mock-treatment or dosing with ammonium sulfate.
C3-Total urine collected from controls after second mock-treatment with water.
P -Total urine collected during poisoning.

[b]Erythrocytes reported negative for none observed and positive for present.

[c]Albumin reported negative for none detected and positive for positive test.

[d]Glucose reported negative for none detected and positive for positive test.

Table 11. (Continued)

Animal number	Sample[a]	Color	Erythrocytes[b]	Albumin[c]	Glucose[d]
28	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
29	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	negative
30	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	pale yellow	negative	negative	negative
31	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	pale yellow	negative	negative	negative
32	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	C3	yellow	negative	negative	negative
33	C1	pale yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	C3	yellow	negative	negative	negative

ammonium sulfate. As found with the ammonium sulfate group, the blood chloride levels did not increase. Acidosis and hyperglycemia were found with this group similar to that found in the other two groups. The non-protein nitrogen was increased in these sheep to about the same degree found in the other two groups. The urea and amino acid fractions accounted for most of the increase.

Urine findings The urine findings for this group of sheep are listed in Table 14. Hematuria and albuminuria were found in all of the

Table 12. Respiration, pulse and rumen motility rates, and body temperature of sheep poisoned with the ammonium salt mixture[a] and of controls

Animal number	Respiration rate per min.		Pulse rate per min.		°F. body temperature		Rumen motility rate per min.	
	Before[b]	After[c]	Before[b]	After[c]	Before[b]	After[c]	Before[b]	After[d]
34	40	60	100	212	104.0	105.4	1	0
35	32	36	88	212	104.2	107.0	1	0
36	40	48	84	196	103.8	110.0	1	0
37	40	40	80	220	103.6	107.0	2	0
38	44	62	96	256	104.6	108.7	2	0
39	64	140	76	160	104.0	108.6	1	0
40	32	44	80	196	103.4	106.4	1	0
41	44	48	84	220	102.4	106.4	1	0
				controls				
42[e]	64	52	76	80	104.0	104.0	1	1
43[e]	32	36	96	84	103.8	103.6	1	1
44[e]	40	44	80	80	102.8	103.0	1	1
45[e]	48	44	96	88	103.8	103.8	2	1

[a]Ammonium salt mixture, a mixture of equal quantities by weight of ammonium carbonate, chloride, phosphate, and sulfate.

[b]Observed just before the administration of ammonium salts.

[c]Observed after administration of ammonium salts 5 to 15 minutes before death.

[d]Observed after administration of ammonium salts before convulsive seizures.

[e]Control animals observed before and after receiving 500 ml. of water instead of ammonium salts.

Table 13. Blood chemical findings: sheep poisoned with ammonium salt mixture[a] and controls

Animal number	Sample[b]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
34	C1	7.70	97	7.2	8.5	23.0	590
	C2	7.72	117	6.5	9.1	22.5	590
	P	7.01	228	16.6	16.0	38.7	622
35	C1	7.73	102	5.5	9.9	23.3	448
	C2	7.75	109	5.3	8.6	22.1	452
	P	7.14	181	11.3	14.5	31.1	485
36	C1	7.69	54	6.5	12.6	30.0	504
	C2	7.64	44	6.5	11.9	28.7	505
	P	7.37	122	8.6	20.5	40.8	500
37	C1	7.72	64	6.5	13.0	30.3	453
	C2	7.72	76	5.8	12.9	29.0	464
	P	7.31	172	13.2	18.6	41.0	491
38	C1	7.67	51	6.1	12.7	29.1	468
	C2	7.67	58	5.8	13.2	31.1	479
	P	7.15	163	11.5	16.9	41.6	500

[a]Ammonium salts: a mixture of equal quantities by weight of ammonium carbonate, chloride, phosphate, and sulfate.

[b]Samples: C1-Control sample collected before mock-treatment with water.
C2-Control sample collected before second mock-treatment or before dosing with ammonium salts.
C3-Control sample collected from control animals after second mock-treatment with water.
P -Sample collected from animals administered ammonium salts 5 to 15 minutes before death.

Table 13. (Continued)

Animal number	Sample[b]	pH	Glucose mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Chlorides mg. per 100 ml. as NaCl
39	C1	7.78	60	5.3	8.8	23.1	486
	C2	7.90	77	4.4	3.6	22.2	513
	P	7.55	192	8.3	18.0	37.6	495
40	C1	7.69	51	6.2	12.4	31.4	471
	C2	7.61	72	6.4	12.8	33.0	465
	P	7.38	181	8.4	23.7	46.1	481
41	C1	7.70	55	6.8	11.3	27.9	500
	C2	7.73	64	7.0	11.3	25.1	514
	P	7.21	235	9.1	15.6	33.8	524
42	C1	7.76	97	7.7	8.1	21.2	563
	C2	7.83	99	7.8	8.7	24.0	570
	C3	7.76	64	7.4	8.8	21.3	552
43	C1	7.77	67	5.9	7.0	16.5	465
	C2	7.76	68	5.8	5.9	16.4	465
	C3	7.76	67	6.0	6.5	16.2	475
44	C1	7.64	75	6.2	11.6	27.5	460
	C2	7.73	77	6.0	11.7	25.7	496
	C3	7.63	75	5.9	11.1	26.0	520
45	C1	7.71	54	6.0	12.7	29.6	461
	C2	7.90	62	6.5	12.6	20.2	490
	C3	7.67	72	5.8	13.0	26.6	495

Table 14. Urine findings: ammonium salt mixture[a] poisoned sheep and controls

Animal number	Sample[b]	Color	Erythrocytes[c]	Albumin[d]	Glucose[e]
34	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
		pink	positive	positive	negative
35	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	positive
	P	pink	positive	positive	positive
36	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	P	pink	positive	positive	positive
37	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	positive
38	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	P	amber	positive	positive	positive

[a]Ammonium salts: a mixture of equal quantities by weight of ammonium carbonate, chloride, phosphate, and sulfates.

[b]Samples: C1-Total urine collected during 1½ hours before mock-treatment with water.
C2-Total urine collected during 1½ hours before second mock-treatment or dosing with ammonium salt mixture.
C3-Total urine collected from controls after second mock-treatment with water.
P -Total urine collected during poisoning.

[c]Erythrocytes reported negative for none observed and positive for present.

[d]Albumin reported negative for none detected and positive for positive test.

[e]Glucose reported negative for none detected and positive for positive test.

Table 14. (Continued)

Animal number	Sample[b]	Color	Erythrocytes[c]	Albumin[d]	Glucose[e]
39	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	P	pink	positive	positive	positive
40	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	P	pink	positive	positive	positive
41	C1	amber	negative	negative	negative
	C2	amber	negative	negative	negative
	P	red brown	positive	positive	positive
42	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	yellow	negative	negative	negative
43	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	pale yellow	negative	negative	negative
44	C1	yellow	negative	negative	negative
	C2	yellow	negative	negative	negative
	C3	yellow	negative	negative	negative
45	C1	pale yellow	negative	negative	negative
	C2	pale yellow	negative	negative	negative
	C3	pale yellow	negative	negative	negative

poisoned sheep. Glycosuria occurred in all but one of the animals (number 34). Abnormal findings were not revealed in the control sheep.

Sequence of blood chemical changes during ammonium chloride poisoning

The measurements of the pH, glucose, ammonia nitrogen (NH_3-N), amino acid nitrogen (AAN), urea nitrogen (BUN), and non-protein nitrogen (NPN) made on the blood samples collected at 10-minute intervals from sheep given

poisonous doses of ammonium chloride and the clinical signs observed at each interval are listed in Tables 15, 16, 17, 18, 19, and 20. Measurements made on the control animals are listed in Tables 21 and 22.

Clinical signs of poisoning appeared in each animal with the occurrence of sharp increases of the blood ammonia. Although there was a progressive change in the pH and glucose in each of the poisoned animals, the clinical signs did not appear in conjunction with these changes. Similar observations were made between the clinical signs and the amino acid and urea fractions of the non-protein nitrogen. That the clinical signs observed during poisoning correlated very closely with the fluctuation of the blood ammonia level is exemplified in the case of animal 15. Signs of poisoning occurred with the rise of blood ammonia and then subsided as the ammonia level decreased. During this time, the other components of the blood had not fluctuated sufficiently to warrant consideration. Twenty-four hours later, signs of poisoning were not apparent by physical examination; however, the pH was lower than when severe signs had been seen in the same animal as well as the others, and the non-protein nitrogen was elevated considerably. The ammonia nitrogen was above normal levels at that time but below 1.00 mg. per 100 ml.

Necropsy Findings

The gross and microscopic lesions observed in all of the animals administered ammonium salts were very similar regardless of the salt employed. No consistent difference was found between those receiving ammonium chloride, ammonium sulfate, or the ammonium salt mixture.

Table 15. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 14

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.39	83	0.23	7.7	8.0	21.4	Normal appearance
10	7.45	98	2.06	9.8	10.7	26.4	Very nervous, hyperesthetic
20	7.17	139	2.16	10.4	10.2	26.2	Mild muscle spasms, weakness
30	7.18	183	2.36	10.5	9.9	28.0	Down, unable to rise, severe muscle spasms
40	6.98	126	2.60	10.7	12.6	27.6	Down, unable to rise, severe muscle spasms
50	7.08	215	2.52	11.4	13.0	31.3	Tonic convulsion
60	7.04	222	3.02	11.6	11.7	33.2	Tonic spasms
70	6.90	219	3.36	13.8	13.1	35.1	Tonic convulsion
80	6.87	200	3.65	14.6	14.8	35.0	Tonic spasms, comatose
90	6.86	188	5.04	14.6	14.5	37.2	Repeated tonic convulsions
100	6.92	170	4.40	17.0	14.4	39.4	Repeated tonic convulsions, state of tetany between convulsions
110	6.89	170	5.50	15.6	15.0	43.4	Repeated tonic convulsions
115	ns	ns	ns	ns	ns	ns	Died

Table 16. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 15

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.41	45	0.14	7.8	10.0	26.7	Normal appearance
10	7.45	51	0.31	8.4	9.8	30.4	Normal appearance
20	7.53	55	2.31	8.6	10.2	26.8	Severe muscular trembling
30	7.42	51	1.30	8.7	10.5	27.8	Mild muscle spasms, weakness
40	7.44	51	1.27	8.9	11.1	28.6	Mild muscle spasms, weakness
50	7.40	52	1.20	8.6	10.5	29.5	Mild muscle spasms, down
60	7.55	55.6	1.48	7.8	11.8	30.0	Mild muscle spasms, unable to rise
70	7.45	55.6	1.64	7.5	12.4	29.3	Mild muscle spasms, unable to rise
80	7.47	56.1	1.12	7.6	12.1	30.2	Mild muscular trembling
90	7.41	58.6	1.01	8.1	11.0	30.3	Arose, apprehensive, slight trembling
100	7.31	61.1	0.98	7.5	11.3	30.8	Appears normal, except hyperesthetic
160	ns	ns	ns	ns	ns	ns	Appears normal, eating hay
		Second dose of ammonium chloride given 24 hours after initial dose					
0	6.98	77	0.64	8.4	15.4	41.0	Normal appearance
10	7.00	90	2.18	10.2	16.7	45.8	Severe muscular trembling
20	7.02	101	2.41	10.7	17.2	49.0	Mild muscle spasms, weakness
30	7.00	112	2.45	9.6	17.9	49.9	Severe muscle spasms, down
40	7.06	122	2.06	9.5	18.9	47.5	Severe muscle spasms, unable to rise
50	6.97	132	4.62	12.2	22.7	51.7	Tonic convulsions

Table 16. (Continued)

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
60	6.91	150	4.90	14.1	27.2	53.9	Tonic convulsions, remained in tetany between convulsions
70	6.57	154	6.13	17.3	26.7	61.9	Tonic convulsions, remained in tetany between convulsions
75	ns	ns	ns	ns	ns	ns	Died

Table 17. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 17

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.42	50	0.27	7.1	4.8	14.5	Normal appearance
10	7.45	69	2.27	10.1	6.8	20.8	Apprehensive, severe muscular trembling, weakness
20	7.26	94	3.22	11.3	9.2	22.9	Severe muscle spasms, down
30	7.08	147	3.68	12.0	8.7	25.5	Tonic convulsions, state of tetany between convulsions
40	6.95	171	5.04	13.7	11.2	29.6	Tonic convulsions
50	6.66	173	5.47	15.8	11.7	33.7	Tonic convulsions, vomition
57	ns	ns	ns	ns	ns	ns	Died

Table 18. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 16

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.31	104	0.13	8.6	6.8	22.8	Normal appearance
10	7.34	96	0.88	8.9	8.3	25.0	Apprehensive
20	7.33	99	0.99	8.9	8.5	25.9	Apprehensive, hyperesthetic
30	7.32	92	1.13	8.7	9.2	24.2	Mild muscular trembling
40	7.29	96	1.32	9.2	9.4	29.8	Severe muscular trembling, weakness
50	7.29	108	1.66	9.2	9.9	28.6	Severe muscular trembling, muscle spasms
60	7.16	154	1.32	9.8	10.7	31.0	Down, unable to rise, muscle spasms
70	6.97	192	2.14	11.4	11.2	32.5	Tonic convulsions
80	6.92	199	2.78	11.3	13.4	36.0	Comatose, tonic spasms
90	6.92	200	3.16	11.9	13.3	39.9	Repeated tonic convulsions intermittent and with severe tonic muscle spasms until death
100	6.75	221	3.52	12.8	13.4	40.2	
110	6.70	201	3.88	13.7	15.0	40.7	
120	6.58	200	7.67	15.3	14.8	44.6	
130	6.54	180	5.20	16.1	15.6	43.8	
140	6.59	172	5.04	17.3	15.1	46.2	
150	6.58	175	5.86	16.7	16.5	44.7	
156	ns	ns	ns	ns	ns	ns	Died

Table 19. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 18

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.34	57	0.27	7.9	10.7	26.0	Normal appearance
10	7.33	79	0.86	9.0	11.9	30.3	Hyperesthetic
20	7.23	92	2.05	9.5	12.6	30.6	Severe muscular trembling, weakness
30	7.18	102	2.43	10.2	13.1	31.3	Severe muscle spasms, weakness
40	7.07	122	3.07	10.5	14.1	34.8	Severe muscle spasms, down
50	6.96	158	3.94	12.3	16.1	39.6	Tonic convulsions
60	6.77	144	5.04	15.4	15.4	39.7	Tonic muscle spasms
70	6.60	121	5.73	17.9	18.2	46.6	Repeated tonic convulsions until death, state of tetany between convulsions
80	6.55	110	6.21	20.3	18.3	50.9	
89	ns	ns	ns	ns	ns	ns	Died

Table 20. Blood chemical changes and clinical signs observed during ammonium chloride poisoning: sheep number 19

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.39	65	0.12	7.8	5.8	20.1	Normal appearance
10	7.36	80	0.54	8.0	7.2	20.0	Normal appearance
20	7.50	81	1.04	8.6	6.9	22.3	Mild muscular trembling
30	7.51	84	1.16	7.8	8.4	23.0	Mild muscular trembling, hyperesthetic
40	7.40	96	1.22	8.9	8.5	23.8	Severe muscular trembling, hyperesthetic
50	7.40	111	1.58	9.0	9.9	25.2	Severe muscular trembling, weakness
60	7.40	145	0.78	8.9	10.8	25.6	Mild muscle spasms, down
70	7.32	161	2.09	9.0	12.1	28.9	Severe muscle spasms, unable to rise
80	7.28	197	1.39	10.1	13.4	32.2	Severe muscle spasms
90	7.16	221	1.38	10.7	17.4	30.9	Tonic muscle spasms, opisthotonus
100	7.00	253	3.30	13.2	16.6	35.4	Tonic convulsions
110	6.98	242	4.54	13.8	17.7	36.4	Repeated tonic convulsions until death, state of tetany between convulsions
120	6.96	221	5.50	16.1	19.1	38.5	
130	6.86	210	4.97	17.7	20.5	41.8	
140	6.89	244	6.14	18.0	17.7	40.0	
144	ns	ns	ns	ns	ns	ns	Died

Table 21. Blood chemical and clinical signs observed in untreated controls during ammonium chloride poisoning: control sheep number 20

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.35	42	0.32	7.7	8.1	23.2	Normal appearance during entire 100 minutes
10	7.50	45	0.24	8.3	8.5	22.2	
20	7.43	45	0.27	7.5	8.1	20.9	
30	7.41	46	0.27	7.4	8.0	22.3	
40	7.49	51	0.28	7.2	8.0	21.0	
50	7.49	50	0.29	8.0	8.4	20.4	
60	7.55	51	0.25	7.2	8.0	22.3	
70	7.50	51	0.26	6.8	10.2	23.4	
80	7.55	51	0.27	7.2	9.6	22.8	
90	7.45	53	0.24	8.1	9.1	22.2	
100	7.42	53	0.25	7.4	9.9	22.4	

Table 22. Blood chemical and clinical signs observed in untreated controls during ammonium chloride poisoning: control sheep number 21

Sampling and observation time in min.	pH	Glucose mg. per 100 ml.	NH_3-N mg. per 100 ml.	AAN mg. per 100 ml.	BUN mg. per 100 ml.	NPN mg. per 100 ml.	Clinical signs
0	7.43	63	0.24	9.3	9.8	24.3	Normal appearance during entire 100 minutes
10	7.48	62	0.24	7.8	10.0	24.8	
20	7.54	62	0.22	7.2	9.8	25.7	
30	7.43	57	0.21	7.2	10.5	23.2	
40	7.47	58	0.21	6.9	9.9	23.0	
50	7.47	63	0.19	6.8	9.1	22.7	
60	7.48	58	0.17	7.1	9.7	22.0	
70	7.42	56	0.17	7.2	8.4	23.6	
80	7.57	56	0.21	7.2	8.9	22.3	
90	7.37	52	0.22	6.9	8.4	22.7	
100	7.43	53	0.21	6.9	8.5	23.0	

Cardiovascular system

General passive hyperemia was grossly prominent in all of the poisoned animals. The blood was bright red in some and dark red in others. Petechial and ecchymotic hemorrhages were present in the epicardium and myocardium and along the aorta of each animal. Massive myocardial hemorrhages as shown in Figure 2 were observed in several animals.

On histologic examination of the heart tissues, numerous hemorrhages were found in the myocardium of each animal. The degree of hemorrhage varied from animal to animal within each group. In some, only petechial or small ecchymotic hemorrhages were found while massive hemorrhages were seen in others.

Digestive system

Other than mild hyperemia of the mucosa of the rumen and retioulum, no changes were found in the rumen, reticulum, omasum, and abomasum. The small intestines of all of the poisoned sheep were hyperemic. Petechial hemorrhages were found in the submucosa of the small intestines of half of them.

Microscopically, passive hyperemia occurred in all of the digestive tissues examined. Mild cloudy swelling of the hepatic cells of the centrolobular regions was observed.

Endocrine system

Other than severe passive hyperemia, no changes were observed in the adrenal, pituitary, thyroid, and pancreatic glands.

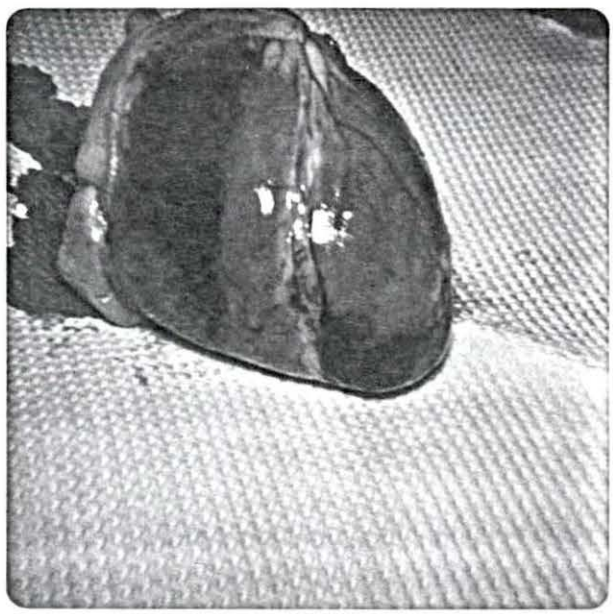


Figure 2. Massive myocardial hemorrhages in the heart of ewe 25. Death occurred 1 hour and 25 minutes after the administration of ammonium sulfate

Lymphatic system

Passive hyperemia was found in all of the lymphatic tissues examined grossly, and most of the lymph nodes contained blood within their sinuses giving the appearance of hemorrhages. Numerous petechial and ecchymotic hemorrhages were present in the thymus gland of each poisoned animal (Figure 3).

On histologic examination, focal accumulations of erythrocytes were found within the sinuses of most of the lymph nodes examined. In view of the numerous hemorrhages observed in various tissues, it is most probable that the erythrocytes were carried into the sinuses by the lymph that drained from the hemorrhagic areas.

Several changes were observed in the thymus glands. Severe passive hyperemia and hemorrhages were present in the centrolobular areas of each of the glands (Figure 4). A number of Hassall's corpuscles had undergone cystic degeneration and necrosis in most of the glands (Figure 5). Accumulations of polymorphonuclear leucocytes and eosinophiles (Figure 6) were present within the lobules of each gland in varying concentrations. Numerous round reticuloendothelial cells having round nuclei and pink-stained cytoplasm that closely resembled large plasma cells were observed in the centrolobular areas of most of the glands. These features were not observed in the glands of the control animals.

Muscular system

The only changes found in the skeletal muscles examined were severe passive hyperemia and varying degrees of hemorrhage.

Figure 3. Numerous hemorrhages in the thymus gland at the base of the heart of sheep 38 poisoned with a mixture of ammonium salts

Figure 4. Passive hyperemia and hemorrhage in the centrolobular area of the thymus gland of sheep 5 poisoned with ammonium chloride
Mayer's hematoxylin and eosin Y. x 100

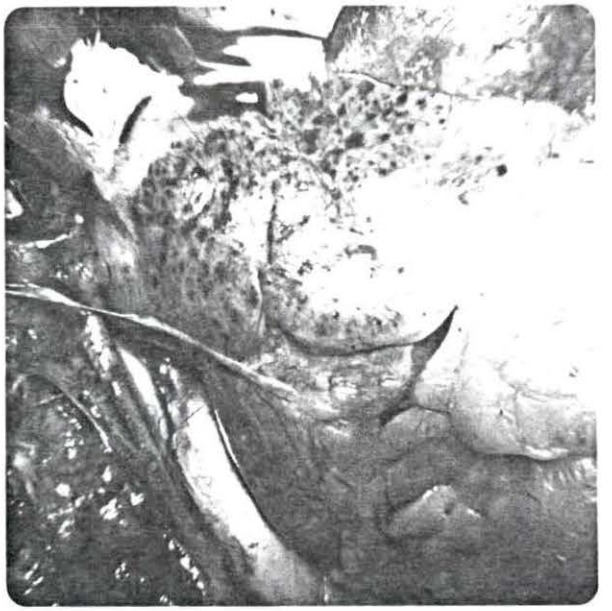
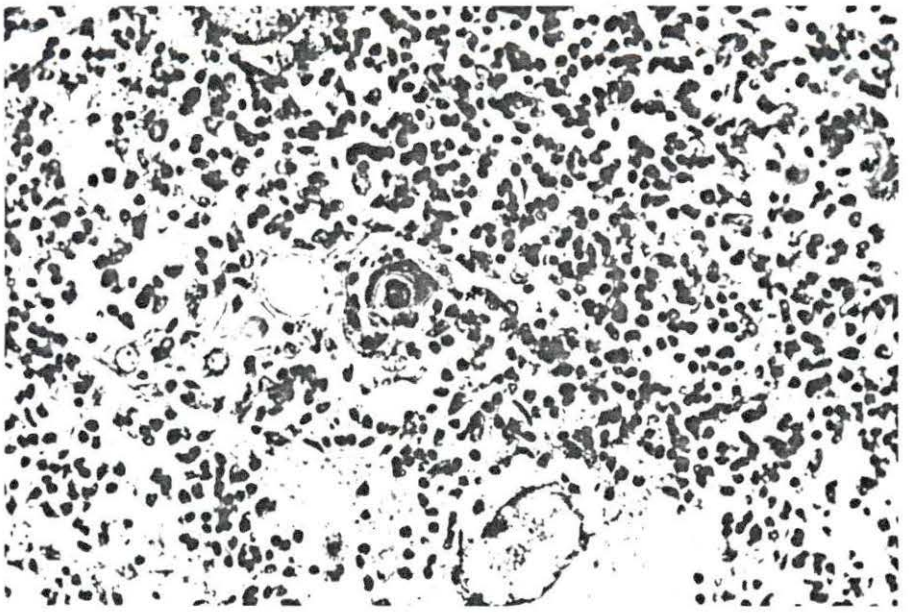
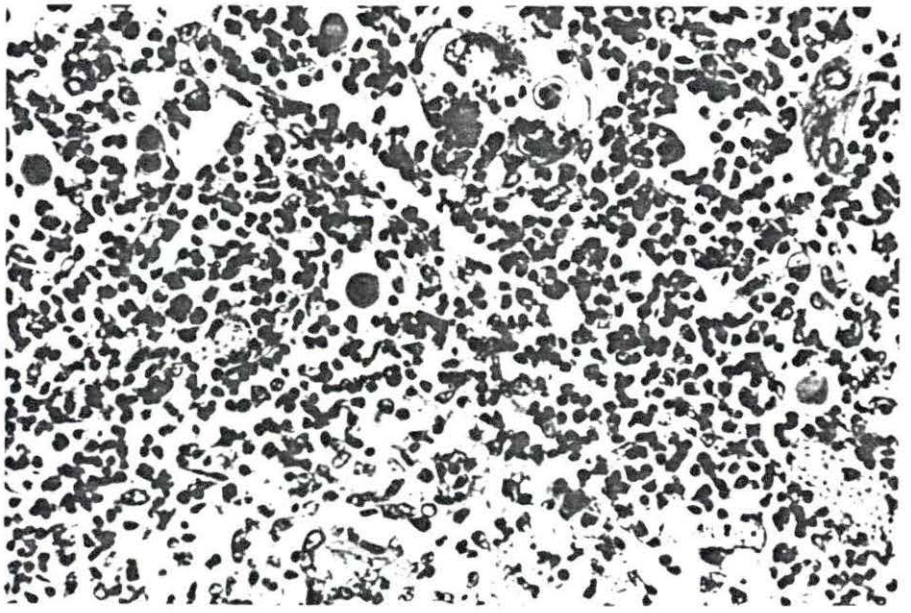


Figure 5. Cystic degeneration and necrosis of Hassall's corpuscles and hemorrhage in the thymus gland of sheep 36 poisoned with a mixture of ammonium salts
Mayer's hematoxylin and eosin Y. x 250

Figure 6. Neutrophiles, eosinophiles, and a degenerated Hassall's corpuscle in the thymus gland of sheep 22 poisoned with ammonium sulfate
Mayer's hematoxylin and eosin Y. x 250





Nervous system

On gross examination, severe passive congestion was observed in the brain and spinal cord of all of the poisoned sheep.

Microscopically, passive hyperemia of the brain and spinal cord was found in each animal. A few contained a number of small hemorrhages (Figure 7).

Respiratory system

Other than general hyperemia and a number of petechiae in the trachea, very little gross change was seen in the upper respiratory tract.

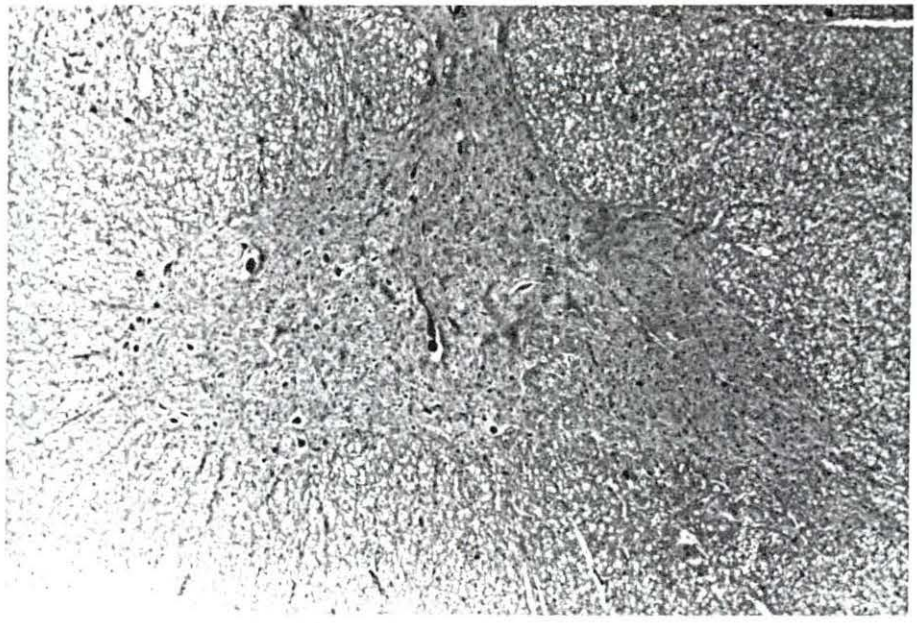
Microscopically, there were a few foci of hemorrhage in the submucosa of the trachea as well as passive hyperemia.

Grossly, the lungs were distended and severely congested with blood. A great number of subserous petechial and ecchymotic hemorrhages were observed (Figure 8). Upon cutting through the lung, a bright red sanguineous fluid drained freely from the cut surface (Figure 9). Numerous hemorrhages were seen in the submucosa of the bronchi. Hemorrhagic fluid was observed in the lumina of the bronchioles of the lungs of several of the animals. Lungs in which hemorrhagic fluids were not observed in the bronchioles contained a small amount of serous fluid but not the amount expected in severe pulmonary edema.

Microscopically, the pulmonary lesions were similar in all of the poisoned animals and were found to be consistent in all lobes of the lungs. The most prominent alteration observed was severe diffuse hyperemia. The alveolar capillaries were extremely distended and filled with erythrocytes to the extent that very little alveolar space remained, thereby presenting

Figure 7. Passive hyperemia in the spinal cord of sheep 11 poisoned with ammonium chloride
Mayer's hematoxylin and eosin Y. x 25

Figure 8. Severe congestion and hemorrhages in a diaphragmatic lobe of the lungs of sheep 27 poisoned with ammonium sulphate



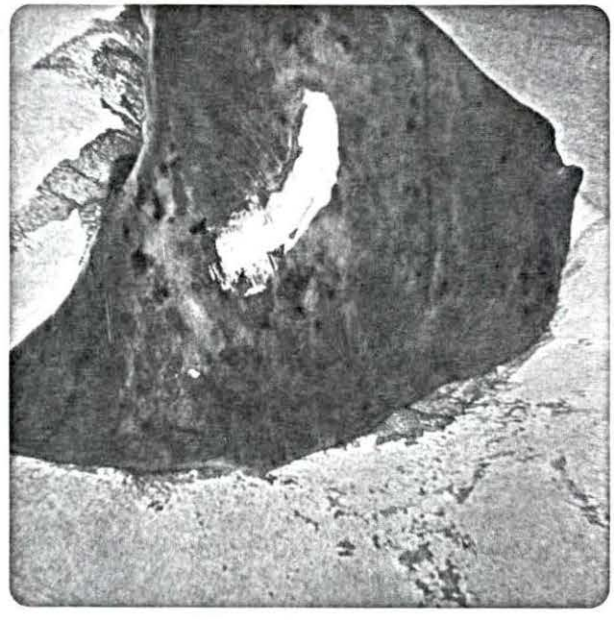
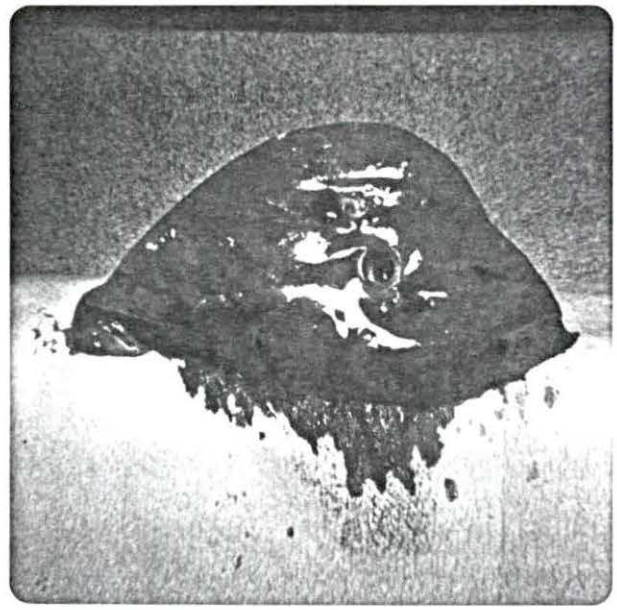
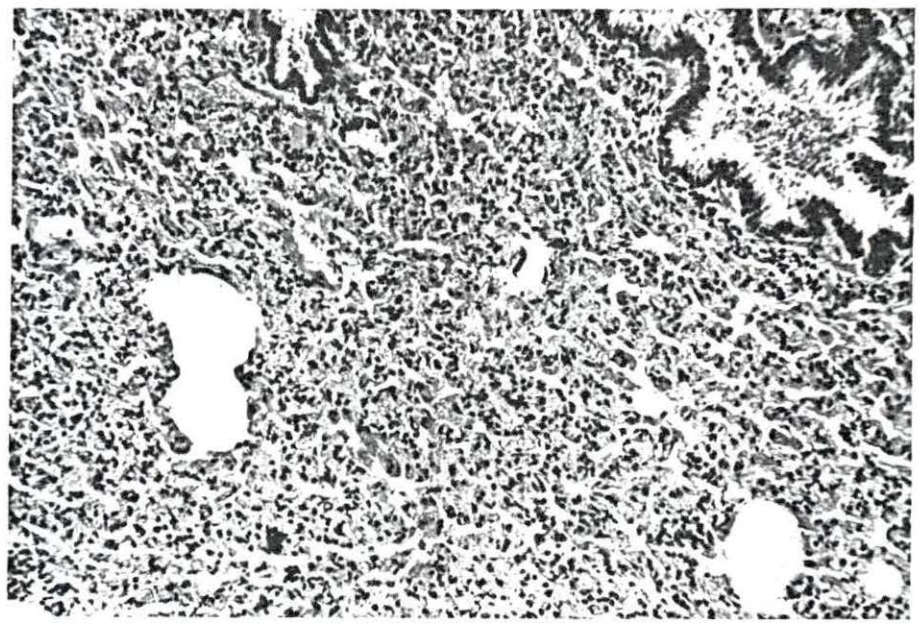


Figure 9. Transverse section of the lung observed in Figure 8

Figure 10. Active hyperemia, atelectasis, and alveolar emphysema in the lung of sheep 37 poisoned with a mixture of ammonium salts
Mayer's hematoxylin and eosin Y. x 100





the appearance of atelectasis (Figure 10). Alveolar hemorrhages were found in most of the lungs, and in a few, hemorrhagic fluid was found in the lumina of the bronchioles (Figure 11). Alveolar emphysema was present in varying degrees in all lobes of each of the lungs. There was alveolar edema in many of the lungs (Figure 12) but some contained very little. The musculature of the bronchi and bronchioles was slightly hyperplastic.

Urogenital system

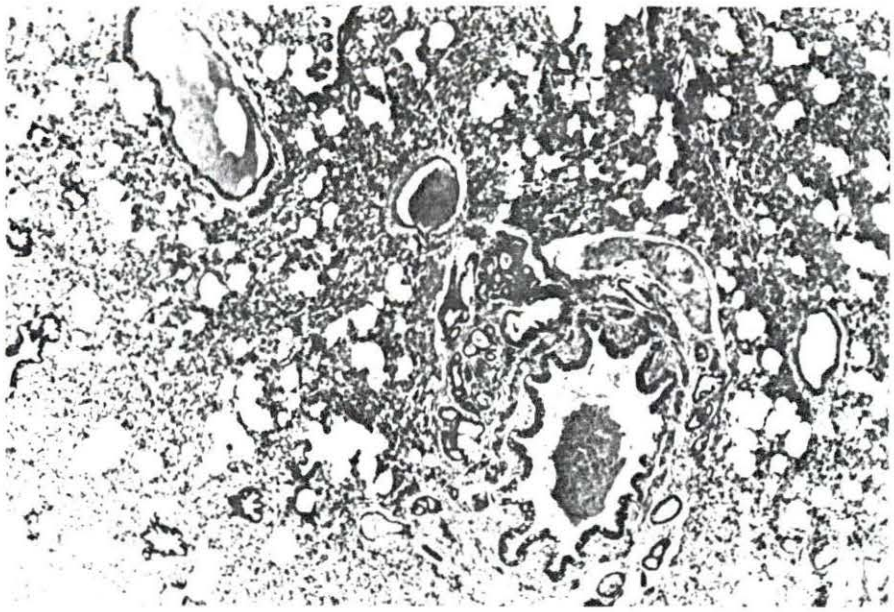
Other than passive hyperemia, no gross or microscopic lesions were observed in the urinary bladders, ovaries, and uteri of the barren ewes or the few that were pregnant. The fetuses of the latter were well developed, without wool growth, and estimated to be at about the 110th to 120th day of fetation. The fetal membranes were severely congested, and numerous small subcutaneous hemorrhages occurred in the fetuses.

On gross examination, cloudy swelling and severe passive hyperemia were obvious in the kidneys of the majority of the poisoned ewes, and petechiae were found in some of them.

Microscopic examination of the kidneys revealed multiple focal areas of cloudy swelling and early coagulative necrosis of the proximal convoluted tubules, marked diffuse hyperemia of the glomerular tufts, and degeneration of the glomerular tuft cells (Figures 13 and 14). The glomerular tufts were greatly distended with blood, and the glomerular filtrate contained an amorphous coagulum and a few erythrocytes (Figures 15 and 16). Many of the cells of the glomerular tufts were vacuolated, presenting the appearance of hydropic swelling.

Figure 11. Hemorrhage into the lumina of bronchiole and a bronchus, hyperemia, and alveolar emphysema in the lung of sheep 8 poisoned with ammonium chloride
Mayer's hematoxylin and eosin Y. x 25

Figure 12. Hyperemia, alveolar edema, and alveolar emphysema in the lung of sheep 24 poisoned with ammonium sulfate
Mayer's hematoxylin and eosin Y. x 100



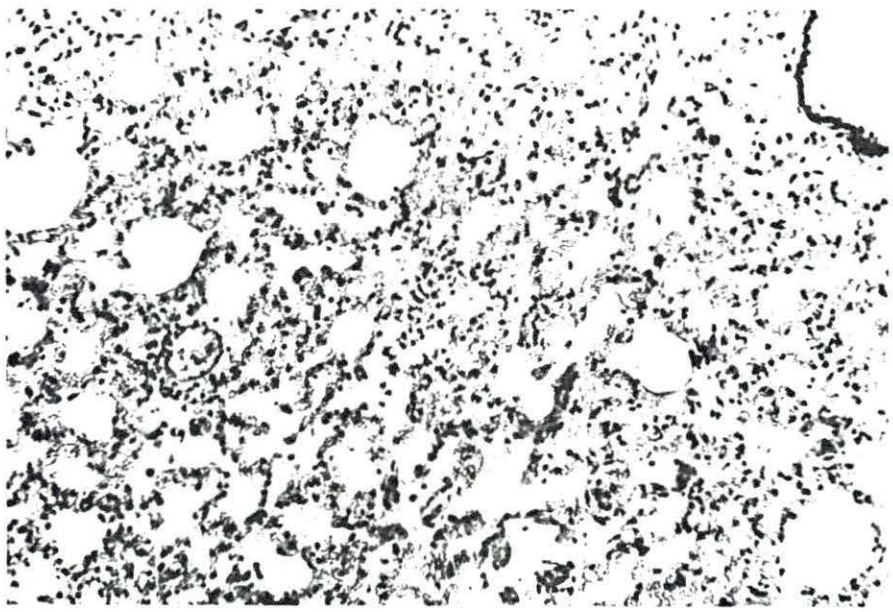
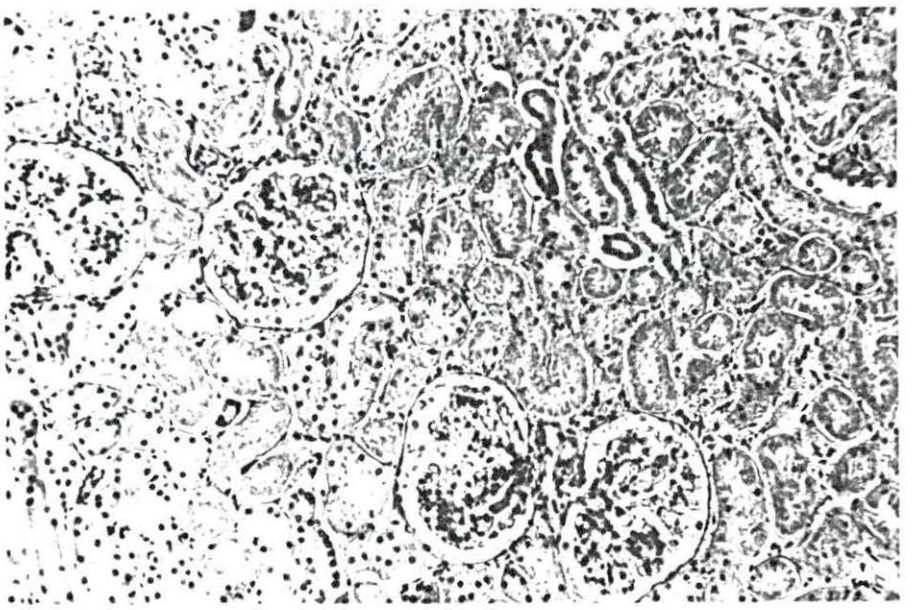


Figure 13. Severe cloudy swelling and early coagulative necrosis of the proximal convoluted tubules, hyperemia of the glomerular tufts, degeneration of the glomerular tuft cells, and amorphous deposits within the glomerulli in a kidney of sheep 4 poisoned with ammonium chloride
Mayer's hematoxylin and eosin Y. x 100

Figure 14. Severe cloudy swelling and early coagulative necrosis of the proximal convoluted tubules, hyperemia of the glomerular tuft, dilation of the glomerular capillary, and amorphous deposits and an occasional erythrocyte within the glomerulus in a kidney of sheep 8 poisoned with ammonium chloride
Mayer's hematoxylin and eosin Y. x 400



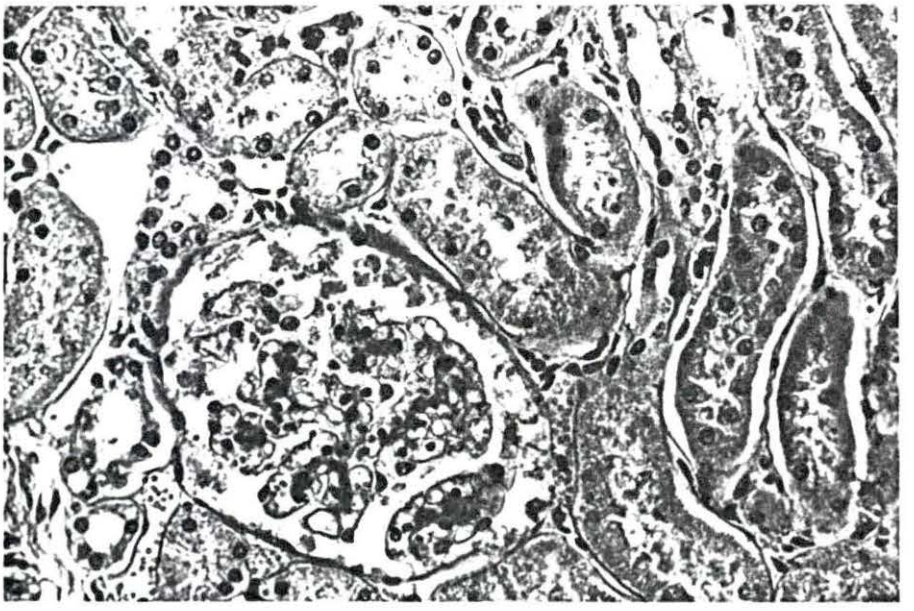
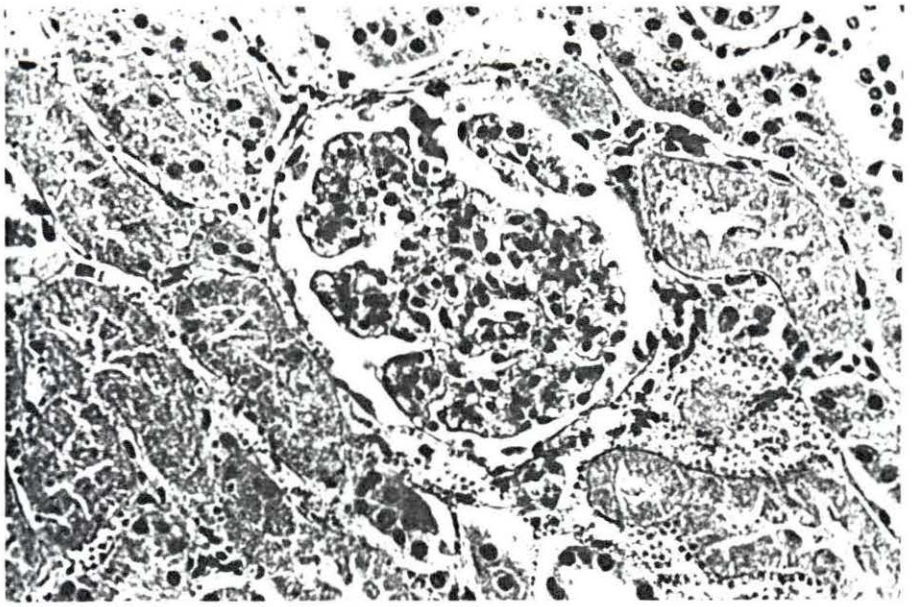
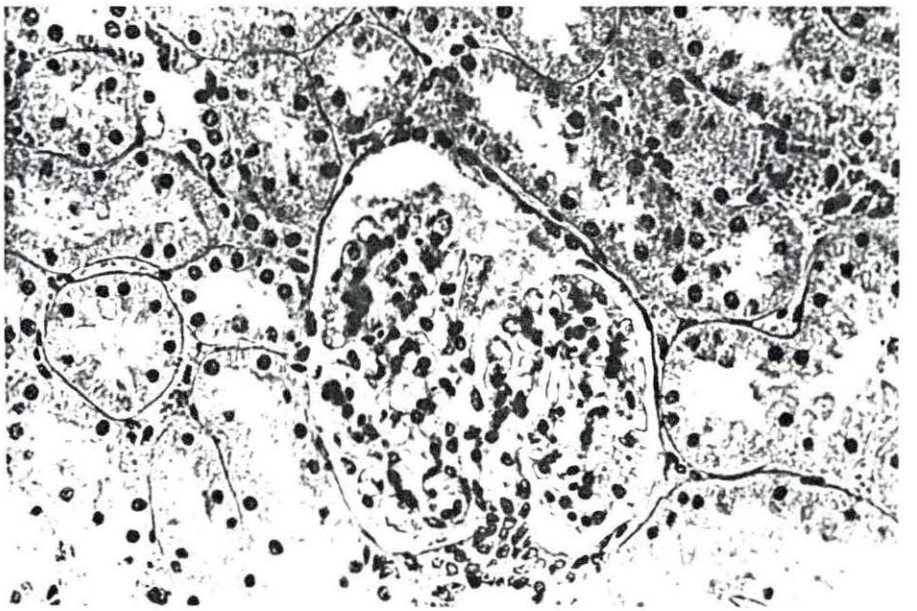


Figure 15. Severe cloudy swelling and early coagulative necrosis of the proximal convoluted tubules, hyperemia of the glomerular tuft, and dilation of the glomerular capillary in a kidney of sheep 37 poisoned with a mixture of ammonium salts
Mayer's hematoxylin and eosin Y. x 400

Figure 16. Hyperemia of the glomerular tuft, dilation of the glomerular capillary, and amorphous deposits and an occasional erythrocyte within the glomerulus in a kidney of sheep 28 poisoned with ammonium sulfate
Mayer's hematoxylin and eosin Y. x 400





DISCUSSION

It was evident from these studies that the quantity of ammonium salts required to cause poisoning is variable from animal to animal. In some sheep, a single dose caused severe clinical signs and death within as little as 30 minutes; in others, additional doses were required to cause death. The early observations of Walley (66) that ammonium salts could cause severe nervous signs in livestock lasting for 20 to 30 minutes and that the animals would recover completely without apparent confusion and seek food were confirmed in these experiments, especially with sheep 1, 2, and 39.

Whether or not the damage to metabolic function and tissues would have been repairable in animals that recovered from acute poisoning is not revealed in this study. That kidney damage did occur was demonstrated by the hematuria and albuminuria observed in those that were severely poisoned but appeared normal 24 hours later.

The clinical signs observed during these experiments did not differ from those described by Lewis (37), Oltjen et al. (51), and Repp et al. (55). Body temperatures were not discussed in their reports. Koval and Vas'ko (36) reported that the body temperature of cattle during urea poisoning was subnormal, but they did not indicate the stage of poisoning that the temperatures had been taken. The body temperature of the sheep in these studies consistently increased sharply during the 5 to 10 minutes preceding death (Tables 5, 6, and 7).

The poisoning syndrome brought about by ammonium salts appeared to be related directly to the concentration of ammonia nitrogen in the peripheral blood. Clinical signs of poisoning appeared when the blood ammonia nitro-

gen reached 0.80 to 1.00 mg. per 100 ml. and became more severe as the concentration increased (Tables 11-16). At concentrations above 2.00 to 2.50 mg. per 100 ml., tonic convulsions occurred. These observations are in close agreement with those reported by Lewis (37) and Repp et al. (55).

The changes observed in the pH values of the blood did not appear to be related to the clinical signs of poisoning. Although the pH changed to lower values in all cases and the changes observed in sheep 38, 40, 41, and 42 (Tables 11, 13, 14, and 15) decreased with the appearance and progress of the signs of poisoning, this correlation did not occur in sheep 39 and 43 (Tables 12 and 16). In fact, the acidosis observed in sheep 39 twenty-two hours after it recovered from severe poisoning was more marked than it was during the period when the signs of poisoning were observed. Nevertheless, acidosis is one of the consistent changes that occurred during inorganic ammonium salt poisoning. It must be given consideration as an important part of the syndrome that may require corrective measures when therapy is applied to animals poisoned with ammonium salts.

The sharp 2- to 4-fold increase of blood glucose observed in all of the poisoned animals must also be considered a constant feature of ammonium salt poisoning. Although there was a close correlation between the rise of blood glucose levels and the development of clinical signs of poisoning in sheep 38, 40, 41, 42, and 43 (Tables 11, 13, 14, 15, and 16), this correlation was not observed in sheep 39 (Table 12). The blood glucose was not elevated in sheep 39 during the first 110 minutes of poisoning nor 22 hours later, but when the second dose of ammonium chloride was given 24 hours after the initial dose, the blood glucose promptly increased. In evaluating these observations, two major questions arise: 1) What was the cause of

the rise in blood glucose and 2) what is its significance in the poisoning syndrome?

On first evaluation, it would seem logical to consider this rise in glucose as the result of glucose mobilization brought about by adrenalin release due to the severe nervous state of the animal. However, Satake (57) has demonstrated that ammonium chloride given subcutaneously produced hyperglycemia in adrenalectomized rabbits to the same degree as in those with intact adrenal glands. While it is possible that this may not be the case in sheep, there is evidence supporting the premise that the hyperglycemia observed was not strictly due to the mobilization of glucose by adrenalin release.

Adrenalin release would have been expected in many of these animals because of the excitement incident to the handling, the collection of urine and blood samples, and the administration of fluids intraruminally. Apparently this was the case with many of the sheep if one considers an elevated blood glucose level as an indicator of increased adrenalin release. Accordto Dukes (12a, p. 49) the normal values for blood glucose in sheep fall within a range of 30 to 50 mg. per 100 ml. of blood. The blood chemical values recorded in Tables 7, 10, 13, and 15 through 22 reveal that 24 of the sheep had levels above this range prior to receiving the ammonium salts. Nine of the 12 control sheep also had levels above this range during one or more of the sample collection periods. Although these values were far lower than those found when the animals were poisoned, it may be argued that the

higher values found during poisoning were related to the degree of excitation or stimulation caused by the severe nervous state of the poisoned animals. While this appears to be a logical possibility, this assumption does not agree with the findings in sheep 1, 2, and 39 (Tables 8 and 14). Those sheep were severely poisoned by the initial dose of ammonium chloride and recovered. The blood samples collected during the time that severe signs of poisoning were observed did not have appreciably elevated glucose levels. After the second dose of ammonium chloride 24 hours later, the blood glucose levels increased to about three times the previous levels in each of the sheep. The great difference in the blood glucose levels observed in these three sheep cannot be easily explained as a glucose mobilization phenomenon. The differences do provide support for a premise that other factors are involved as the cause of the hyperglycemia.

The prime factor that must be taken into consideration as a cause of the hyperglycemia is the possible effect of blood ammonia on the metabolism of glucose. There is an interrelationship between the metabolic cycles involving ammonia and glucose. Ammonia is metabolized in the liver by way of the urea cycle as portrayed in Figure 17. Fumaric acid and urea are two products that are not directly reintroduced into the cycle. Fumaric acid is also a component of the tricarboxylic acid cycle portrayed in Figure 18 through which pyruvate derived from glucose is metabolized. If there is an overabundance of one of the products, such as fumaric acid, the activity of the cycle will decrease, thereby decreasing the metabolism of glucose. Furthermore, fumaric acid is converted to malic acid by the addition of water in a reaction that is catalyzed by the enzyme fumarase. An overabundance of malic acid inhibits the dehydrogenation of succinic acid of

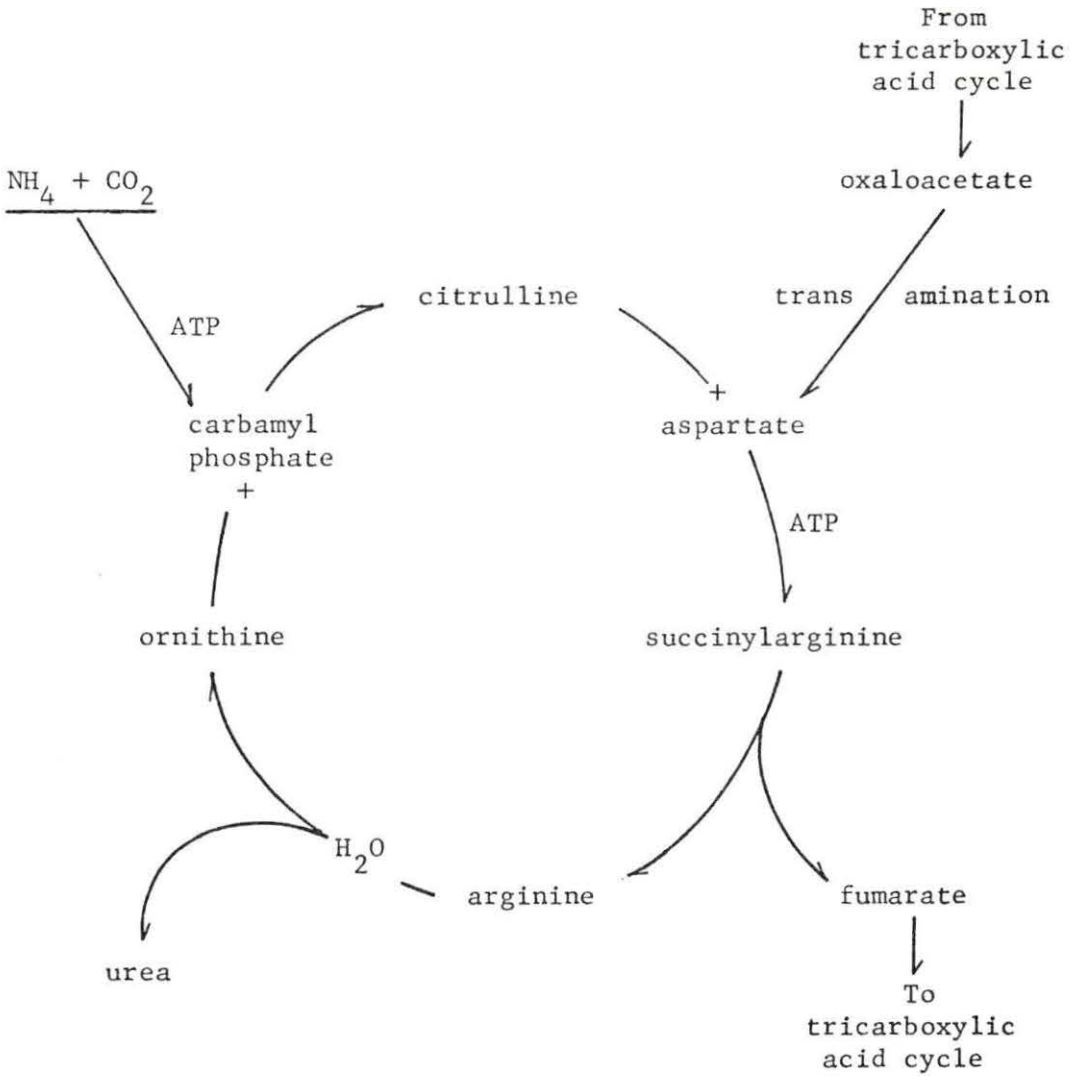


Figure 17. The conversion of ammonia into urea by way of the urea cycle (Karlson, 31b, p. 158)

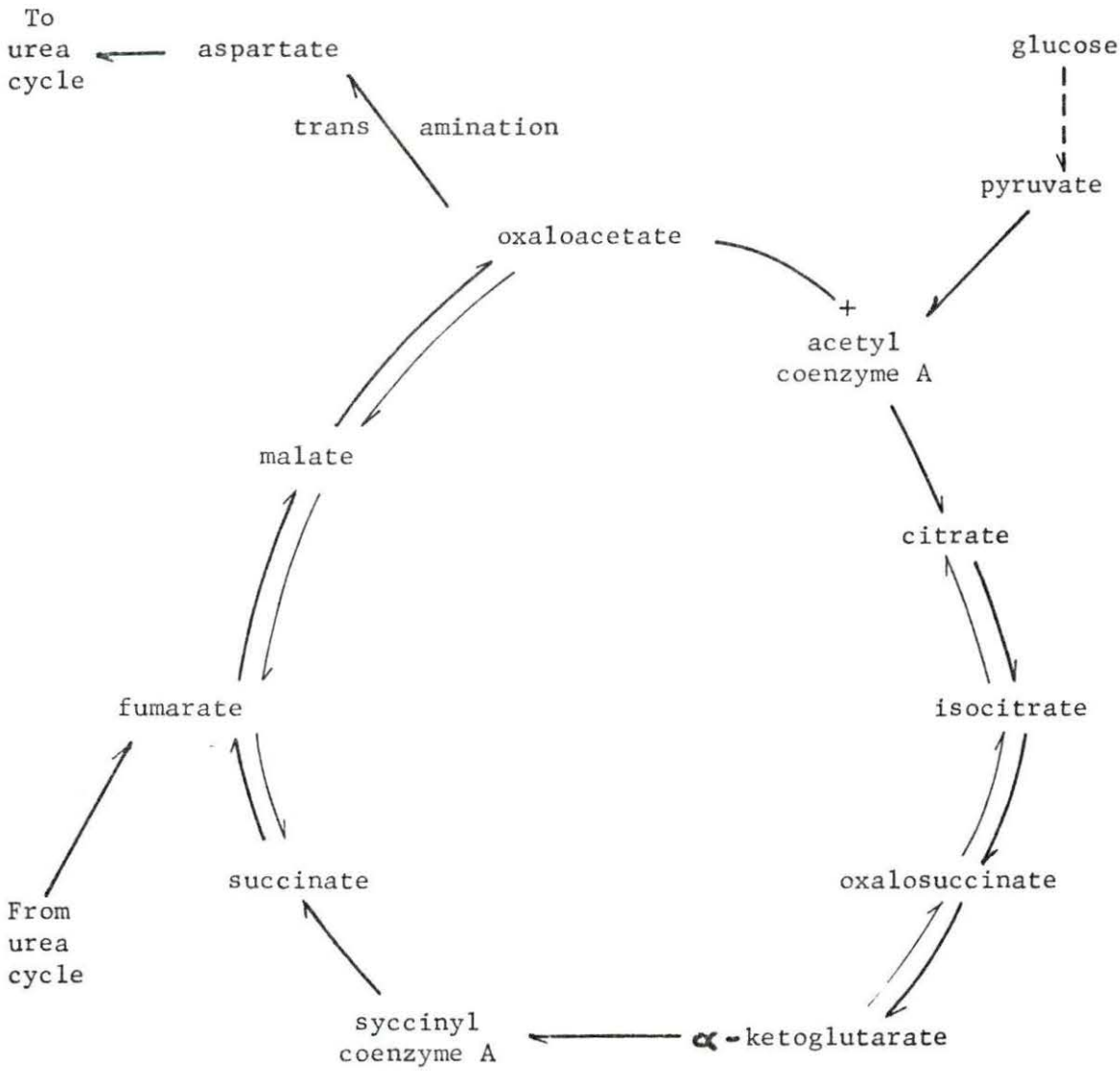


Figure 18. The tricarboxylic acid cycle (Karlson, 31b, p. 206)

the tricarboxylic acid cycle by competing with succinic acid as a substrate for succinic acid dehydrogenase (31b, pp. 205-211). This would result in an increase in the blood glucose concentration.

That overloading of the tricarboxylic acid cycle during ammonium salt poisoning did occur is indirectly supported. For every mole of urea formed in the urea cycle, a mole of fumaric acid is formed. Since the blood urea levels nearly doubled during poisoning in each animal in these studies, it may be assumed that an equivalent increase in fumaric acid production also occurred. The increased amount of fumaric acid would upset the equilibrium of the tricarboxylic acid cycle, and the increased amount of malic acid subsequently formed from the fumaric acid would inhibit the dehydrogenation of succinic acid, thereby impairing a part of the cycle's function. This would lead to a considerable decrease of the metabolism of glucose by way of the tricarboxylic acid cycle, thereby permitting an accumulation of glucose. That the increases in blood glucose levels of sheep 38 through 43 correlate closely with the increases of blood urea nitrogen also appear to support this premise. The reverse effect was demonstrated in man by Brown et al. (6): Glucose administered orally or intravenously brought about a significant rise in blood ammonia. They attributed this rise in ammonia to the interference of glucose with the urea cycle through the common product, fumaric acid.

The increase in the amino acids in the blood also parallels the increase in the urea nitrogen concentration. This reflects an increased

activity of the urea cycle and of the transamination reactions of tricarboxylic acid cycle intermediates indicated in Figure 15.

The cause of the hyperglycemia may, therefore, be attributed primarily to a reduction in glucose metabolism due to an imbalance of the tricarboxylic acid cycle brought about by the great activity of the urea cycle and partially to glucose mobilization caused by adrenalin release. The significance of the hyperglycemia to the poisoning is not revealed in these experiments except that it is a constant feature of ammonium salt poisoning.

The tissue damage observed primarily involves the vascular, respiratory, and urinary systems. Severe congestion accompanied by hemorrhages of the various organs and the musculature was a constant observation. Examination of the vascular system within the various tissues leaves the impression that direct damage has occurred to the endothelial lining of the vessel walls so that capillary membranes would break down and hemorrhages would occur. The fact that the glomerular tuft cells of the kidneys had undergone degenerative changes that permitted the passage of albumin and erythrocytes into the urine (Tables 8, 11, and 14) lends some support that direct damage to the vascular endothelial cells had occurred. It is possible that the hyperemia and hemorrhages might have been the result of other factors; the question will have to be resolved by further study.

The hyperemia and hemorrhages found in the lungs of these sheep were similar to the lesions observed during the early stages of pneumonia caused by chemical irritants. It has been demonstrated in dogs by Robin et al. (56) and Jacquez et al. (29) that the partial pressure of gaseous ammonia in the alveolar air of the lungs is equal to that in the arterial blood. The ammonium ion does not readily penetrate cell membranes and is

relatively non-toxic, but un-ionized ammonia (NH_3) passes through cell membranes freely (Milne et al. (45) and Gaertner and von Englehardt (17)). Warren (69, 70) attributed the toxicity of ammonium salts to the amount of un-ionized ammonia present in the blood. While the free ammonia was not measured in the exhaled air of the animals of these experiments, its transport across the alveolar membrane from the blood in sheep does not appear improbable. Its passage through the alveolar cells and its unbuffered presence in the alveolar air may account for the irritant effect observed.

The damage to the kidneys appeared to be due to the action of a toxic agent on the glomerular tuft cells and the renal tubules. The hematuria and albuminuria appeared to have had their origin from the glomerular tufts. Urinary ammonia is produced as a deamination product by the renal tubules cell, from which it diffuses into the fluid within the tubular lumen (Milne et al. (45)), its diffusion gradient dependent upon the pH of the urine and the concentration of the ammonia in the urine. As the amount of ammonia in the tubular fluid increases, less ammonia can diffuse out of the cells, and the direction of the diffusion may be reversed. It appears that the early coagulative necrosis that occurred in these sheep may have been caused by the accumulation of toxic quantities of ammonia in the cells.

SUMMARY

A study was made of the clinical and pathologic alterations found in sheep that had been poisoned with ammonium salts. Forty-five sheep were used in the study; 33 were poisoned with ammonium salts and 12 were employed as controls. The experimental method and results of administering toxic quantities of different ammonium salts intraruminally to sheep and of mock-treatment of controls are described.

Toxic quantities of ammonium chloride, of ammonium sulfate, or of a mixture of ammonium chloride, carbonate, phosphate, and sulfate resulted in muscular trembling, tonic muscle spasms, muscular weakness, tonic convulsions, and subsequent death. Ruminal stasis, rapid pulse, labored breathing, and a high body temperature were constant clinical findings.

Examination of the blood and urine disclosed sharp increases in the amino acid and urea levels of the blood, hyperammonemia, severe acidosis, hyperglycemia, hematuria, and albuminuria in each of the poisoned animals. The hyperglycemia appeared to be due to a disturbance in the metabolism of glucose.

A study of the sequence of blood chemical changes and the correlation of the changes with clinical signs of poisoning was conducted on six of the sheep administered toxic quantities of ammonium chloride. The clinical signs were correlated with increases in blood ammonia levels but not with changes in the amino acid, urea, or glucose levels or of the pH. Clinical signs of poisoning appeared when the blood ammonia level reached approximately 1.0 mg. per 100 ml. of blood. Convulsions occurred with levels

above 2.5 - 3.0 mg. per 100 ml. and terminal blood ammonia levels were above 5.0 mg. per 100 ml.

General passive hyperemia and numerous petechial and ecchymotic hemorrhages in the body musculature, heart, thymus gland, and lungs were constant alterations observed on gross examination.

On microscopic examination, the pulmonary lesions consisted of severe hyperemia, hemorrhages, alveolar edema, and alveolar emphysema. Degeneration and necrosis of Hassall's corpuscles and centrolobular hemorrhages were found in the thymus glands. Lesions in the kidneys consisted of severe cloudy swelling and multiple foci of early coagulative necrosis of the proximal convoluted tubules, general hyperemia of the glomerular tufts, and degeneration of the glomerular tuft cells.

With the exception of elevated blood chloride levels found in the group of sheep that received ammonium chloride, differences in the clinical and pathologic alterations were not found between any of the groups receiving ammonium salts.

LITERATURE CITED

1. Annison, E. F. Nitrogen metabolism in the sheep. Protein digestion in the rumen. Biochem. J. 64: 705-714. 1956.

2. Armed Forces Institute of Pathology. Manual of histologic and special staining technics. 2nd ed. New York, N.Y., The Blakiston Division, McGraw-Hill Book Company, Inc. 1960.

3. Bassini, B. and Ferrante, A. Sulla tossicita dell'ione ammonico. Arch. fisiol. 37: 180-189. 1937.

4. Bessman, S. P. and Bessman, A. N. The cerebral and peripheral intake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. J. Clin. Invest. 34: 622-628. 1955.

5. Bloomfield, R. A., Kearly, E. O., Creach, D. O. and Muhrer, M. E. Ruminal pH and absorption of ammonia and VFA (Abstract). J. of Animal Science 22: 833. 1963.

6. Brown, H., O'Hara, E., Brown, M. E., Covelli, V. H., Konda, D. and McDermott, W. V. Relation of blood glucose to blood ammonia and urea cycle enzymes. J. Am. Med. Assn. 199: 641-646. 1967.

7. Clark, R., Oyaert, W. and Quin, J. I. Studies on the alimentary tract of the Merino sheep in South Africa. XXI. The toxicity of urea to sheep under different conditions. Onderstepoort J. Vet. Research 25: 73-78. 1951.

8. Conway, E. J. and Byrne, A. LXI. An absorption apparatus for the micro-determination of certain volatile substances. 1. The micro-determination of ammonia. Biochem. J. 27: 419-429. 1933.

9. Coombe, J. B. and Tribe, D. E. The feeding of urea supplements to sheep and cattle. J. Agri. Sci. 55: 125-141. 1962.

10. Davis, G. K. and Roberts, H. F. Urea toxicity in cattle. Fla. Agr. Expt. Sta. Bul. 611. 1959.

11. Dinning, J. S., Briggs, H. M., Gallup, W. D., Orr, H. W. and Butler, R. Effect of orally administered urea on the ammonia and urea concentration in the blood of cattle and sheep, with observations on blood ammonia levels associated with symptoms of alkalosis. Am. J. Physiol. 153: 41-46. 1948.

12a. Dukes, H. H. The physiology of domestic animals. 7th ed. Ithaca, N.Y., Comstock Publishing Associates. 1955.

12b. Fazekas, I. Gy. v. Histologische Veranderungen des Zentralnervensystems bei experimentellen Ammoniakvergiftungen. Deut. Z. gerichtl. Med. 25: 102-111. 1935.

13. Fenton, H. J. H. On the limited hydration of ammonium carbamate. Proc. Royal Soc. London 39: 386-393. 1885.

14. Fingerling, G., Hientzsch, B., Kunze, H. and Reifgerst, K. Ersatz des Nahrungseiweisses durch Harnstoff beim wachsenden Rinde. Ladew. Vers.-Sta. 128: 221-235. 1937.

15. Fujimoto, Y. and Tajima, M. Pathological studies on urea poisoning. Jap. J. Vet. Sci. 15: 133-134. 1953.

16. Gaertner, K. Untersuchungen in vitro über die Ammoniakpassage durch die Pansenschleimhaut von Rindern und deren Beeinflussung durch pH-Wert und antidiuretisches Hormon. Zentr. Veterinärmed. 10: 11-20. 1963.

17. _____ and von Engelhardt, W. Untersuchungen über den Resorptionsmechanismus von Ammoniak an der Pensenschleimhaut. Deut. Tierärztl. Wochenschr. 71: 57-60. 1964.

18. Gallop, W. D., Pope, L. S. and Whitehair, C. K. Urea in rations for cattle and sheep. Okla. Agr. Expt. Sta. Bul. B-409. 1953.

19. Gangolli, S. and Nicholson, T. F. The determination of blood ammonia. Clin. Chim. Acta 14: 585-592. 1966.

20. Gigon, A. Kohlenhydratstoffwechsel und ammoniakbildung im Blute. Schweiz. med. Wochenschr. 57: 294-296. 1927.

21. Govan, A. D. T. and Parkes, J. Observations on experimental ammonium chloride acidosis. J. Path. Bact. 58: 505-515. 1946.

22. Hale, W. H. and King, R. P. Possible mechanism of urea toxicity in ruminants. Proc. Soc. Exptl. Biol. Med. 89: 112-114. 1955.

23. Hart, E. B., Bohstedt, G., Deobald, H. J. and Wegner, M. I. The utilization of simple nitrogenous compounds such as urea and ammonium bicarbonate by growing calves. J. Dairy Sci. 22: 785-798. 1939.

24. Hazard, R. and Vaille, C. L'ion ammonium, facteur d'acidose vraie et d'hyperglycemie. Compt. rend. soc. biol. 123: 576-578. 1936.

25. Head, M. J. Protein utilization by the dairy cow. Proc. Nutr. Soc. 18: 108-112. 1959.

26. Hogan, J. P. The absorption of ammonia by the rumen of the sheep. Australian J. Biol. Sci. 14: 448-460. 1961.

27. Horvath, A. A. Ammonia and blood sugar. J. Biol. Chem. 70: 289-296. 1926.

28. Howell, S. F. and Sumner, J. B. The specific effects of buffers upon urease activity. J. Biol. Chem. 104: 619-626. 1934.

29. Jacquez, J. A., Poppell, J. W. and Jeltsch, R. Partial pressure of ammonia in alveolar air. Science 129: 269-270. 1959.

30. Juhász, B. The effect of ammonia on rumen peristalsis (Translated title). Acta Vet. Acad. Sci. Hung. 15: 317-333. 1965. Original not available; abstracted in Nutritional Abstracts and Reviews 36: 6289. 1966.

31a. Kaishio, Y., Higaki, S., Horii, S. and Awai, Y. On the transition of the given urea in the body of ruminants. Bull. Natl. Inst. Agr. Sci., Ser. G, 2: 131-139. 1951.

31b. Karlson, P. Introduction to modern biochemistry. 2nd ed. New York, N.Y., Academic Press. 1965.

32. Kita, T., Shibanai, D., Takahashi, M. and Yasuda, Y. Studies on urea poisoning in ruminants. I. J. Jap. Vet. Med. Assn. 12: 302-306. 1959.

33. Koenig, H. and Koenig, R. Acute pulmonary edema produced by ammonium salts. Fed. Proc. 7: 67. 1948.

34. _____ and _____. Production of acute pulmonary edema by ammonium salts. Proc. Soc. Exptl. Biol. Med. 70: 375-380. 1949.

35. _____ and _____. Pulmonary changes produced by ammonium salts (Abstract). Anat. Rec. 100: 684. 1948.

36. Koval, M. P. and Vas'ko, I. V. Poisoning of cows with carbamide (urea) (Translated title). Veterinariya (Moscow) 41: 49-50. 1964. Original available but not translated; abstracted in Veterinary Bulletin 35: 1425. 1965.

37. Lewis, D. Ammonia toxicity in the ruminant. J. Agr. Sci. 55: 111-117. 1960.

38. _____, Hill, K. J. and Annison, E. F. Studies on the portal blood of sheep. I. Absorption of ammonia from the rumen of the sheep. Biochem. J. 66: 587-592. 1957.

39. McDonald, I. W. The absorption of ammonia from the rumen of the sheep. Biochem. J. 42: 584-587. 1948.

40. Mack, E. and Villars, D. S. The action of urease in the decomposition of urea. J. Am. Chem. Soc. 45: 505-510. 1923.

41. Mann, F. C. and Bollman, J. L. The formation of ammonia following complete removal of the liver. Am. J. Physiol. 85: 390-391. 1928.

42. Marfori, P. Ueber die Ammoniakmengen, welche der Organismus in Harnstoff umzuwandeln vermag. Arch. Exptl. Path. Pharmakol. 33: 71-78. 1893.

43. Masamune, H. The influence of different anions of the ammonium salts on the sugar in the blood and urine (Translated title). Fukuoka-Ikwadaigaku-Zasshi 20: 511-531. 1929. Original not available; abstracted in Chemical Abstracts 23: 2216. 1929.

44. Maynard, L. A. Animal nutrition. 2nd ed. New York, N.Y., McGraw-Hill Book Company, Inc. 1947.

45. Milne, M. D., Scribner, B. H. and Crawford, M. A. Nonionic diffusion and the excretion of weak acids and bases. Am. J. Med. 24: 709-729. 1958.

46. Miyarua, R. The mechanism of hyperglucemia produced by ammonium carbonate and by urea. I. Hyperglucemia by injections of ammonium carbonate and urea and carbohydrate tolerance (Translated title). Sei-i-kai Med. J. 54: 1544-1553. 1935. Original not available; abstracted in Chemical Abstracts 30: 3098. 1936.

47. Miyoshi, M. Uber den Einfluss des Ammoniumchlorides auf den Blutzucker und den Gehalt des Muskels an Glycogen und den verschiedenen Phosphaten am Kaninchen. Mitt. med. Akad. Kioto 13: 837-867. 1935. Original not available; abstracted in Ber. ges. Physiol. exptl. Pharmakol. 87: 569-570. 1935.

48. Morita, S. Die Hyperglykämie und Glycosurie durch Ammoniumchlorat und magnesiumsulfat bei der Katze. Tohoku J. Exptl. Med. 3: 363-413. 1922.

49. Nix, R. R. and Anthony, W. B. Urea - lethal dose and toxic syndrome for sheep (Abstract). J. Animal Science 24: 286. 1965.

50. Nunn, J. A. Veterinary toxicology. New York, N.Y., William R. Jenkins Company. 1907.

51. Oltjen, R. R., Waller, G. R., Nelson, A. B. and Tillman, A. D. Ruminant studies with diammonium phosphate and urea. J. Animal Science 22: 36-42. 1963.

52a. Oser, B. L. Hawk's physiological chemistry. New York, N.Y., McGraw-Hill Book Co. 1965.

52b. Phillips, G. B., Schwartz, R., Gabuzda, G. B. and Davidson, C. S. The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. New Engl. J. Med. 247: 239-246. 1952.

53. Priouzeau, M. Purpura hémorragique des Bovidés, consécutif a l'ingestion de sulfate d'ammonique. Rec. Med. Vet. 127: 287-295. 1951.

54. Reis, P. J. and Reid, R. L. In vitro studies on the effect of pH and of glucose on ammonia accumulation in the rumen of sheep. Australian J. Agr. Research 10: 71-80. 1959.

55. Repp, W. W., Hale, W. H., Cheng, E. W. and Burroughs, W. The influence of oral administration of non-protein nitrogen feeding compounds upon blood ammonia and urea levels in lambs. J. Animal Science 14: 118-131. 1955.

56. Robin, E. D., Travis, D. M., Bromberg, P. A., Forkner, C. E., Jr. and Tyler, J. M. Ammonia excretion by mammalian lung. Science 129: 270-271. 1959.

57. Satake, Y. Chlorammonium-Hydroglykämie beim nebennierenlosen Kaninchen. Tohoku J. Exptl. Med. 8: 26-36. 1926.

58. Snyder, C. D. Recent advances in knowledge of the liver. Physiol. Rev. 22: 54-73. 1942.

59. Soejima, M., Sugawara, K., Kikuchi, E. and Shimura, K. Mechanism of urea utilization by ruminants. IV. Absence of ammonium carbamate during urea hydrolysis by urease. Nippon Nogei-Kagaku Kaishi 33: 91-96. 1959. Original not available; abstracted in Chemical Abstracts 56: 5114i. 1962.

60. Sumner, J. B., Hand, D. B. and Holloway, R. G. Studies of the intermediate products formed during the hydrolysis of urea by urease. J. Biol. Chem. 91: 333-341. 1931.

61. Szwabowicz, A. Urea poisoning in cattle (Translated title). Med. Wet. 18: 330-333. Original available but not translated; abstracted in Veterinary Bulletin 32: 3897. 1962.

62. Thamm, H., Ihlenburg, H. and Lange H. Zur Verfüterung synthetischer Stickstoffverbindungen nichteiweissartiger Natur. Arch. Exptl. Veterinärmed. 16: 393-411. 1962.

63. Tolone, S. Hyperglycemia from convulsive seizures (Translated title). Boll. soc. ital. biol. sper. 20: 295-297. 1945. Original not available; abstracted in Chemical Abstracts 40: 6163. 1946.

64. Topchyan, M. S. and Averyanova, K. G. Toxicity of ammonium nitrate for animals (Translated title). Trudy armyan. nauchno-issled. vet. Inst. 8: 179-185. 1955. Original not available; abstracted in Veterinary Bulletin 27: 1882. 1957.

65. Underhill, F. P. and Kapsinow, R. The comparative toxicity of ammonium salts. J. Biol. Chem. 54: 451-457. 1922.

66. Walley, P. Ammonia and the stimulative system of treatment in disease. Vet. J. 9: 223-235. 1879.

67. Wang, J. H. and Tarr, D. A. On the mechanism of urease action. J. Am. Chem. Soc. 77: 6205-6206. 1955.

68. Wantoch, H. Über den Einfluss verschiedener Substanzen auf den Zucker und Ammoniakgehalt des Blutes. Arch. Exptl. Path. Pharmakol. 143: 337 357. 1929.

69. Warren, K. S. The differential toxicity of ammonium salts. J. Clin. Invest. 37: 497-501. 1958.

70. _____ and Nathan, D. G. The passage of ammonia across the blood-brain-barrier and its relation to blood pH. J. Clin. Invest. 37: 1724 1728. 1958.

71. White, L. P., Phear, E. A., Summerskill, W. H. J. and Sherlock, S. Ammonium tolerance in liver disease: observations based on catheterization of the hepatic veins. J. Clin. Invest. 34: 158-168. 1955.

72. Windle, W. F., Koenig, H. and Jensen, A. V. Histological study of the brain in experimentally induced acidosis. Arch. Neurol. Psychiat. 56: 428-433. 1946.

ACKNOWLEDGMENTS

This study was conducted under project number ADPa 7-45 of the Veterinary Toxicology and Entomology Research Laboratory of the United States Department of Agriculture.

The author wishes to thank Dr. R. D. Radeleff for advice and guidance during this study.

Appreciation is expressed to Dr. F. K. Ramsey for his encouragement and valuable counsel.

Appreciation is also expressed to the late Dr. Robert McCarty and to Dr. H. E. Smalley for their able assistance during the various phases of the study.

Thanks are extended to the staff of the Toxicological Research Laboratory, United States Department of Agriculture, Kerrville, Texas, for their aid in obtaining and housing the sheep used in this study and for a portion of the laboratory assistance.