OBSERVATIONS ON THE EFFECTS OF SODIUM GLUTAMATE, SODIUM SUCCINATE AND L-ARGININE·HCl ON AMMONIA INTOXICATION IN LAMBS

by

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

Major Subject: Veterinary Physiology

Approved:

Signatures have been redacted for privacy

versity
Of Science and Technology
Ames, Iowa

1961

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INTRODUCTION

The use of urea and other non-protein nitrogen feeding compounds as a partial replacement for the protein nitrogen in ruminant rations is now an established practice.

The amount of this non-protein nitrogen feeding compound recommended for general use in ruminant rations is limited to low levels principally because of the possibility of toxicity developing when high levels are fed. This toxicity is believed to be due to the rapid liberation of ammonia in the rumen resulting from the hydrolysis of the urea and the subsequent absorption of large quantities of ammonia into the portal blood resulting in an ammonia intoxication. From the available information it would seem that the more useful non-protein nitrogen feeding compounds, including urea, are in general the ones with the highest degree of ammonia toxicity.

In view of the widespread use of these non-protein nitrogen feeding compounds, knowledge of the ammonia toxicity syndrome and of methods of preventing or alleviating this condition becomes a matter of paramount importance not only to the practicing veterinarian but also the nutritionist as well.

In attempting to arrive at the single dose toxic level of each of the optically pure amino acids and of mixtures thereof, Gullino *et al.* (1956) found that l-arginine·HCl afforded a protective mechanism in adult male rats when it was present in toxic mixtures of amino acids. Greenstein

et al. (1956) found that four mM of l-arginine·HCl per kilogram of body weight prevented ammonia toxicity when administered intraperitoneally to adult male rats 60 to 90 minutes prior to a lethal dose of ammonium acetate.

The presence of a protective mechanism whereby ammonia is removed from the body by converting glutamic acid to glutamine has also been shown by Krebs (1935) and Tigerman and MacVicar (1951).

Succinic acid was incorporated into this study since it is a readily available metabolite in the Krebs citric acid cycle and could quite conceivably enhance the detoxification of ammonia as shown in Figure 1.

With these facts in mind, 21 healthy, yearling, wether lambs were obtained for study from the Department of Animal Husbandry of Iowa State University and studies were initiated to determine what role, if any, l-arginine·HCl, glutamic acid and succinic acid play in the detoxification of ammonia in lambs.

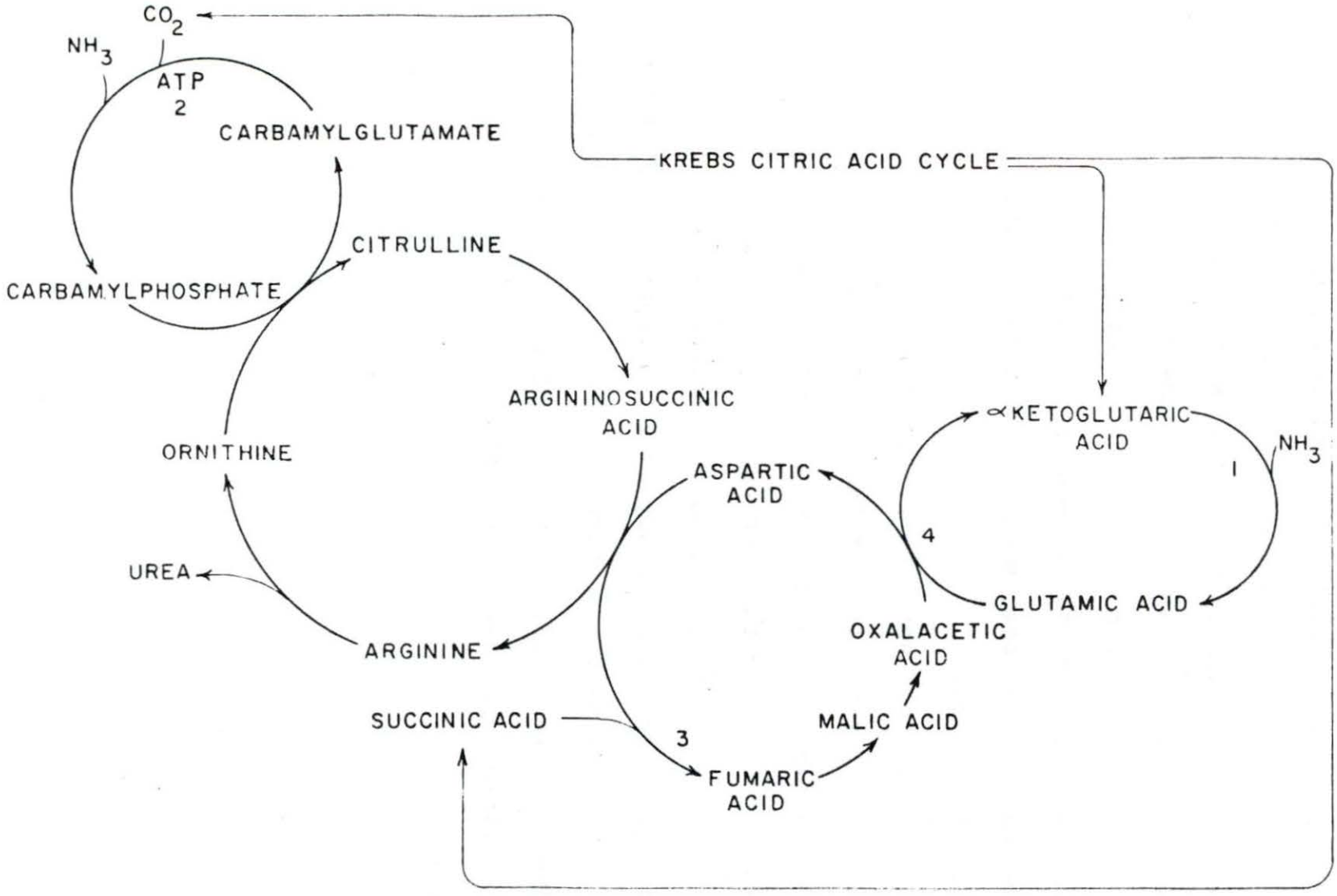

Figure 1. The urea cycle and its relationship to the Krebs citric acid cycle

REVIEW OF LITERATURE

The economic importance of urea and other non-protein nitrogen feeding compounds has increased enormously over the past one and one-half decades. Research literature which justifies the acceptance of these compounds as a partial replacement for the protein nitrogen in ruminant rations is voluminous and was ably reviewed by Reid (1953) and Repp (1955).

Limitations and Toxicity of Urea

As a result of the widespread use of urea a vast amount of research on this and other non-protein nitrogen feeding compounds has been carried on, thus accounting for the knowledge of the limitations and toxicity of these compounds. Hart et al. (1939) did the first intensive experimental work in the United States on the nutritional value of urea. It was noted that when urea was fed to cattle at the rate of 4.3 per cent of the dry matter of the ration, diuresis resulted and kidney damage was found at necropsy. They concluded from their observations that kidney damage was not observed if urea constituted less than 2.8 per cent of the dry matter of the ration. These studies were extended by Work et al. (1943) and it was found that the harmful level of urea feeding for cattle may be between 2.29 and 2.8 per cent of the dry matter of the ration. Evidence that these findings do not

apply to other species, at least to sheep, was shown by Harris and Mitchell (1941), who found that rations containing as much as 3.16 per cent urea on a dry weight basis did not exert any detrimental effects on lambs over a feeding period of 110 days. Work by Rupel et al. (1943) suggests that urea should not be fed to dairy cattle at a rate exceeding one per cent of the dry matter of the ration. Repp et al. (1953) encountered toxicity in lambs when urea was administered as a drench at the rate of 40 grams per 100 pounds of body weight. The treated lambs were able to tolerate blood ammonia levels as high as 1.0 milligram per 100 milliliters of whole blood.

It is a rather universal belief that because of its unpalatability ruminants, will not consume enough urea to cause toxicity symptoms. The assumption that urea poisoning is unlikely except in gluttonous or fasted animals, or those unaccustomed to urea feeds, has been made in an anonymous report from the Oklahoma Experiment Station (1953). Also Dinning et al. (1949) stated in their work that it was not possible to induce steers to eat a sufficient quantity of urea to produce symptoms of urea toxicity. However, Bullington et al. (1955) reported three cases of suspected urea poisoning in Tennessee involving 16 head of dairy cattle of which 10 died. In all three cases inadequate mixing of the feed was held responsible for the observed symptoms of urea toxicity.

It would seem logical based on this literature review to assume that with the ever increasing use of urea and other non-protein nitrogen feeding compounds other cases of ammonia intoxication are certain to appear unless measures are made available to prevent them.

Effect of Time and Rate of Administration of Urea

It is of interest here to note the effects of time and the rate of administration on the toxicity of urea and other ammonium-containing compounds. Karr and Hendricks (1949) studied the use of ammonium chloride as a therapeutic agent in human medicine and observed that the occurrence of toxicity was due entirely to the ammonium ion and was dependent upon the rate of administration and virtually independent of total amount administered. Payne made the same observations with dogs and sheep. (Dr. Loyal C. Payne, Department of Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University of Science and Technology, Ames, Iowa. The Physiological Response to Ammonium Carbonate. Private communication. 1956.) Pearson and Smith (1943) stated that the urease activity of rumen ingesta in dairy cattle is so great at all times of the day and remains so little affected by relatively large amounts of urea that all the urea ever likely to be fed, even to high production cows in full lactation, would readily be converted to ammonia within one hour.

These facts would account for the findings of Dinning et al. (1948) who noted that urea in amounts exceeding 100 grams was fatal to steers when given orally as a drench, yet amounts up to 400 grams produced no ill effects when administered over a period of 24 hours as feed mixed with other concentrates.

Toxicity Syndrome

Repp et al. (1955) described the typical urea toxicity syndrome as being characterized by the following clinical symptoms in the order of their appearance: restlessness, ataxia, labored breathing, muscular spasms, tetany, collapse and death. Clark et al. (1951) noted a decrease or cessation of rumen motility and a sharp rise in rumen pH when sub-lethal doses of urea were introduced into the rumen of sheep. The previously cited report from the Oklahoma Experiment Station (1953) also states that the oral administration of 20 grams of urea per 100 pounds of body weight may give rise to colic, incoordination, tetany and bloat with death in three hours. Bullington et al. (1955) observed profuse sweating and salivation in three field cases of urea poisoning in dairy cattle. In a study of urea toxicity Fujimoto and Tajima (1953) sacrificed five goats by drenching individually with 50 grams of urea and also drenching 10 rabbits with varying amounts of urea.

Necropsy findings showed lesions of acute catarrhal gastro-enteritis, bronchitis, peribronchial and intra-alveolar hemorrhages with congestion and nephrosis. Hemorrhages and degenerative changes in the central nervous system were also present.

Dinning et al. (1948) also stated in their paper that ataxia appears in steers when the ammonia nitrogen of the systemic blood reaches a level of approximately 2.5 milligrams per 100 milliliters of whole blood and symptoms of alkalosis followed by death at a level of about 4.0 milligrams per 100 milliliters of blood.

The syndrome of hepatic coma in human medicine parallels that of ammonia intoxication or so-called urea poisoning in our domestic ruminant animals. It has been shown by Riddell et al. (1954) that the condition of hepatic coma is actually caused by an ammonia intoxication. Schwartz et al. (1953) and Singh et al. (1954) found elevated blood ammonia levels in patients with cirrhosis of the liver. Webster and Davidson (1954) were able to induce hepatic coma by the administration of ammonium salts. Phillips et al. (1952) reported that symptoms clinically indistinguishable from impending hepatic coma resulted from the administration of several ammonium containing compounds including urea and ammonium chloride in nine chronic alcoholics with advanced cirrhosis of the liver. They noted a positive correlation between the blood ammonia concentration and the observed toxicity syndrome.

Therapeutic Value of Glutamic Acid

The defense of the organism to high levels of blood ammonia may follow several pathways as shown by Manning (1957). One important route may be the storage of ammonia as a nontoxic substance. Krebs (1935) and Tigerman and MacVicar (1951) have shown that glutamic acid has the ability to combine with and store ammonia as the non-toxic salt, glutamine. Work by McIlwain (1951) suggests that the anti-convulsant action of glutamic acid may be due, in part, to its ability to decrease the brain tissue content of energy-rich creatine phosphate thus lowering its metabolic response to electrical stimulation.

Further evidence that hepatic coma is in part an ammonia intoxication has been shown by McDermott et al. (1955) who classified 28 patients with hepatic coma as falling into one of three groups: spontaneous, exogenous or chronic encephalopathy. In the exogenous and chronic groups ammonia intoxication was found to be the predominant feature and glutamic acid was shown to be an effective adjunct in treatment.

Walshe (1953) treated five episodes of hepatic coma in three patients suffering from sub-acute or chronic liver injury with sodium glutamate. On each occasion a return of consciousness closely followed the administration of the drug. Alexander and Porter (1954) observed a rapid recovery in a patient in severe hepatic coma with convulsions when

treated with intravenous sodium glutamate and adrenocorticotrophic hormone. Walley (1954) noted definite improvement in a patient in hepatic coma upon treatment with intravenous injection of sodium glutamate. Price et al. (1943) observed that epileptic seizures of the petit mal and psychomotor types were decreased in frequency when glutamic acid was used in conjunction with known anticonvulsant therapy. The authors suggested that the favorable response to this drug may be due to the detoxification of ammonia in the brain.

Richter et al. (1948) showed that ammonia is liberated in the brain on stimulation. Their work suggested that in conditions such as epilepsy, in which the brain is abnormally irritable, the toxic action of ammonia may play a significant role. Torda (1953) states that an accumulation of the ammonium ion in the brain is the result of increased cerebral activity but is not necessarily the factor initiating convulsions.

Conversely, Webster et al. (1954) found that sodium glutamate had little, if any, beneficial effects on hepatic coma or impending coma. It also failed to prevent onset of impending coma induced by ammonium salts. They noted no consistent changes in plasma ammonia concentrations following glutamate therapy. Singh et al. (1954) also observed the failure of glutamic acid to lower elevated blood ammonia levels to normal in patients with hepatic cirrhosis. These negative findings may be explained on the basis of observa-

tions made by McDermott et al. (1955), previously cited. In his classification those cases representing spontaneous encephalopathy were cases of true hepatic decompensation in which the disorder of ammonia metabolism was only one of many biochemical defects and to which glutamic acid had only transient effects. Najarian and Harper (1956b) found that sodium glutamate was only slightly effective in preventing a rise in blood ammonia when given concomitantly with glycine and was completely ineffective in reducing the elevated blood ammonia when administered one hour after the commencement of glycine infusion in dogs.

Saperstein (1943) found that glutamic acid gave complete protection from the convulsant effects of ammonium chloride in rabbits.

Therapeutic Value of Arginine

Another clinically significant mode of defense against ammonis intoxication is to increase the capacity of the urea cycle for the removal of ammonia by the use of one or more of the substrate components of this cycle.

Gullino et al. (1956), Greenstein et al. (1956) and du Ruisseau et al. (1956), in a series of experiments, have shown that l-arginine·HCl affords complete protection to rats against $LD_{99.99}$ doses of ammonium acetate. Najarian and Harper (1956b) induced elevated blood ammonia levels in dogs

by intravenous infusion with glycine. When arginine was administered concurrently with glycine no significant rise in blood ammonia was evidenced. When arginine was given one hour after the start of glycine administration, it effected a prompt reduction of blood ammonia levels. The rise in blood urea which accompanied the marked fall in blood ammonia when arginine was given indicated that this amino acid mediated its effect on the blood ammonia through its influence on the production of urea. Najarian and Harper (1956a) also noted the ability of arginine to decrease the elevated blood ammonia levels in 15 patients afflicted with several disease entities. The decrease in blood ammonia was always accompanied by a significant rise in the blood urea nitrogen. Manning and Delp (1957) utilized intravenous infusions of l-arginine·HCl in the treatment of hepatic coma in three patients with excellent results.

Clementi (1951) estimated the effect of different rations upon the excretion levels of uric acid, urea and ammonia in the fowl. It was noted that excretions of both urea and ammonia relative to total nitrogen ingested tended to be higher in birds on legume rations, which were relatively rich in arginine, than in those on cereal rations which were low in this amino acid.

EXPERIMENTAL

Pilot Studies with White Mice

A series of screening experiments was carried out to determine what effect, if any, one might expect from sodium glutamate, sodium succinate and l-arginine·HCl when administered singly and in combination to lambs concomitantly or prior to an injection of a toxic dose of ammonia. Mice were chosen for this part of the study because of their adaptability from the standpoint of being readily available, inexpensive, easy to handle and because of the low total dosages of l-arginine·HCl required to afford protection. This was quite important due to the limited supply of this rather expensive amino acid.

Materials and methods

For the initial screening experiments adult CFW albino mice weighing approximately 20 grams each were used. The mice were allotted at random into groups of 10 mice each.

The dose level of all the compounds tested, including that of ammonium chloride, was calculated on the basis of milligrams of the compound per kilogram of body weight. This amount was diluted with distilled water to 10 milliliters and administered intraperitoneally at the level of 0.01 milliliter per gram of body weight.

The lethal dose of ammonium chloride was determined

experimentally by injecting lots of 10 mice each intraperitoneally with added increments of this compound. When one or more deaths were observed per treatment succeeding increments were adjusted to one milligram over and above that given the preceding lot. The minimum dose of ammonium chloride that is lethal to 99+ per cent of the mice was defined as being that dose wherein all the mice in a lot of 20 succumbed to treatment. This dose was found to be 625 milligrams of ammonium chloride per kilogram of body weight and is hereafter referred to as LD_{99}.

Glutamic acid and succinic acid were administered intraperitoneally as the sodium salts since they are less irritating and are more readily soluble than the acid forms. Protective levels of sodium glutamate and sodium succinate were calculated on the assumption that one equivalent of either glutamic acid or succinic acid as their sodium salts would detoxify two equivalents of ammonis. This reasoning was based on the fact that each time the urea cycle passes through its sequence of reactions free ammonia is removed directly at Step 1 by glutamic acid and at Step 2 by carbamylphosphate as illustrated in Figure 1. The calculated dosages of sodium glutamate and sodium succinate were then doubled and rounded off to 1800 and 3000 milligrams, respectively, per kilogram of body weight to assure levels of a sufficient magnitude to provide protection. Practically no toxicity was encountered at these levels except for a transient depression of short

duration in the case of sodium succinate.

l-arginine·HCl was administered intraperitoneally at the same level as that shown by Greenstein et al. (1956) to give complete protection against ammonia intoxication in rats namely, four millimoles or 843 milligrams per kilogram of body weight.

Preliminary studies were carried out to determine the optimum time to administer the sodium glutamate, sodium succinate and l-arginine·HCl prior to the injection of an LD_{99} dose of ammonium chloride. Accordingly, for each of the three compounds designated above four lots of 10 mice each were injected intraperitoneally with a protective dose of the appropriate drug. One lot in each of the four groups represented a control and hence was not injected with ammonium chloride. The remaining three lots in each group were injected at 0, 30 and 60 minutes, respectively, prior to the injection of the ammonium chloride. As indicated in Table 1 it was found that the greatest degree of protection was provided when the detoxifying agent was administered 30 minutes prior to the injection of the ammonium chloride. As the result, all mice treated with the test compounds were injected 30 minutes prior to the administration of the LD_{99} dose of ammonium chloride.

In the cases where animals succumbed to treatment, death generally occurred within one hour and usually within 30 minutes following the injection of ammonium chloride. Clinical symptoms of ammonia intoxication preceding death con-

Table 1. Protective effect of sodium glutamate, sodium succinate and l-arginine·HCl when administered singly at different times prior to the injection of an LD_{99} dose of ammonium chloride[a]

Detoxifying agent	Dosage (mg/kilo)	Lot no.	Time admin. prior to NH_4Cl (min.)	No. survived
Sodium glutamate	1800	control	--	10/10
	"	1	0	2/10
	"	2	30	3/10
	"	3	60	3/10
Sodium succinate	3000	control	--	10/10
	"	1	0	2/10
	"	2	30	4/10
	"	3	60	0/10
l-arginine·HCl	843[b]	control	--	10/10
	"	1	0	3/10
	"	2	30	5/10
	"	3	60	2/10

[a]625 milligrams of ammonium chloride per kilogram of body weight.

[b]Equivalent to 4 mM of l-arginine·HCl per kilogram of body weight.

sisted of: depression, dyspnea, protrusion of the eyeballs, spastic muscular contractions and tetany.

Results and discussion

In Table 1 are recorded the effects of single doses of sodium glutamate, sodium succinate and l-arginine·HCl when they were administered intraperitoneally concomitantly and prior to a lethal dose of ammonium chloride. No clinical

symptoms of toxicity from the test compounds were observed. The animals in the control lot would immediately commence eating as soon as they were injected. However, in the case of sodium succinate the mice exhibited a brief period of depression which lasted for approximately 10 to 15 minutes after which they appeared normal in all respects. A definite trend seemed to exist in these data with greater survival at the 30 minute time interval; however, statistical analysis indicates that differences were due to random variation.

It will be noted that in all three groups a definite degree of protection against ammonia intoxication was provided when the test compounds were administered concurrently with the lethal dose of ammonium chloride. Sodium glutamate and l-arginine·HCl also provided some protection when injected one hour prior to the ammonia challenge, however, sodium succinate appeared of no value when administered at this time interval (one hour). The greatest degree of resistance to ammonia in all three groups was encountered when the administration of the detoxifying compounds preceded the ammonium chloride by 30 minutes. In the case of sodium glutamate there appeared to be no difference in this respect between 30 and 60 minute time intervals. However, subsequent experiments incorporating various combinations of the test compounds were conducted at the 30 minute time interval.

As shown in Table 1 the effectiveness of the test compounds in preventing ammonia toxicity when administered up to 30 minutes prior to a lethal dose of ammonium chloride was found to be greatest when l-arginine·HCl was used, with sodium glutamate being the least effective.

Similar studies with various combinations of the test compounds were conducted utilizing identical dosages of the appropriate drugs as that used in the singly administered experiments namely: 1800, 3000 and 843 milligrams (4 mM) per kilogram of body weight, respectively, for sodium glutamate, sodium succinate and l-arginine·HCl. The results of this study are recorded in Table 2. Eight lots of 10 mice each were allotted at random into four groups. One lot in each group was injected with the appropriate compounds followed by an identical treatment in the remaining lot the following day. The test compounds were not mixed but rather were administered intraperitoneally immediately following one another and 30 minutes prior to the injection of an LD_{99} dose of ammonium chloride. As in the case when the detoxifying agents were administered singly, no toxic symptoms to the test compounds developed except for a transient depression of short duration when sodium succinate was present in the mixture.

Table 2 reveals that a combination of sodium glutamate and sodium succinate did not demonstrate an efficacy which was comparable to that exhibited by other combinations studied

Table 2. Protective effect of various combinations of sodium glutamate, sodium succinate and l-arginine·HCl when administered 30 minutes prior to the injection of an LD_{99} dose of ammonium chloride[a]

Detoxifying agents	Dosage (mg/kilo)	Lot no.	Time admin. prior to NH_4Cl (min.)	No. survived	Replicates
		control	--	10/10	
Sodium glutamate Sodium succinate	1800 3000	1	30	5/10	3/10
		control	--	10/10	
Sodium glutamate l-arginine·HCl	1800 843[b]	2	30	8/10	8/10
		control	--	10/10	
Sodium succinate l-arginine·HCl	3000 843	3	30	9/10	8/10
		control	--	10/10	
Sodium glutamate Sodium succinate l-arginine·HCl	1800 3000 843	4	30	10/10	10/10

[a]625 milligrams of ammonium chloride per kilogram of body weight.

[b]Equivalent to 4 mM of l-arginine·HCl per kilogram of body weight.

although a marked protective action was nevertheless evidenced. The data indicate a 40 per cent survival rate when this combination of drugs was employed. All of the mice which survived this treatment exhibited depression and dyspnea following the injection with ammonium chloride and two mice suffered mild spastic muscular contractions.

Combinations of sodium glutamate and l-arginine·HCl were approximately equal to sodium succinate and l-arginine·HCl in their ability to defend the organism against ammonia intoxication. Survival rates of 80 and 85 per cent, respectively, were indicative of a vigorous propensity to detoxify ammonia. Clinical symptoms of ammonia toxicity in the mice surviving this treatment included depression and dyspnea. All of the surviving mice appeared normal in all respects within 30 minutes.

The data of lot 4 in Table 2 indicated 100 per cent survival when a combination of sodium glutamate, sodium succinate and l-arginine·HCl was administered 30 minutes prior to a lethal dose of ammonium chloride. Clinical symptoms of ammonia intoxication were limited to a short period of depression and dyspnea. The fact that more total chemicals were given to the mice in this lot may have contributed some added protective effect.

Statistical analysis of these data shows a high degree of significance between the treatments of lots 1 versus 2, 1 versus 3, and 1 versus 4. The effectiveness of the agents

used in lot 4 as opposed to those used in lot 2 was found to be significant at the 0.05 level whereas the difference observed between the treatments employed in lots 3 and 4 only approached significance at the 0.05 level. No significant difference was found between lots 2 and 3.

The postulation that one may enhance the formation of urea by introducing substrate components of the urea cycle seems well founded. It has not been shown whether glutamic acid exerts its effect by transferring its nitrogen to oxalacetic acid in the synthesis of aspartic acid as shown in Step 4, Figure 1, or in simply combining with and storing ammonia as the non-toxic salt, glutamine. Further work in the future on the value of aspartic acid as a prophylactic agent in the prevention of ammonia intoxication would seem to be indicated.

The discrepancy shown in this study, in the failure of l-arginine·HCl to provide 100 per cent protection against lethal doses of ammonia, and that of Greenstein et al. (1956) may be explained by 1) the fact that the calculated LD_{99} dosage of ammonia reported here was slightly higher than that found in Greenstein's work and 2) by species differences in metabolic rates. Optimum time for administering arginine to white mice prior to an ammonia challenge was decreased by 50 per cent over the time used with rats in Greenstein's study. This might indicate a significant difference in rates of

metabolism between the two species.

Summary

Three metabolic compounds or their sodium salts were administered intraperitoneally singly and in combination to adult white mice for the purpose of determining what effect they might have on ammonia intoxication. Observations were made on the effect of time between treatment and administration of a lethal dose of ammonium chloride. The highest degree of protection was afforded when the administration of the detoxifying agents preceded the ammonia challenge by 30 minutes. In this respect l-arginine·HCl proved to be the most efficient followed by sodium succinate and sodium glutamate in that order. The effects of various combinations of the above listed compounds on ammonia toxicity were also studied. Combinations of sodium glutamate and sodium succinate were least effective in preventing ammonia intoxication and provided a survival rate of only 40 per cent. Sodium glutamate and l-arginine·HCl were approximately equal in value to sodium succinate and l-arginine·HCl in this respect when administered 30 minutes prior to the injection of a lethal dose of ammonium chloride. Survival rates in these two treatments were 80 and 85 per cent, respectively. Concomitant administration of all three test compounds 30 minutes prior to the ammonia challenge resulted in 100 per cent survival.

An indication of a protective mechanism against ammonia intoxication from sodium glutamate, sodium succinate and l-arginine·HCl when administered intraperitoneally either singly or in combination was demonstrated.

Experimental Studies with Lambs

With the information gained from the pilot studies with white mice, experiments were conducted with yearling lambs for the purpose of determining the efficiency of sodium glutamate, sodium succinate and l-arginine·HCl in preventing symptoms of ammonia intoxication in ruminants. A second purpose was to gain more information regarding the mechanism of ammonia detoxification in ruminants.

Preliminary studies were carried out to determine 1) the sub-lethal dose of ammonium chloride that would produce irrefutable symptoms of toxicosis and 2) the effect of such doses of ammonium chloride on ammonia nitrogen levels of the systemic blood. Observations were also made on the detoxifying effects of the above listed compounds when administered singly and in various combinations concomitantly and prior to a toxic dose of ammonium chloride.

Materials and methods

For this part of the study 21 yearling, wether lambs weighing approximately 85 pounds each were used. All lambs

were fed a ration consisting of 0.25 pound soybean oil meal, 0.50 pound cracked shell corn and all the brome-alfalfa hay of medium quality they would consume. In determining the sub-lethal toxic dose of ammonium chloride six lambs, allotted at random, were used. For the remaining studies all 21 lambs, including the six mentioned, were used.

In view of the limited number of test animals available it was necessary to use the animals more than once. All lambs were given a seven day rest period between treatments to enable their metabolic processes to return to normal and after the second treatment they were allowed a recuperative period of not less than four weeks. Feed was withheld for 18 hours prior to treatment but water was available at all times.

Dosage levels of sodium glutamate, sodium succinate and l-arginine·HCl were calculated on the basis of grams per kilogram of body weight. The assumption that one equivalent of either glutamic acid or succinic acid, as their sodium salts, would detoxify two equivalents of ammonia has been previously cited. Therefore, protective levels of 0.592, 0.945 and 0.737 grams per kilogram of body weight, respectively, for sodium glutamate, sodium succinate and l-arginine·HCl were calculated. Experimentation demonstrated that these levels of sodium glutamate and sodium succinate were toxic to the extent that they caused the animals to become depressed and a few animals refused to stand. Since it was desirable that the animals be

in an upright position to facilitate blood withdrawal, all injections of the test compounds were made at one-half of the calculated levels shown above. Since theoretically the calculated dosage should provide 100 per cent protection against ammonia intoxication, it was assumed that levels one-half as great would affect a decided decrease in expected blood ammonia nitrogen levels and mitigate or prevent the anticipated syndrome of ammonia intoxication.

The animals were bled just prior to treatment and at intervals of 10, 15, 30, 45, 60, 75 and 90 minutes thereafter. Blood samples were taken from the jugular vein and were analyzed for ammonia nitrogen by a microdiffusion method, Seligson (1951). With the exception of the normal pre-injection blood sample, all samples were transferred to the diffusion flasks within three minutes.

Intraperitoneal injections of the compounds used were made at intervals of 30, 60 and 120 minutes prior to the injection of ammonium chloride. Normal blood samples were withdrawn just priot to the administration of the test compounds but were not placed in diffusion flasks until after the administration of the ammonium chloride because there was a tendency for a precipitate to form in the whole blood and potassium carbonate mixture if left standing for any extended period of time. The blood samples were kept in tightly corked test tubes but may not have been completely free from the absorption of some free ammonia from the environment with the

result that the normal blood ammonia nitrogen values reported throughout this study tended to be somewhat higher than one would expect to find in the systemic blood of healthy animals. However, the data of Tables 6, 7, 8, 9 and 10 reveal respective average normal blood ammonia nitrogen values of 325, 284 and 276 micrograms per 100 milliliters, when the blood samples were kept for periods of 30, 60 and 120 minutes prior to placing them in the diffusion flasks. These facts would indicate either 1) the diffusion of ammonia nitrogen from the blood to the environment rather than the more widely accepted opposite view of absorption of environmental ammonia or 2) that amounts of ammonia nitrogen, absorbed from the surrounding air by whole blood left standing in tightly corked vessels for periods up to 120 minutes, if present at all, are indeed insignificant.

Ammonium chloride was used as a source of ammonia nitrogen because of its relatively non-irritating qualities when administered intraperitoneally and its stability when in aqueous solution. The appropriate amount of each drug, including ammonium chloride, was diluted to 50 milliliters in distilled water and injected intraperitoneally via the paralumbar fossa. Precaution was taken to insure that the needle was inserted into the peritoneal cavity.

A restraining crate was constructed which enabled one operator to perform all injections and blood withdrawals. In

instances where animals exhibited convulsions and/or tetany they were removed from the crate and placed on the floor. In these cases it was sometimes impossible to obtain all of the blood samples at the appropriate times. All results are reported as micrograms of ammonia nitrogen. Colorimeteric readings were taken with a Klett-Summerson photoelectric colorimeter employing a blue filter with an approximate spectral range of 400 to 465 millimicrons. Figures 2 and 3 represent standard curves of ammonia nitrogen used in determining the amount of this element present in the whole blood samples. Figure 2 illustrates the regression of optical density of the standard solutions of ammonium sulfate upon the micrograms of ammonia nitrogen present in the standard solutions. The regression was found to be linear within the range of 600 to 5000 micrograms of ammonia nitrogen per 100 milliliters of standard solution. Amounts of ammonia nitrogen below this range were determined by plotting optical density readings on a line connecting the linear regression line at 600 micrograms of ammonia nitrogen and the point of origin as shown in Figure 3. The fact that this lower range actually presents a curvilinear regression was disregarded as being insignificant to this study since 1) very few normal blood samples fell below 300 micrograms of ammonia nitrogen per 100 milliliters of whole blood and 2) this study was concerned more intimately with relative changes in amounts of

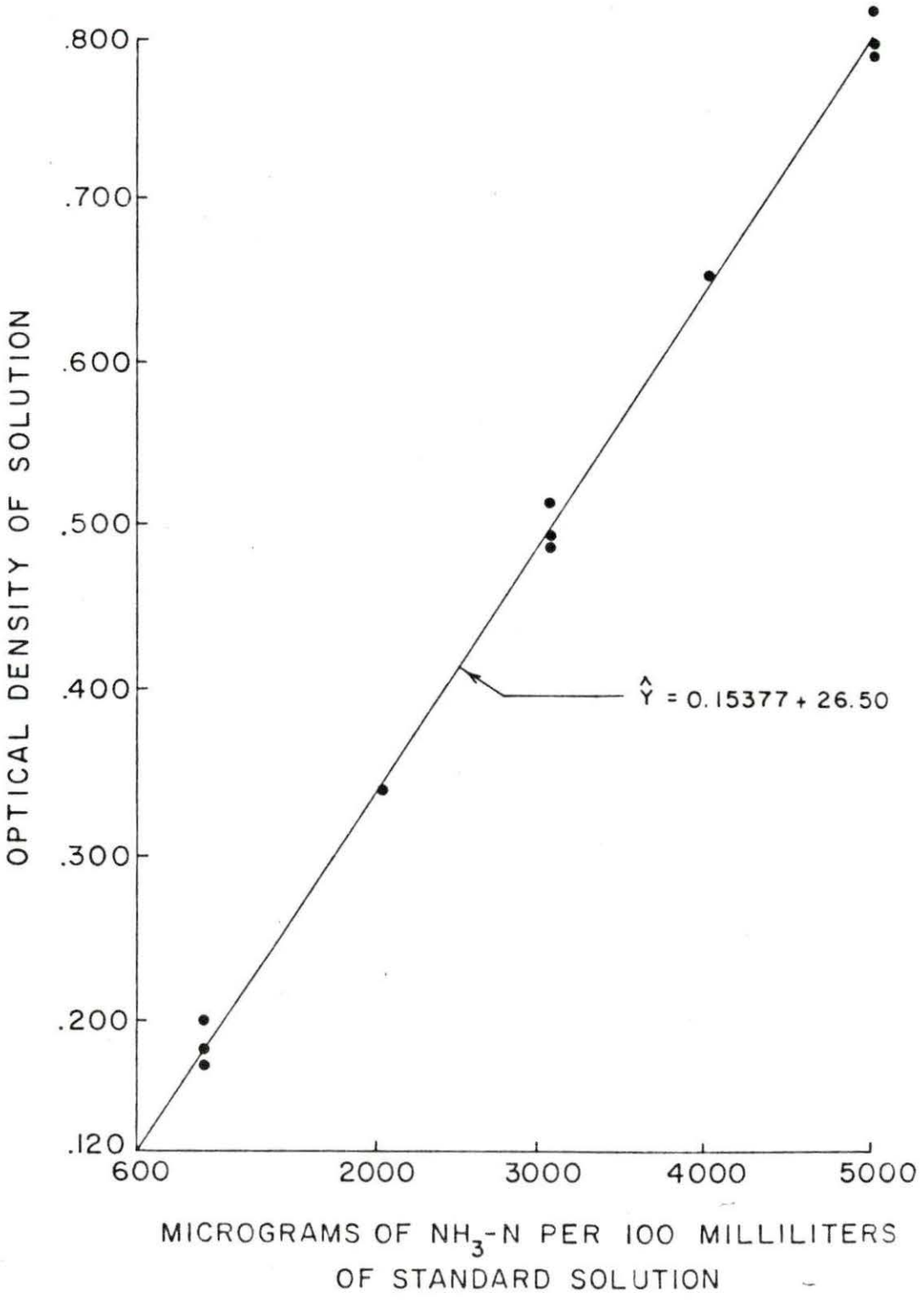

Figure 2. Regression of optical density on micrograms of NH_3-N in standard solutions of ammonium sulfate

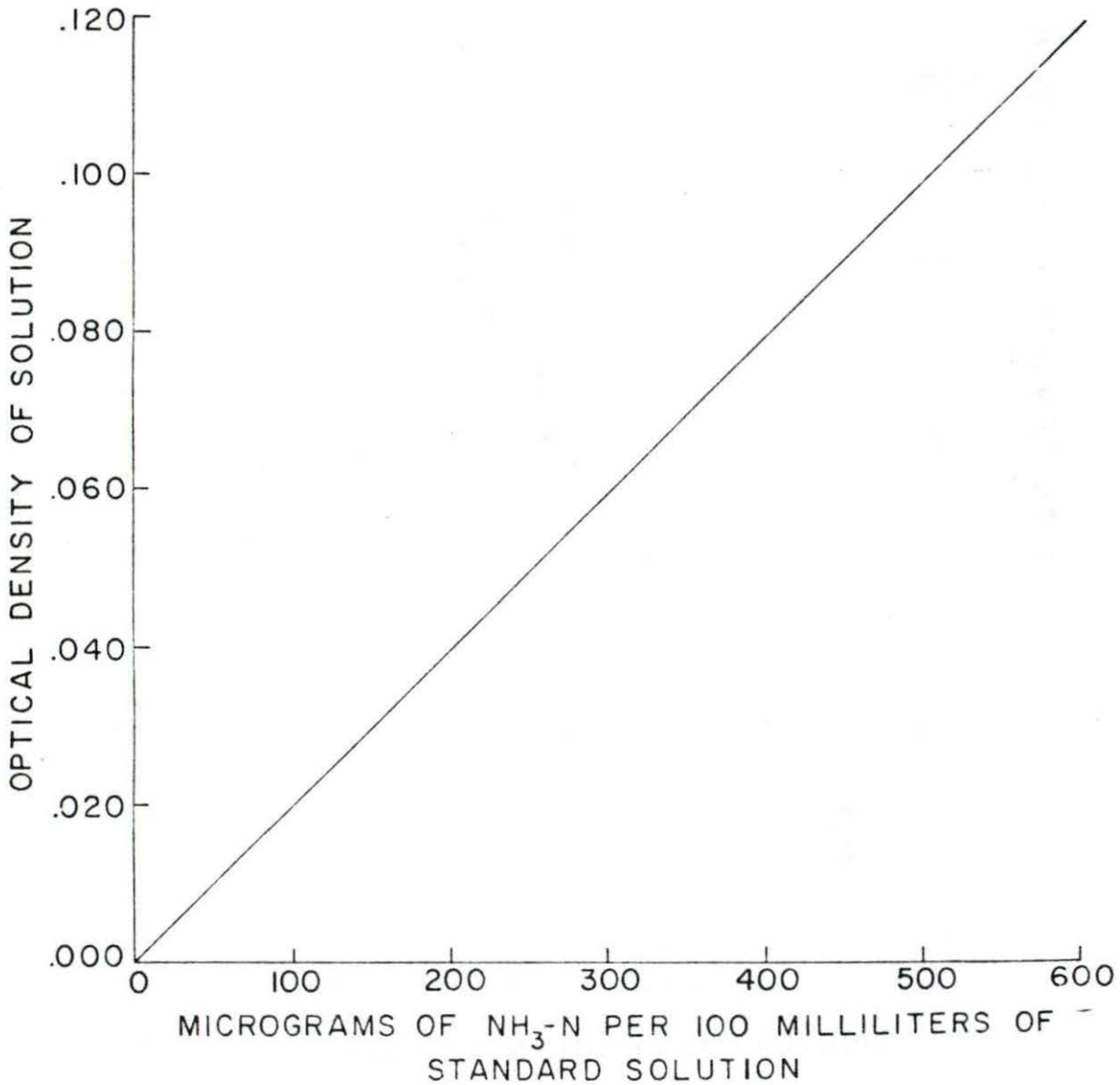

Figure 3. Graphic representation of approximate NH_3-N values based on optical density readings

blood ammonia nitrogen rather than in actual amounts.

The characteristic symptoms observed in determining the sub-lethal toxic dose of ammonia nitrogen were nervousness, increased salivation with frothing, dyspnea, muscular tremors and ataxia. As illustrated in Table 3, when 49 milligrams of ammonia nitrogen, as ammonium chloride, were injected intraperitoneally clinical symptoms of nervousness, profuse salivation and frothing, dyspnea, and muscular tremors were exhibited. A pronounced ataxia of the hind quarters was observed in one case and in another case convulsions and tetany with recovery in 70 minutes. Approach of toxic levels seemed to stimulate micturition and defecation. Therefore it was concluded that 49 milligrams was the sub-lethal toxic dose of ammonia nitrogen and this figure was used throughout the remainder of this study.

Results and discussion

In Table 3 are recorded the blood ammonia nitrogen values obtained at the more significant levels of ammonium chloride administration as well as the average values for each of the three dose levels. The blood ammonia nitrogen levels at which clinical symptoms of ammonia intoxication occurred are also indicated in this table. It will be noted that clinical symptoms of depression, dyspnea and increased salivation did not occur until the blood ammonia nitrogen level had risen to

Table 3. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of varying amounts of ammonium chloride

Body weight (kilograms)	Milligrams of NH_3-N per kilogram of body weight	Grams of ammonium chloride administered		Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
36.8	45	6.32		310	787	1020[a]	840	820	740	660	425
39.5	45	6.79		378	890	1211[a]	1065	878	645	589	450
			Av.	344	839	1116	953	849	693	625	438
42.7	49	7.99		345	1401[b]	1584	1492	1128	634	595	395
41.4	49	7.75		415	1610[b]	1519	1180	816	647	490	405
40.0	49	7.49		312	1535[b]	1640	1423	978	680	521	440
37.3	49	6.98		375	2013	2793[c]	2351	2325	1649	1440	1128
			Av.	362	1640	1884	1612	1312	903	762	592
38.6	53	7.81		367	3824[c]	3796	3480	2963	2522	2012	1778
40.5	53	8.20		280	3665[c]	3912	3118	2780	2312	1922	1649
			Av.	324	3745	3854	3299	2872	2417	1967	1714

[a]Exhibited clinical symptoms of depression, dyspnea and salivation.

[b]Exhibited clinical symptoms of profuse salivation and frothing, dyspnea, muscular tremors and/or ataxia.

[c]Exhibited clinical symptoms of convulsions and tetany.

approximately 1000 micrograms per 100 milliliters of blood. This was the approximate maximum blood ammonia nitrogen level attained following the intraperitoneal administration of 45 milligrams of ammonia nitrogen per kilogram of body weight. These criteria of ammonia intoxication were deemed non-significant and of little value in this study. Injection levels of 49 milligrams of ammonia nitrogen per kilogram of body weight resulted in maximum blood ammonia nitrogen values of approximately 1600 micrograms per 100 milliliters of blood with one exception. This lamb showed a maximum blood ammonia nitrogen level of 2793 micrograms per 100 milliliters and exhibited extreme clinical symptoms of convulsions and tetany. It seems doubtful that this was a normal response to this level of ammonia nitrogen administration but rather was a result of an error in dosage calculation. It will also be noted that in all cases, with the exception of the lamb mentioned above, blood ammonia nitrogen levels had returned to approximately normal at the end of 90 minutes. Clinical symptoms observed at this level included profuse salivation and in some cases frothing, dyspnea, muscular tremors and ataxia. These were assumed to be dependable symptoms of ammonia intoxication and were used as criteria throughout this study. Dose levels of 53 milligrams of ammonia nitrogen or higher were found to be unsatisfactory because of the consequent convulsions and tetany and the disadvantages of obtaining periodic blood

samples under these conditions. Maximum blood ammonia nitrogen levels associated with this dose (53 mg.) of ammonia nitrogen were found to be approximately 3900 micrograms per 100 milliliters of blood. Blood ammonia nitrogen levels remained relatively high and clinical symptoms of toxicity were still present at the end of 90 minutes with eventual recovery.

The effects of single doses of sodium glutamate, sodium succinate and l-arginine·HCl on normal blood ammonia nitrogen levels are recorded in Table 4. In all but two cases there was a considerable decrease in blood ammonia nitrogen levels after the administration of the test compounds and in one lamb no change was noted. By employing the "Sign Test" of Dixon and Mood (1946) these data were found to be statistically significant at the 0.05 level. These two exceptions viz. 405 micrograms per 100 milliliters of blood obtained 60 minutes following the administration of sodium succinate, and 764 micrograms per 100 milliliters of blood obtained 60 minutes following the administration of l-arginine·HCl might have been due to the presence of minute quantities of calcium on the walls of the colorimeter tubes resulting in the formation of a cloudy precipitate of calcium hydroxide and hence a higher than expected colorimetric reading, despite extreme preventative precautions.

Normal blood ammonia nitrogen levels were decreased by an average of 17, 21, and 10 per cent, respectively, for

Table 4. Micrograms of NH_3-N per 100 milliliters of whole blood before and after the intraperitoneal administration of single doses of sodium glutamate, sodium succinate and l-arginine·HCl

Compound administered	Normal	Time after administration of test compounds (minutes) +30	+60	+120
Sodium glutamate[a]	270	200		
	330	250		
	210			155
	270	—		200
Average	270	225		178
Sodium succinate[b]	385	290		
	260	190		
	310		220	
	280		405	
	290			270
	290	—	—	230
Average	302	240	312	250
l-arginine·HCl[c]	385	300		
	290	220		
	395		764	
	210		135	
	330		210	
	250			250
	230			145
	230	—	—	180
Average	290	260	370	192

[a] 0.296 grams per kilogram of body weight.

[b] 0.473 grams per kilogram of body weight.

[c] 0.368 grams per kilogram of body weight.

sodium glutamate, sodium succinate and l-arginine·HCl 30 minutes after their intraperitoneal administration. Elimination of the two values mentioned above results in average percentage decreases of 27 and 40 per cent, respectively, in the normal blood ammonia nitrogen level 60 minutes after the intraperitoneal administration of sodium succinate and l-arginine·HCl. When the concentration of blood ammonia nitrogen was determined 120 minutes after the administration of sodium glutamate, sodium succinate and l-arginine·HCl, average decreases in the level of systemic blood ammonia nitrogen of 34, 17 and 34 per cent, respectively, were noted.

In Table 5 are recorded the blood ammonia nitrogen levels obtained at 30, 60 and 120 minute intervals after the administration of a combination of sodium glutamate and sodium succinate and a combination of sodium glutamate, sodium succinate and l-arginine·HCl. Here 18 cases exhibited a significant decrease in blood ammonia nitrogen following the intraperitoneal administration of the test compounds which was shown statistically to be a definite departure from randomness. Of the four remaining cases one showed an increase and three showed no change in the blood ammonia nitrogen level. Normal blood ammonia nitrogen levels were lowered by an average of 20, 13 and 12 per cent, respectively, 30, 60 and 120 minutes after the administration of a combination of sodium glutamate and sodium succinate. When a combination of sodium glutamate,

Table 5. Micrograms of NH_3-N per 100 milliliters of whole blood before and after the intraperitoneal administration of combinations of sodium glutamate, sodium succinate and l-arginine·HCl

Compounds administered	Normal	Time after administration of test compounds (minutes) +30	+60	+120
Sodium glutamate[a] and Sodium succinate[b]	300	230		
	310	200		
	325	200		
	230		435	
	190		190	
	230		210	
	125		85	
	260		210	
	300			300
	375			200
	230	---	---	190
Average	261	210	226	230
Sodium glutamate[a] Sodium succinate[b] and l-arginine·HCl[c]	365	290		
	395	355		
	355	270		
	280	220		
	230		125	
	445		345	
	145		145	
	355		270	
	375			290
	240			175
	270	---	---	180
Average	314	284	221	215

[a]0.296 grams per kilogram of body weight.

[b]0.473 grams per kilogram of body weight.

[c]0.368 grams per kilogram of body weight.

sodium succinate and l-arginine·HCl was administered the average decrease in the blood ammonia nitrogen levels were 10, 30 and 32 per cent, respectively, at post-injection times of 30, 60 and 120 minutes. However, the t-test showed treatment effects to be nonsignificant.

The blood ammonia nitrogen levels obtained when sodium glutamate was administered intraperitoneally at intervals of 30, 60 and 120 minutes prior to a sub-lethal toxic dose of ammonia nitrogen are recorded in Table 6. Within 15 minutes after being challenged with the ammonia nitrogen all lots exhibited average blood ammonia nitrogen levels which were greater than 1884 micrograms per 100 milliliters which was the maximum value anticipated from this amount of ammonium chloride injected as shown in Table 3. Lamb 11 reached a maximum blood ammonia nitrogen level of 2845 micrograms per 100 milliliters 15 minutes after the administration of a toxic dose of ammonium chloride. This lamb exhibited convulsions and went into tetany seven minutes after being challenged with ammonia nitrogen and remained in a recumbent position for 70 minutes. At the end of 90 minutes the blood ammonia nitrogen level of this lamb was still far above the normal value of 210 micrograms per 100 milliliters; however, he showed no clinical symptoms of distress at this time other than lethargy and depression and eventually recovered. The remaining lambs manifested blood ammonia nitrogen levels which

Table 6. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of sodium glutamate at different time intervals prior to a sub-letahl toxic dose of ammonium chloride[a]

Lamb no.	Kilograms of body weight	Grams of sodium glutamate administered[b]	Time before administering ammonium chloride		Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
3	45.9	13.59	30		330	1519[c]	1724	1232	725	490	405	290
8	39.5	11.69	30		270	2052[c]	2338	1961	1401	1089	660	310
				Av.	300	1786	2031	1597	1063	790	533	300
8	40.0	11.84	60		310	1935[c]	1922	1505	1180	816	738	435
12	42.3	12.52	60		480	2052	2091[d]	1453	842	425	355	230
				Av.	395	1994	2007	1479	1011	621	547	333
11	42.0	12.43	120		210	2572[e]	2845	1948	1753	1519	1011	767
16	45.9	13.59	120		270	1545	1831[d]	1479	1011	673	605	320
				Av.	240	2059	2338	1714	1382	1096	808	544

[a]0.18725 grams of ammonium chloride per kilogram of body weight.

[b]0.296 grams per kilogram of body weight.

[c]Exhibited clinical symptoms of profuse salivation, dyspnea, muscular tremors and/or ataxia.

[d]Exhibited clinical symptoms of convulsions.

[e]Exhibited clinical symptoms of convulsions and tetany.

approached normal or below 90 minutes after the administration of the ammonium chloride. Lambs 12 and 16 exhibited maximum blood ammonia nitrogen values of 2091 and 1831 and were in coordinate convulsions at 13 and 12 minutes, respectively, following the intraperitoneal administration of ammonium chloride. At the end of 90 minutes following the challenge with ammonia nitrogen the blood ammonia nitrogen level of lamb 16 approached the normal value of 270 micrograms per 100 milliliters while that of lamb 12 was less than half of the normal value taken just prior to the administration of sodium glutamate. It will be noted that clinical symptoms of convulsions did not occur until the blood ammonia nitrogen level had risen to approximately 2000 micrograms per 100 milliliters of whole blood. While this was not true in all cases reported in this study it was found to be fairly reliable in most of them.

In Table 7 are recorded the blood ammonia nitrogen values obtained when sodium succinate was administered intraperitoneally at various time intervals prior to a sub-lethal toxic dose of ammonium chloride. Lambs 6 and 17 which exhibited maximum blood ammonia nitrogen levels of 1050 and 816 micrograms per 100 milliliters, respectively, appeared to benefit from the action of sodium succinate. Whether this was a direct consequence of the detoxifying effects of sodium succinate seems dubious in view of the results obtained with the

Table 7. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of sodium succinate at different time intervals prior to a sub-lethal toxic dose of ammonium chloride[a]

Lamb no.	Kilograms of body weight	Grams of sodium succinate administered[b]	Time before administering ammonium chloride	Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
5	45.0	21.28	30	260	2390[c]	2585	2078	1610	933	435	320
6	46.6	22.04	30	385	777	985[d]	1050	777	621	500	345
			Av.	323	1584	1785	1564	1194	777	468	333
17	43.2	20.41	60	310	816[e]	500	395	270	250	210	200
20	46.8	22.11	60	280	2273	2468[c]	1922	1388	816	565	405
			Av.	295	1545	1484	1159	829	533	388	303
9	33.6	15.89	120	290	1857[f]	2416[c]	1818	1857	1831	1701	1388
13	41.4	19.58	120	290	1662[f]	1740	1571	1115	777	540	310
			Av.	290	1760	2078	1695	1486	1304	1121	849

[a]0.18725 grams of ammonium chloride per kilogram of body weight.

[b]0.473 grams per kilogram of body weight.

[c]Exhibited clinical symptoms of convulsions and tetany.

[d]Exhibited clinical symptoms of depression, dyspnea and salivation.

[e]Exhibited no clinical symptoms of ammonia toxicity.

[f]Exhibited clinical symptoms of profuse salivation, dyspnea, muscular tremors and/or ataxia.

remaining four lambs in this lot. None of the lambs, with the exception of lamb 17, exhibited blood ammonia nitrogen levels which approximated normal before 90 minutes following the administration of the ammonium chloride. Lamb 9 became ataxic at eight minutes following the ammonia nitrogen challenge and was in tetany at 24 minutes. This lamb exhibited a blood ammonia nitrogen level of 1388 micrograms per 100 milliliters at the end of 90 minutes and refused to stand for an additional 20 minutes but recovered. Lambs 5 and 20 manifested convulsions and tetany at 11 and 19 minutes, respectively, at which time their blood ammonia nitrogen levels approximated 2500 micrograms per 100 milliliters. Only lamb 13 responded to the ammonia nitrogen challenge as predicted in Table 3 based on results obtained when ammonium chloride alone was given, i.e., reached a peak blood ammonia nitrogen level of approximately 1884 micrograms per 100 milliliters.

The results obtained with lambs 5, 9 and 20 would indicate not only that sodium succinate was without value in the prevention of ammonia intoxication in lambs but also that sodium succinate had a detrimental influence in that it aggravated and augmented the adverse blood ammonia nitrogen picture. Whether this premise is valid or rather that the unexpected high blood ammonia nitrogen levels manifested by these lambs were the result of individual differences in response to this level of ammonia nitrogen administration is not

known. Since it has been shown that sodium succinate, in amounts approximating one gram per kilogram of body weight, exerts a depressing effect on lambs it is possible that in so doing it influences the normal detoxifying mechanisms of the animal in such a way as to be detrimental rather than beneficial.

The blood ammonia nitrogen values and their associated clinical symptoms following the intraperitoneal administration of l-arginine·HCl at intervals of 30, 60 and 120 minutes prior to a sub-lethal toxic dose of ammonium chloride are recorded in Table 8. The data do not suggest the enhancement of any protective mechanism in lambs against ammonia toxicity by l-arginine·HCl. Although lamb 17 did not manifest a maximum blood ammonia nitrogen level as great as would be expected, it would seem more probable that this was an individual response to this dose of ammonia nitrogen and not an indication of a protective mechanism. The blood ammonia nitrogen level of this lamb had returned to normal 75 minutes after the challenge with ammonium chloride. At no time were clinical symptoms of toxicosis more severe than a mild transient attack of dyspnea and ptyalism.

The data suggest a positive correlation between the severity of the toxicity syndrome and the length of time elapsing between the administration of l-arginine·HCl and ammonium chloride. Although lamb 11, which was injected with

Table 8. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of l-arginine·HCl at different time intervals prior to a sub-lethal toxic dose of ammonium chloride[a]

Lamb no.	Kilograms of body weight	Grams of l-arginine ·HCl administered[b]	Time before administering ammonium chloride	Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
11	41.8	15.38	30	385	1805[c]	2039[d]	1688	1206	764	490	385
17	42.3	15.57	30	290	1128[c]	1636[c]	1453	946	615	445	260
			Av.	338	1467	1838	1571	1076	690	468	323
17	43.2	15.92	60	210	1102[c]	1349	842	520	310	210	165
18	44.5	16.38	60	330	1883[e]	1714	1388	1050	699	460	290
			Av.	270	1493	1532	1115	785	505	335	228
5	43.2	15.92	120	230	2442[f]	2260	1154	712	480	310	270
15	36.8	13.56	120	230	2247[e]	1974[f]	1766	1323	725	575	280
			Av.	230	2345	2117	1460	1018	603	443	275

[a]0.18725 grams of ammonium chloride per kilogram of body weight.

[b]0.368 grams per kilogram of body weight.

[c]Exhibited clinical symptoms of depression, dyspnea and salivation.

[d]Exhibited clinical symptoms of profuse salivation, dyspnea, muscular tremors and/or ataxia.

[e]Exhibited clinical symptoms of convulsions.

[f]Exhibited clinical symptoms of convulsions and tetany.

l-arginine·HCl 30 minutes prior to the ammonium chloride, had a maximum recorded blood ammonia nitrogen level of 2039 micrograms per 100 milliliters, the level at which one would expect convulsions to occur, he exhibited symptoms of toxicity which were no more severe than mild muscular tremors and ataxia of the front quarters. Lamb 18 which was injected with l-arginine·HCl 60 minutes before being subjected to the ammonium chloride had a peak blood ammonia nitrogen level which was considerably below that of lamb 11 and yet had symptoms which were much more severe. Lambs 5 and 15 which were injected 120 minutes prior to the ammonium chloride administration had both the highest blood ammonia nitrogen levels and the most severe clinical symptoms of toxicosis. Respective maximum blood ammonia nitrogen values of 2442 and 2247 micrograms per 100 milliliters of blood together with clinical symptoms of convulsions and tetany were noted.

When the administration of l-arginine·HCl preceded that of ammonium chloride by 30 or 60 minutes the blood ammonia nitrogen returned to normal or below 90 minutes following the injection of ammonium chloride. When 120 minutes elapsed between the two injections the blood ammonia nitrogen had not returned to normal at the end of 90 minutes although they did approach these values.

In Table 9 are recorded the blood ammonia nitrogen values obtained when a combination of sodium glutamate and sodium

Table 9. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of a mixture of sodium glutamate and sodium succinate at different time intervals prior to a sub-lethal toxic dose of ammonium chloride[a]

Lamb no.	Kilograms of body weight	Grams of sodium glutamate administered[b]	Grams of sodium succinate administered[c]	Time before administering ammonium chloride	Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
3	44.5	13.17	21.05	30	300	2247[d]	2364[d]	1870	1545	1206	1011	803
3	45.2	13.41	21.43	30	310	1922[d]	2104	1375	907	595	480	290
6	45.9	13.59	21.71	30	325	2130[d]	2247[e]	1948	1545	959	550	425
				Av.	312	2100	2238	1731	1332	920	680	506

[a]0.18725 grams of ammonium chloride per kilogram of body weight.

[b]0.296 grams per kilogram of body weight.

[c]0.473 grams per kilogram of body weight.

[d]Exhibited clinical symptoms of profuse salivation, dyspnea, muscular tremors and/or ataxia.

[e]Exhibited clinical symptoms of convulsions.

Table 9. (Continued)

Lamb no.	Kilograms of body weight	Grams of sodium glutamate administered	Grams of sodium succinate administered	Time before administering ammonium chloride	Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
15	35.9	10.63	16.98	60	190	1401[d]	1688[e]	1336	686	445	290	210
16	45.2	13.41	21.40	60	125	1961[f]	2169	2221	1571	--[g]	--[g]	355
18	43.6	12.90	20.60	60	230	1141[h]	1154	764	595	460	385	345
				Av.	182	1501	1670	1440	951	453	338	303
9	35.5	10.51	16.78	120	375	2949[f]	2494[f]	2299	2013	1649	1466	1102
11	42.3	12.52	19.99	120	230	1857[d]	2052[f]	1453	998	605	460	310
14	43.0	12.73	20.34	120	300	1011[h]	1102	816	660	405	270	230
				Av.	302	1939	1883	1523	1224	886	732	547

[f]Exhibited clinical symptoms of convulsions and tetany.

[g]Unable to obtain a blood sample due to convulsions and tetany.

[h]Exhibited clinical symptoms of depression, dyspnea and salivation.

succinate was administered intraperitoneally at various time intervals prior to a sub-lethal toxic dose of ammonium chloride.

Of nine lambs so treated only two, lambs 14 and 18, gave any indication of a protective mechanism attributable to this combination of drugs. For the most part the results were quite similar to those obtained when only one of the two compounds was administered. Maximum blood ammonia nitrogen values in excess of amounts predicted from Table 3 when ammonium chloride alone was administered, i.e. 1884 micrograms per 100 milliliters of blood, were noted. Lamb 9 which exhibited a maximum recorded blood ammonia nitrogen level of 2949 micrograms went into extreme tetanic seizures beginning 10 minutes after the administration of ammonium chloride. Regurgitation of approximately 50 milliliters of rumen ingesta at 15 minutes raised some doubts as to whether this lamb would survive and consequently an attempt to relieve the tetanic contractions with intraperitoneal pentobarbital sodium was initiated. Following the administration of 10 milliliters of this anaesthetic a transient period of about 10 minutes duration, in which there was complete muscular relaxation, was noted followed by a return of the original symptoms. The intraperitoneal injection of a supplementary 10 milliliters of pentobarbital sodium inhibited the symptoms for an additional 13 minutes after which there was a short period of

relatively mild muscular contractions mainly of the extensor muscles of locomotion. This lamb was standing and appeared to be normal in outward appearances 85 minutes after the initial convulsions and tetany. While it was not always possible to obtain blood samples from lambs that were in convulsions and tetany, no undue difficulty was encountered in withdrawing all of the samples from this lamb. When it was found to be relatively impossible to restrain an animal sufficiently, when in convulsions or tetany, to obtain blood samples then the animal was returned to the holding pen for a rest period of at least seven days. An obvious exception to this was lamb 16. Despite repeated attempts it was not possible to obtain blood samples at the 60 and 75 minute time intervals; however, in this case the data from the remaining readings were recorded and used in this study.

The average values of blood ammonia nitrogen for the various bleeding intervals which are shown in this table are of interest in showing that one could expect lower maximum blood ammonia nitrogen values when a combination of sodium glutamate and sodium succinate is administered 60 minutes prior to a sub-lethal dose of ammonium chloride than when they are administered either 30 or 120 minutes previous to the ammonium chloride. Apparently the blood ammonia nitrogen values are only a rough index of the severity of the associated symptoms since the clinical symptoms exhibited in this

lot were equally as severe as those produced when the test compounds were administered either 30 or 120 minutes prior to the ammonium chloride.

The micrograms of ammonia nitrogen per 100 milliliters of whole blood following the intraperitoneal administration of a mixture of sodium glutamate, sodium succinate and l-arginine·HCl at different time intervals prior to a sub-lethal toxic dose of ammonium chloride are given in Table 10.

It will be noted from the average blood ammonia nitrogen values calculated in this table that the maximum blood ammonia nitrogen levels attained when the test compounds were administered either 30 or 60 minutes prior to the ammonium chloride were substantially lower than those shown in Table 3 when ammonium chloride was administered alone, i.e. 1884 micrograms per 100 milliliters.

It is not known whether the unexpectedly low blood values shown by lamb 14 are valid. They may possibly be explained by the inadvertent injection of the ammonium chloride into the perirenal fat resulting in a greatly reduced rate of absorption into the systemic blood. The average peak blood ammonia nitrogen values obtained when 120 minutes were allowed to elapse between the injection of the test compounds and the ammonium chloride were significantly higher than one would expect if, in fact, there was no prophylactic effect from the test compounds. The reason for this is not clear, however, it would seem logical to assume from this and previous tables

Table 10. Micrograms of NH_3-N per 100 milliliters of whole blood following the intraperitoneal administration of a mixture of sodium glutamate, sodium succinate and l-arginine·HCl at different time intervals prior to a sub-lethal toxic dose of ammonium chloride[a]

Lamb no.	Kilograms of body weight	Grams of sodium glutamate administered[b]	Grams of sodium succinate administered[c]	Grams of l-arginine·HCl administered[d]	Time before administering ammonium chloride		Bleeding intervals (minutes) 0	+10	+15	+30	+45	+60	+75	+90
6	43.2	12.79	20.43	15.90	30		365	1063	1440[e]	1375	816	550	425	325
6	45.55	13.47	21.52	16.74	30		280	1610[e]	1974[f]	1753	1154	790	480	325
15	34.5	10.20	16.30	12.70	30		395	1440[e]	1779	1128	565	480	330	375
						Av.	347	1371	1731	1419	845	607	412	342
14	41.8	12.38	19.75	15.40	60		445	816[g]	660	405	365	330	345	330
14	43.2	12.79	20.41	15.92	60		145	1167	1519[e]	1141	621	450	300	200
20	46.6	13.79	22.04	17.15	60		355	2078[e]	2390[f]	1792	1271	855	550	395
						Av.	315	1354	1523	1113	752	545	398	308
6	46.8	13.85	22.14	17.22	120		240	1519[e]	1896	1414	946	565	490	300
9	33.2	9.83	15.69	12.23	120		270	2351[h]	2325	1011	565	500	405	330
20	45.9	13.59	21.71	16.89	120		375	2312[e]	2156	1831	1479	907	673	510
						Av.	295	2061	2126	1419	997	657	523	380

[a]0.18725 grams of ammonium chloride per kilogram of body weight.

[b]0.296 grams per kilogram of body weight.

[c]0.473 grams per kilogram of body weight.

[d]0.368 grams per kilogram of body weight.

[e]Exhibited clinical symptoms of profuse salivation, dyspnea, muscular tremors and/or ataxia.

[f]Exhibited clinical symptoms of convulsions.

[g]Exhibited no clinical symptoms of ammonia toxicity.

[h]Exhibited clinical symptoms of convulsions and tetany.

that whatever benefit may be derived from these test compounds, whether given singly or in combination, has been completely exhausted by 120 minutes following their injection.

Statistical analyses of data in Tables 6 through 10 are presented under the section titled "General Discussion".

Summary

The sodium salts of two metabolic compounds namely, glutamic acid and succinic acid, and l-arginine·HCl were administered intraperitoneally singly and in combination to yearling wether lambs for the purpose of determining their prophylactic value in preventing or alleviating the syndrome of ammonia intoxication. Observations were made on the effect of time between treatment and the administration of a sub-lethal toxic dose of ammonium chloride. The sub-lethal toxic dose of ammonium chloride which, when administered intraperitoneally, would produce irrefutable symptoms of toxicosis was found to be 0.18725 grams per kilogram of body weight which is equivalent to 49 milligrams of ammonia nitrogen. Maximum blood ammonia nitrogen levels were reached approximately 15 minutes following the injection of this amount of ammonium chloride.

The average normal blood ammonia nitrogen value for yearling wether lambs was found to be 300 micrograms per 100 milliliters of whole blood with a range of 125 to 480 micrograms.

When the lambs were not challenged with ammonium chloride a very definite decrease in blood ammonia nitrogen from the normal was observed when the test compounds were given either singly or in combination. Of the three compounds tested only sodium succinate and l-arginine·HCl exhibited any detoxifying properties against high blood levels of ammonia, however, this effect was not consistently observed in all cases. The ability of these compounds to exert this effect was found to be greatest when they were administered 60 minutes prior to the toxic dose of ammonium chloride. This was also true in all mixtures of the test compounds in which sodium succinate and l-arginine·HCl were component parts. No effect was noted when the test compounds were administered two hours prior to the ammonium chloride undoubtedly due to the fact that the major portion of the test compounds was completely metabolized at the time of the ammonia challenge.

GENERAL DISCUSSION

The production of livestock for meat purposes in the United States is a vast and dynamic industry. Workers in the fields of education and industry as well as those people who are intimately connected with the production of livestock, such as ranchers and cattle feeders, are constantly at work on new and more efficient methods of producing high quality animals at the lowest possible cost. Among the principal areas of endeavor have been the fields of animal breeding, nutrition, management, and sanitation and disease control.

In the field of animal nutrition economics assumes a position of great importance and consequently workers are constantly on the alert for new sources of relatively inexpensive, high quality feedstuffs. In ruminant nutrition this problem has been partially allayed through the use of several non-protein nitrogen feeding compounds of which urea has been the most prominent in past years as well as at the present time.

The widespread use of urea has been based on its ability to release ammonia when it is acted upon by the enzyme, urease, which is present in the rumen of sheep and cattle. The liberated ammonia is then utilized by the rumen microflora in the synthesis of cellular protoplasm. The host animal is able to benefit from this symbiotic relationship when these bacteria are digested and broken down in the abomasum and

small intestine to the basic constituent amino acids which are then absorbed and utilized in the synthesis of body protein by the host.

The ideal non-protein nitrogen feeding compound would be one which is non-toxic, inexpensive, completely utilizable by the rumen microflora and highly palatable. When used at recommended levels urea fulfills the first three of these requirements but unfortunately problems have arisen through its indiscriminate use as well as from inadequate mixing of the ration and improper adaptation of the animals and their rumen microorganisms to this type of feedstuff. When administered at levels above that which can be utilized by the rumen microflora, the excess ammonia is absorbed into the portal blood producing the typical syndrome of ammonia intoxication.

These clinical symptoms were found to include, in the order of their appearance: apprehension, depression, polypnea, micturition and defecation, muscular tremors, hyperesthesia, increased salivation with frothing, dyspnea, ataxia, convulsions, tetany, bloat, coma and death.

Necropsy examination of the lambs that succumbed to treatment, were performed by the Iowa State University Diagnostic Laboratory. Due to the acute nature of this condition very few lesions were found. The basic findings consisted primarily of numerous petechial hemorrhages and a considerable amount of edema throughout the cadaver. In one case a

small myocardial infarct was found in the wall of the left ventricle near the apex. In most every case the hemoglobin and myoglobin displayed a brownish discoloration suggestive of the presence of methemoglobin.

Similar toxicity problems seem unavoidable with the use of other non-protein nitrogen feeding compounds, such as ammonium acetate, ammonium formate and propionamide. This thesis problem was initiated with the hope that a means could be found of reducing the toxicity and associated hazards of feeding non-protein nitrogen feeding compounds to ruminant animals.

Preliminary studies employing adult CFW albino mice were most encouraging. A very definite protective effect was shown by all three of the test compounds with l-arginine·HCl exhibiting the greatest effect followed by sodium succinate and sodium glutamate. The greater degree of protection afforded when combinations of sodium glutamate and l-arginine·HCl, sodium succinate and l-arginine·HCl or sodium glutamate, sodium succinate and l-arginine·HCl were used as opposed to a combination of sodium glutamate and sodium succinate was found to be statistically significant at the 0.01 level. It will be noted that in all three of these combinations l-arginine·HCl was a component part which would further substantiate the claim that this compound possesses a relatively vigorous propensity to detoxify ammonia. When a combination

of all three of the test compounds was administered complete protection was provided against a lethal dose of ammonium chloride.

Essentially the same principles embodied in the experimental design used with mice were employed in the lamb study with the exceptions being that 1) a sub-lethal toxic dose of ammonium chloride was used, 2) blood ammonia nitrogen levels together with clinical symptoms were used as criteria of toxicity and 3) observations were made on the effect of varying the time between administration of the test compounds and the toxic dose of ammonium chloride.

Due to the highly divergent responses obtained from some of the lambs to the dosage of ammonium chloride used in this study some difficulty was encountered in arriving at a set of valid conclusions. The conclusions which were reached through a statistical analysis of the data are therefore open to some question and will be discussed at the appropriate time.

The reason for the wide deviations in the response of some of the lambs to an equivalent dose of ammonium chloride is not known. It would seem unlikely that this is a normal variation due to individual differences in susceptibility to ammonium chloride. It also seems improbable that it was due to faulty technique in making the intraperitoneal injections since more often than not the observed blood ammonia nitrogen

level far exceeded the expected level. A possible explanation may lie in the presence of only limited amounts of arginase in the hepatic cells of these animals which would seriously impede the synthesis of urea and consequently the detoxification of ammonia. A measurement of blood urea at various time intervals following the administration of a toxic dose of ammonium chloride would give an indirect measurement of the arginase activity of the liver. Since blood urea determinations were not obtained from the lambs in this study, this point is justifiably open to criticism.

A summation of the analysis of variance is presented in the Appendix. Obviously significant differences were found among the blood ammonia nitrogen levels at the various bleeding intervals following the administration of ammonium chloride. The maximum blood ammonia nitrogen levels were recorded 10 to 15 minutes following the administration of the toxic dose of ammonium chloride and were found to range from a low of 816 to a high of 2949 micrograms per 100 milliliters. The treatment effects were also found to be significant at the .01 level which is, perhaps, not as obvious from a casual glance at the data. Within treatments the time at which the test compounds were given prior to the toxic dose of ammonium chloride was also found to be highly significant. It would appear from the data that the optimum time to administer these compounds, prior to the ammonium chloride, would be somewhere

between 30 and 120 minutes. Further studies to determine this optimum time would appear to be indicated.

The differences observed between the maximum blood ammonia nitrogen levels attained following the administration of the test compounds and those of the controls were found to be significant at the .05 level.

A more critical analysis of the data was obtained through the use of the new multiple range test, Duncan (1955). Those treatments which were found to be significantly better, at the .05 level, in their ability to detoxify ammonia in lambs were: l-arginine·HCl administered 60 minutes prior to the challenging dose of ammonia nitrogen, sodium succinate administered at the 60 minute interval, a combination of sodium glutamate and sodium succinate administered at 60 minutes and a combination of all three of the test compounds when administered at either 30 or 60 minutes prior to the ammonium chloride.

The facts, however, would refute these statistical findings. To say that these are valid conclusions is unwarranted and misleading and they must be tempered with discretion and good judgment. The average maximum blood ammonia nitrogen level reached when l-arginine·HCl was administered 60 minutes prior to a toxic dose of ammonium chloride was found to be 1532 micrograms per 100 milliliters which is 352 micrograms below the average for the controls. However, the data reveal that one of the two lambs injected at this time interval

actually exhibited a maximum blood ammonia nitrogen level which was only one microgram below the average maximum level attained in the control animals when only ammonium chloride was administered. The remaining lamb in this lot had an unexpectedly low blood ammonia nitrogen level, the reason for which is unknown.

The same situation exists in the data obtained when sodium succinate was administered 60 minutes prior to the sub-lethal toxic dose of ammonium chloride with the exception that one of the two lambs in this lot exhibited blood ammonia nitrogen levels which far exceeded the controls. This lamb showed clinical symptoms of convulsions and tetany 15 minutes after the injection of ammonium chloride.

The average maximum blood ammonia nitrogen levels recorded when a combination of sodium glutamate and sodium succinate was injected 60 minutes prior to the ammonium chloride were found to be below that of the controls and yet here again one of the lambs had a maximum blood ammonia nitrogen level which was considerably higher than the controls. Similar findings were noted when a combination of sodium glutamate, sodium succinate and l-arginine·HCl was administered at either 30 or 60 minutes prior to the ammonium chloride.

Despite the fact that no evidence was found in any of the three test compounds indicative of any appreciable amount of protection against ammonia intoxication in lambs there is

evidence in the data of two significant trends. In all cases, except when sodium glutamate was administered alone, the average maximum blood ammonia nitrogen levels were below those of the controls when the test compounds were administered 60 minutes prior to the ammonium chloride. A combination of all three of the test compounds appeared to exert the most influence on blood ammonia nitrogen levels when administered either 30 or 60 minutes prior to a sub-lethal toxic dose of ammonium chloride.

SUMMARY

The sodium salts of two metabolic compounds, (glutamic acid, succinic acid) and l-arginine·HCl were administered intraperitoneally to both white mice and yearling lambs for purposes of determining their prophylactic value in the prevention of ammonia intoxication.

In the pilot studies with white mice survival rates of 30, 40 and 50 per cent, respectively, were obtained when sodium glutamate, sodium succinate and l-arginine·HCl were administered singly 30 minutes prior to a minimum lethal dose of 625 milligrams of ammonium chloride per kilogram of body weight. When combinations of sodium glutamate and sodium succinate, sodium glutamate and l-arginine·HCl, sodium succinate and l-arginine·HCl and sodium glutamate, sodium succinate and l-arginine·HCl were administered intraperitoneally 30 minutes prior to a lethal dose of ammonium chloride, respective survival rates of 40, 80, 85 and 100 per cent were noted. Statistical analysis of these data revealed a high degree of significance between each of the latter three combinations, all of which contained l-arginine·HCl, as opposed to a mixture containing only sodium glutamate and sodium succinate. The detoxifying properties of a combination of all three of the test compounds were found to be statistically greater at the 0.05 level than that shown by a combination containing only sodium glutamate and l-arginine·HCl.

A degree of protection against ammonia intoxication was demonstrated by all three of the test compounds with l-arginine·HCl being the most active and sodium glutamate the least.

The second part of this thesis problem was concerned with the effects of the three test compounds on ammonia intoxication in lambs. The criteria of ammonia intoxication used in this study were based on clinical symptoms and blood ammonia nitrogen levels. The sub-lethal toxic dose of ammonium chloride which would produce incontrovertible symptoms of toxicity was found to be 0.18725 grams of ammonium chloride per kilogram of body weight which is equivalent to 49 milligrams of ammonia nitrogen per kilogram of body weight. In general, symptoms of toxicity associated with this level of ammonia nitrogen, listed in the order of their appearance, were: apprehension, depression, polypnea, micturition and defecation, muscular tremors, hyperesthesia, profuse salivation with frothing, dyspnea and ataxia. Jugular blood samples were taken at 0, 10, 15, 30, 45, 60, 75 and 90 minute intervals following the intraperitoneal administration of the ammonium chloride and were analyzed for blood ammonia nitrogen by a microdiffusion method, Seligson (1951). Clinical manifestations of toxicosis were first noted when the blood ammonia nitrogen level approached 1000 micrograms per 100 milliliters. At levels of approximately 2000 micrograms convulsions were observed with fatalities occurring at blood

levels of approximately 4000 micrograms. Normal blood ammonia nitrogen levels were found to average 300 micrograms per 100 milliliters with a range of 125 to 480 micrograms.

Observations were made on the effects of time between treatments and the administration of the toxic dose of ammonium chloride. All treatments with the test compounds were given at three different time intervals of 30, 60 and 120 minutes prior to the toxic dose of ammonium chloride. Peak blood ammonia nitrogen levels were attained approximately 15 minutes following the injection of ammonium chloride. A significant decrease in the normal blood ammonia nitrogen level was found when the test compounds were administered singly or in combination; however, no evidence indicative of the presence of a marked protective mechanism against high levels of blood ammonia nitrogen was observed in any of the experiments following the injection of a sub-lethal toxic dose of ammonium chloride. There appears to be a wide variation in the response of individual lambs to this dosage level of ammonium chloride. Lesions noted at necropsy of those animals that succumbed to treatment were limited by the acute nature of the condition and primarily consisted of generalized petechiation, anasarca and a brownish discoloration of the myoglobin and hemoglobin suggesting the presence of methemoglobin.

LITERATURE CITED

Alexander, J. W. and Porter, C. E. 1954. The treatment of a patient in hepatic coma with intravenous sodium glutamate. Gastroenterology. 26: 926-929.

Bullington, T. H., Byrd, C. E. and Harris, T. W. 1955. Urea poisoning in the bovine. N. Amer. Vet. 36: 107-109.

Clark, R., Oyaert, W. and Quin, J. I. 1951. 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. Sci. 25: 73-78.

Clementi, A. 1951. La produzione dell'urea nell' organismo dei polli in rapporto al contenuto in arginina delle proteine presenti nei semi delle piante alimentari. Arch. Sci. Biol. Bologna. 35: 167-176. (Original available but not translated; abstracted in Nutr. Abstr. 21: 512. 1952.)

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

_____, _____, _____, Ross, O. B., Moe, L. H. and Butler, R. L. 1949. Urea in livestock feeding. J. Amer. Vet. Med. Ass. 144: 90-92.

Dixon, W. J. and Mood, A. M. 1946. The statistical sign test. J. Amer. Statist. Ass. 41: 557-566.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.

Du Ruisseau, J. P., Greenstein, J. P., Winitz, M. and Birnbaum, S. M. 1956. Studies on the metabolism of amino acids and related compounds in vivo. IV. Blood ammonia and urea levels following intraperitoneal administration of amino acids and ammonium acetate, and the effect of arginine thereon. Arch. Biochem. Biophys. 64: 355-367.

Fujimoto, Y. and Tajima, M. 1953. Pathological studies on urea poisoning. Jap. J. Vet. Sci. 15: 125-132. (English summary. 15: 133-134.)

Greenstein, J. P., Winitz, M., Birnbaum, S. M. and Otey, M. C. 1956. Studies on the metabolism of amino acids and related compounds in vivo. III. Prevention of ammonia toxicity by arginine and related compounds. Arch. Biochem. Biophys. 64: 342-354.

Gullino, P., Winitz, M., Birnbaum, S. M., Cornfield, J., Otey, M. C. and Greenstein, J. P. 1956. Studies on the metabolism of amino acids and related compounds in vivo. I. Toxicity of essential amino acids, individually and in mixtures, and the protective effect of l-arginine. Arch. Biochem. Biophys. 64: 319-332.

Harris, L. E. and Mitchell, H. H. 1941. The value of urea in the synthesis of protein in the paunch of the ruminant. II. In growth. J. Nutr. 22: 183-196.

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

Karr, N. W. and Hendricks, E. L. 1949. The toxicity of intravenous ammonium compounds. Amer. J. Med. Sci. 218: 302-307.

Krebs, H. A. 1935. Metabolism of amino acids. IV. The synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. Biochem. J. 29, Pt. 3: 1951-1969.

Manning, R. T. 1957. Ammonia intoxication: The theoretical basis for therapy with arginine. J. Kans. Med. Soc. 58: 163-165.

Manning, R. T. and Delp, M. 1957. Hepatic coma: Use of a new drug, arginine, in its treatment. J. Kans. Med. Soc. 58: 18-20.

McDermott, W. V., Wareham, J. and Riddell, A. G. 1955. Treatment of "hepatic coma" with l-glutamic acid. New Engl. J. Med. 253: 1093-1102.

McIlwain, H. 1951. Glutamic acid and glucose as substrates for mammalian brain. J. Ment. Sci. 97: 674-680.

Najarian, J. S. and Harper, H. A. 1956a. A clinical study of the effect of arginine on blood ammonia. Amer. J. Med. 21: 832.

_____ and _____. 1956b. Comparative effect of arginine and monosodium glutamate on blood ammonia. Proc. Soc. Exp. Biol. 92: 560-563.

Pearson, R. M. and Smith, J. A. B. 1943. The utilization of urea in the bovine rumen. II. The conversion of urea to ammonia. Biochem. J. 37: 148-153.

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

Price, J. C., Waelsch, H. and Putnam, T. J. 1943. DL-glutamic acid hydro-chloride in treatment of petit mal and psychomotor seizures. J. Amer. Med. Ass. 122: 1153-1156.

Reid, J. T. 1953. Urea as a protein replacement for ruminants: A review. J. Dairy Sci. 36, Pt. 3: 955-996.

Repp, W. W. 1955. Relative toxicity of several non-protein nitrogen feeding compounds for lambs. Unpublished Ph. D. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Repp, W. W., Hale, W. H. and Cheng, E. W. 1953. Blood urea and ammonia levels in lambs following oral administration of graded amounts of five experimental non-protein nitrogen feeding compounds. (Abstract.) J. Anim. Sci. 12: 953.

_____, _____, _____ and Burroughs, W. 1955. The influence of oral administration of non-protein nitrogen feeding compounds upon blood ammonia and urea levels in lambs. J. Anim. Sci. 14: 118-131.

Richter, D. and Dawson, R. M. C. 1948. The ammonia and glutamine content of the brain. J. Biol. Chem. 176: 1199-1210.

Riddell, A. G., Kopple, P. N. and McDermott, W. V. 1954. Etiology of meat intoxication in Eck fistula dog. Surgery. 36: 675-684.

Rupel, I. W., Bohstedt, G. and Hart, E. B. 1943. The comparative value of urea and linseed meal for milk production. J. Dairy Sci. 26: 647-664.

Saperstein, M. R. 1943. Effect of glutamic acid on the central action of the ammonium ion. Proc. Soc. Exp. Biol. 52: 334.

Schwartz, R., Phillips, G. B., Gabuzda, G. J. and Davidson, C. S. 1953. Blood ammonia and electrolytes in hepatic coma. J. Lab. Clin. Med. 42: 499-508.

Seligson, D. and Seligson, H. 1951. A microdiffusion method for the determination of nitrogen liberated as ammonia. J. Lab. Clin. Med. 38: 324-330.

Singh, I. D., Barclay, J. A. and Cooke, W. T. 1954. Blood ammonia levels in relation to hepatic coma and the administration of glutamic acid. Lancet. 266: 1004-1007.

Tigerman, H. and MacVicar, R. 1951. Glutamine, glutamic acid, ammonia administration and tissue glutamine. J. Biol. Chem. 189: 793-799.

Torda, C. 1953. Ammonium ion content and electrical activity of the brain during the preconvulsive and convulsive phases induced by various convulsants. J. Pharmacol. 107: 197-203.

Urea in rations for cattle and sheep. A summary of experiments at the Oklahoma Agricultural Experiment Station. 1953. Okla. Agr. Expt. Sta. Bull. No. B-409.

Walley, R. V. 1954. Treatment of hepatic coma with sodium glutamate. Lancet. 266: 157.

Walshe, J. M. 1953. The effect of glutamic acid on the coma of hepatic failure. Lancet. 264: 1075-1077.

Webster, L. T. and Davidson, C. S. 1954. Hepatic coma: effect of sodium glutamate. J. Clin. Invest. 33: 971.

Work, S. H., Hamre, C. J., Henke, L. A. and Harris, L. E. 1943. A note on the effect on the kidneys and livers of feeding urea to steers fattening in dry lot and on pasture. J. Anim. Sci. 2: 166-169.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to the members of his committee for their counsel and assistance in the performance of this study.

He is especially grateful to Dr. Loyal C. Payne for his direction and wise counsel in initiating and conducting the research work reported in this thesis and to Dr. William H. Hale for his aid in the pursuance of this project. He is also grateful to Dr. Earl A. Hewitt for his counsel and help in the writing of this thesis.

The author expresses his appreciation for the research funds provided by the Veterinary Medical Research Institute and to Dr. Wise Burroughs for the financial assistance made available from the Department of Animal Husbandry at Iowa State University.

He is also grateful to Dr. Melvin J. Swenson for re-kindling the spark of enthusiasm and for his valuable counsel and guidance in the writing of this thesis and to Dr. Ervin P. Smith for the statistical analysis of these data.

The author wishes to express his appreciation to Dr. Joseph G. Graca for his valuable assistance and for his generosity in supplying the white mice used in this study and to Mr. Victor Applegate for help in feeding and caring for the experimental animals.

He also wishes to express his profound appreciation to

his wife whose patience, fortitude, understanding and encouragement helped him greatly in completing this research and to his daughter for generously relinguishing her playtime.

The cooperation and generosity of the Armour Laboratories, Chicago, Illinois in furnishing the l-arginine·HCl used in this study are very much appreciated.

APPENDIX

Table 11. Analyses of variance of experimental data from the lamb studies

Source of variation	d.f.		S.S.	M.S.	F
Intervals	7		106146338	15163763	75.55**
Treatments	15		8283548	552237	2.75**
Agents vs. control		5	2327682	465536	2.32*
Control vs. agents		1	413329	413329	2.06
Agents		4	1914353	478588	2.38
Times		2	4484296	2242148	11.17**
Agents vs. times		8	1471570	183946	0.92
Intervals vs. treatment	105		5236721	49874	0.25
Error	190		38133815	200704	
Total	317		157800422		

**Denotes significance at 0.01 level.

*Denotes significance at 0.05 level.

Table 12. Duncan's new multiple range test for significance within treatments

	Treatment	$\bar{x}$	$\bar{x}-\bar{t}_{16}$	$\bar{x}-\bar{t}_{15}$	$\bar{x}-\bar{t}_{14}$	$\bar{x}-\bar{t}_{13}$	$\bar{x}-\bar{t}_{12}$	$\bar{x}-\bar{t}_{11}$
1.	S_{120}	1322.68	540.06*	534.14*	505.93*	468.01*	438.64*	351.74
2.	G_{120}	1272.44	489.92*	483.90*	455.69*	417.77	388.40	301.50
3.	$G+S_{30}$	1227.42	444.80*	438.88*	410.67	372.75	343.38	256.48
4.	Control	1133.09	350.47	344.55	313.34	278.42	249.05	162.15
5.	$G+S_{120}$	1129.42	346.80	340.88	312.67	274.75	245.38	158.48
6.	A_{120}	1061.12	278.50	272.58	244.37	206.45	177.08	90.18
7.	$G+S+A_{120}$	1057.08	274.46	268.54	240.33	202.41	173.04	86.14
8.	G_{30}	1049.75	267.13	261.21	233.00	195.08	165.71	78.81
9.	G_{60}	1048.06	265.44	259.52	231.31	193.39	163.02	77.12
10.	S_{30}	1003.18	220.56	214.64	186.43	148.51	119.14	32.24
11.	A_{30}	970.94	188.32	182.40	154.19	116.27	86.90	--
12.	$G+S+A_{30}$	884.04	101.42	95.05	67.29	29.37	--	--
13.	$G+S_{60}$	854.67	72.05	66.13	37.92	--	--	--
14.	S_{60}	816.75	34.13	28.21	--	--	--	--
15.	$G+S+A_{60}$	788.54	5.92	--	--	--	--	--
16.	A_{60}	782.62	--	--	--	--	--	--

*Denotes significance at 0.05 level.