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Blood Gases and Certain Energy Metabolite Concentrations in Ammonia Intoxicated Sheep

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BLOOD GASES AND CERTAIN ENERGY METABOLITE CONCENTRATIONS IN AMMONIA INTOXICATED SHEEP

by

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BLOOD GASES AND CERTAIN ENERGY METABOLITE CONCENTRATIONS

IN AMMONIA INTOXICATED SHEEP

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

It has been known for almost a century that ruminant animals have the unique ability to convert nonprotein nitrogen (NPN) to protein. Weiske *et al.* (1879) may have been the first to suggest the use of NPN compounds as protein substitutes in ruminant rations.

Urea has been and continues to be the most widely used NPN compound in ruminant rations. According to Visek (1972), no enzyme system is known whereby urea can be converted to host or microbial protein without intervening hydrolysis to ammonia and carbon dioxide. Ammonia, in fact, is the common denominator in the utilization of all NPN substances by ruminants. Ammonia intoxication, therefore, is one of the problems associated with the use of urea, as well as other forms of NPN, in ruminant rations.

Ingested urea is hydrolyzed in the gastrointestinal tract of ruminants by bacterial urease enzymes. Ammonia released by hydrolysis is used in the formation of microbial protein. This protein is in turn broken down and absorbed as amino acids by the ruminant host. All ammonia formed in the rumen would be used in this manner with maximum dietary efficiency. Urea hydrolysis, however, often occurs much more rapidly than the resulting products can be utilized and excess ammonia diffuses into the hepatic portal circulation. Normally this is not serious as the liver has the ability to detoxify ammonia by converting it into urea. Problems arise when the concentrations of ammonia reaching the liver become excessive. Under these conditions the liver's ability to detoxify ammonia is exceeded, ammonia levels in

the peripheral circulation become abnormally high, and ammonia intoxication may result.

Nutritionists currently seek to avoid ammonia intoxication by carefully controlling dietary intake of urea by animals. Continued investigations of the ammonia toxicity problem may help to alleviate the hazards of urea use in ruminant rations. Additional information may also aid in development of methods whereby increased amounts of urea can be safely and efficiently utilized in ruminant rations.

The objectives of this study were to measure the changes occurring in certain blood parameters during acute ammonia intoxication. It was hoped that these changes would reflect and thereby provide insight into the effects of acute ammonia intoxication on metabolic processes. Blood pH, pO_2, and pCO_2 were measured in order to observe possible metabolic disturbances as reflected in the acid-base balance. Glucose was measured due to its importance as a basic energy source and metabolic intermediate. Volatile fatty acids were measured because they serve as an important energy source in ruminants. Non-esterified fatty acids and triglycerides were measured because they are important intermediates in lipid metabolism.

LITERATURE REVIEW

Ammonia Toxicity

Becker (1924) performed one of the earliest experiments involving ammonia toxicity. Rabbits fed urea showed severe toxic symptoms and death resulted. The cause of death was cited as a primary specific toxicity. Visek (1972) summarized the effects of urea hydrolysis on metabolism. He stated that toxic manifestations appear when normal detoxification processes become impaired, ammonia formation is too rapid, ammonia quantities are excessive, and when hydrogen ion and electrolyte relationships in body fluids raise the concentration of nonionic ammonia (NH_3) relative to ammonium ion (NH_4^+). Visek further stated that toxicity is believed to result from the action of ammonia at intracellular sites.

Ammonia Metabolism

There were four processes responsible for the removal of ammonia from blood in vivo, according to Rosado *et al.* (1962) and Wilson *et al.* (1968). The body responded to high ammonia concentrations by the following sequential processes. Initially, ammonia was rapidly removed from the circulation by muscle uptake. Glutamine was synthesized, primarily in the liver and brain. Urea synthesis, taking place mainly in the liver, utilized ammonia gradually released from muscle and from enzymatic hydrolysis of glutamine. Concurrently, synthesis of glutamic acid from alpha-ketoglutarate removed small amounts of ammonia.

Mechanism of Ammonia Toxicity

Acid-base balance

Several studies have explored the effects of ammonia intoxication on the acid-base balance. Roberts et al. (1956) measured blood pH and carbon dioxide tension in dogs after infusing them with ammonium acetate. The decrease in carbon dioxide and the increase in pH noted were indicative of respiratory alkalosis.

An initial metabolic alkalosis followed by a distinct metabolic acidosis were noted by Roller (1966) when toxic doses of urea were given to cattle. Death was attributed to metabolic acidosis stemming from interference with metabolic reactions by high ammonium levels.

Rash et al. (1968) reported acidosis of respiratory origin in urea fed ruminants. Blood carbon dioxide tension increased near death while oxygen tension decreased. Lloyd (1970) noted similar results in cattle and sheep dosed with urea.

Apland (1969) observed a steady decrease in pH in rabbits acutely intoxicated with urea. Blood pCO_2 levels initially decreased then increased as toxicity progressed. Blood pO_2 levels were observed to increase to a plateau.

Davidovich et al. (1977) stated that metabolic alkalosis occurred in cattle during the early stages of ammonia intoxication. Arterial blood pH increased from the time of dosing with urea until the initial signs of toxicity appeared. Blood pO_2 and pCO_2 remained unchanged during this period. Clifford et al. (1969b) induced severe toxicity in rats by injecting crystalline urease. The toxicity observed resulted from uresse hydrolysis of endogenous urea. Blood pH, pCO_2,

and pO_2 remained within normal limits in the test animals.

Blood parameter changes

Many investigators have demonstrated a rise in blood glucose in response to acute ammonia intoxication. Lloyd (1970) reported severe hyperglycemia in cattle dying from ammonia toxicity. Singer (1969) observed hyperglycemic conditions in acutely intoxicated sheep. He attributed the high glucose levels to reduced glucose utilization caused by an imbalance in tricarboxylic acid (TCA) cycle metabolism. This disruption of the TCA cycle was, according to Singer, brought about primarily by overloading of the urea cycle. Hepatic glycogenolysis caused by adrenalin release was also cited as contributing to high glucose levels.

Prior et al. (1970) reported that blood glucose and insulin levels increased in urease-treated rats. In later studies, Prior et al. (1971) tested the effects of insulin on hyperammonemic rats and concluded that ammonia caused a refractive response to insulin which resulted in decreased peripheral glucose uptake.

Chalupa and Opliger (1969) administered a sublethal dose of urea to sheep. An increase in blood glucose and acetate levels was noted. Non-esterified fatty acids also increased initially then decreased to normal levels as toxicity progressed.

Prior and Visek (1972) observed that all plasma amino acids except phenylalanine decreased in rats acutely intoxicated by urease injections. Plasma pyruvate, citrate, succinate, lactate, and glucose levels all increased during acute ammonia intoxication.

It was suggested that the decrease in plasma amino acids may have been due to glucagon release or to insulin released in response to elevated blood glucose levels.

Clifford et al. (1969b) reported a marked elevation in blood concentrations of glycolytic and TCA cycle intermediates in intoxicated rats. Glucose, pyruvate, and alpha-ketoglutarate levels all increased significantly. Data suggested dramatic alterations in the glycolytic and TCA cycles.

Riedeman (1977) measured the effects of urea-induced acute ammonia toxicity on a variety of blood parameters in sheep. Glucose levels were observed to rise while triglyceride levels fell sharply and remained depressed as toxic conditions progressed.

Liver tissue metabolite changes

Because of its important role in metabolic processes as well as in ammonia detoxication, the liver has been given much attention in ammonia intoxication studies. Experiments by Clifford et al. (1969a) showed a decrease in liver ATP levels in intoxicated rats. Later work by Clifford et al. (1969b) indicated a reduction of reduced pyridine nucleotides in rats. NAD and NADP levels were increased in both liver and blood while NADH and NADPH levels were decreased. Sixty percent of the decrease in reduced pyridine nucleotides could be accounted for by increases in NAD and NADP. The remaining forty percent decrease was ascribed to an increase in oxidative cleavage of reduced nicotinamide to nicotinamide.

Prior et al. (1970) noted decreased concentrations of liver glutamic acid and glycine in urease treated rats. Levels of isoleucine, lysine, methionine, arginine, ornithine, cysteine, alanine, and aspartic acid were observed to increase. Conversion of alpha-ketoglutarate into blood glutamic acid as well as liver glutamic acid and alanine was observed by tracing the movements of injected C^{14} labeled glucose. Stimulation of glycolysis and TCA cycle activity by ammonia during acute intoxication were suggested as possible reasons for these findings.

Brain tissue metabolite changes

Many of the symptoms observed in acute ammonia intoxication suggest derangement of neurological function. Schenker et al. (1967) conducted studies involving rats given ammonium acetate injections. Brain cortex levels of ATP and phosphocreatinine remained unchanged in test animals while their concentrations in basilar areas of the brain fell dramatically. Glucose and glycogen levels were also observed to decrease sharply but by similar amounts in both basilar and cortical areas. Lactate and pyruvate levels increased in both areas. Ammonia levels were determined to be equal in all areas of the brain in these animals. In interpreting these results, Walker and Schenker (1970) suggested that the brain stem was especially sensitive to the toxic effects of ammonia. Increases in lactate and pyruvate levels indicated that metabolism via the TCA cycle was being blocked.

Bessman and Bessman (1955) put forth a widely prevalent hypothesis that ammonia depletes intracellular alpha-ketoglutarate by reductive amination to glutamate. This serves to decrease the amount of alpha-ketoglutarate available for the TCA cycle and led to decreased ATP levels in the brain.

Shorey et al. (1967) failed to demonstrate a decrease in alpha-ketoglutarate levels in the brains of ammonium acetate-treated rats. He did note significantly decreased ATP concentrations in the brain stem area.

MATERIALS AND METHODS

Experimental Animals

Eleven female Southdown sheep, weighing between 50.9 and 68 kg., were used in this study. These animals were randomly divided into two groups. The principal group consisted of seven animals while four animals served as the control group. At least one animal from each group was used on each of two test days. All animals were housed indoors and provided water and a mixture of alfalfa and brome grass hay ad libitum. Feed was withheld 24 hours prior to experimentation. All animals appeared to be alert, active, and in good health on test days.

Collection of Samples

Blood aliquots of approximately 25 ml. each were drawn via jugular venipuncture. After drawing pretreatment samples the principal animals were drenched with a 3.3 M. urea solution prepared with tap water. This represented a dosage rate of 2.5 gm. of urea/kg. of body weight. The control animals were given 12.5 ml. of tap water/kg. of body weight, which approximated the amount of water given to the principal animals in the 3.3 M. urea solution. Blood samples were subsequently drawn at 30-minute intervals for three aliquots and a final sample was collected at the time of death. Final samples for control animals were collected at a time which approximated the average time of death of principal animals.

Each blood aliquot was collected in a series of three test tubes. Two test tubes, previously prepared with sodium heparin, were used for

collection of blood for volatile fatty acid analysis. Ten ml. of blood were collected in each tube followed by gentle inversion to ensure mixing. Tubes were centrifuged[a] at 3500 r.p.m. for 15 minutes and 6 ml. of plasma from each tube was transferred to a corresponding 50 ml. plastic centrifuge tube. One ml. of internal standard[b] was added to each of the tubes which were then stored at -20°C. until analysis for volatile fatty acids.

Five ml. of blood were collected in a third unheparinized test tube. A 1-ml. aliquot of blood for blood ammonium nitrogen (BAN) determination was withdrawn from this tube with a tuberculin syringe and 0.5 ml. was ejected into each of two 5-ml. glass stoppered centrifuge tubes which contained 1 ml. of resin suspension[c]. Each whole blood-resin mixture was immediately mixed for one minute with a mechanical mixer[d]. The laked blood was decanted and the resin washed three times with ammonia-free water. The resin tubes were then stored at 7°C. until analysis for blood ammonium nitrogen.

A 0.5-ml. volume of blood for pH-blood gas analysis was withdrawn from the unheparinized test tube into a heparinized 1-ml. syringe.

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[a]Model PR-2, International Equipment Company, Needham Heights, Massachusetts 02194

[b]Method of Gardner and Thompson (1974)

[c]Method of Jaquette et al. (1974)

[d]Vortex Genie Mixer, Scientific Industries, Inc., Springfield, Massachusetts 01103

The syringes were sealed by pushing the end of the attached needle into a 00 rubber stopper and stored in ice at 7°C. until analysis for blood gases and pH.

The blood remaining in the unheparinized test tube was allowed to coagulate. Clotted blood was centrifuged for five minutes. The serum was drawn off and stored at -20°C. until used for glucose, triglyceride, and free fatty acid analysis.

Analytical Procedures

Blood pH, pO_2, and pCO_2[e]

Blood pH, pO_2, and pCO_2 were determined by using a pH-Blood Gas Analyzer[f]. Blood was introduced directly from the collection syringes into the analyzer. The analyzer was standardized immediately prior to analysis and following analysis of every five samples. All blood samples for pH-blood gas analysis were tested within six hours of collection time.

Blood ammonia

Blood ammonium nitrogen (BAN) was determined using the method described by Jaquette (1974). Ion exchange resin (30-80 mesh)[g] was used in preparing resin tubes. Prior to analysis the whole blood-

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[e]Blood gas and pH analyses were performed at the Brookings Hospital, Brookings, South Dakota 57006

[f]Model 213-05, Instrumentation Laboratory Inc., 9 Galen Street, Watertown, Massachusetts 02172

[g]Baker Reagent No. 4622, J. T. Baker Chemical Company, Phillipsburg, New Jersey 08865

resin mixtures were washed with ammonia-free water until a clear supernatent fluid was obtained. Working ammonium nitrogen standard solution was prepared daily by diluting the standard ammonium nitrogen stock solution. Optical densities of final color mixtures were read in a spectrophotometer[h] at 640 nm. wavelength.

Blood glucose

Blood glucose was determined by using reagent kits and controls obtained from Coulter Diagnostics[i]. Tests were conducted according to procedures described in the reagent kits. A 1:10 saline dilution of all samples, using 0.2 ml. of serum and 2 ml. of 0.87% NaCl solution, was made prior to testing. Five samples were tested in duplicate along with one control during each run. Absorbance was read in a spectrophotometer at 500 nm. wavelength.

Triglyceride

Triglycerides were determined using reagent kits purchased from American Monitor Corporation[j]. Samples were analyzed according to the procedures supplied with the reagent kits. Dispcsable 20-microliter pipettes were used for dispensing samples. Tubes were mixed using a mechanical mixer rather than by inversion. Optical density

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[h]Model DB-G, Beckman Instruments, Inc., Fullerton, California 92634

[i]C-Nyme Glucose No. 6600515, Coulter Enzyme Control-Normal No. 7546236, Coulter Diagnostics, Hialeah, Florida 33010

[j]Enzymatic Triglyceride No. 29228-01, American Monitor Corporation, Indianapolis, Indiana 46268

was read at 610 nm. wavelength. The spectrophotometer was re-zeroed with the reagent blank after reading duplicate determinations for each sample. A standard curve was prepared for the determination of triglyceride concentration.

Free fatty acid

Free fatty acids were analyzed according to the method described by Solani and Sardina (1973). Crystallized human albumin (100% pure)[k] was used without further treatment in preparing purified albumin solution for the fatty acid standard solution. A mechanical mixer was adapted with a head which allowed 12 test tubes to be shaken in a vertical position simultaneously. Filter paper pads, which were used to absorb the aqueous phase from above the chloroform phase as described in the procedure, were cut from Whatman 43 ashless, 12.5 cm. diameter filter papers[l]. Each filter paper was cut into four identical pieces. Individual pieces were then folded into small fluted shapes so that they could be easily inserted into the 16 x 100 test tubes containing the aqueous-chloroform phase mixtures. The chloroform phases were decanted into 16 x 100 mm. test tubes and 1.0 ml. was immediately pipetted into corresponding 12 x 75 mm. test tubes. Final color development was carried out in these stoppered tubes. Absorbance was read at 620 nm. wavelength. Five samples run in duplicate, a water blank, and a fatty acid standard were included in each run.

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[k]Chemical Dynamics Corporation, South Plainfield, New Jersey 07080

[l]W & R Balston Limited, London, England

Volatile fatty acids

Volatile fatty acids were analyzed using a modification of the procedure described by Gardner and Thompson (1974). Formic acid and isopropyl alcohol used were purified by fractional distillation until gas-liquid chromatographic analysis showed them to contain minimal traces of acetic, propionic, and butyric acids which had been present in the original reagents. The internal standard solution used contained n-hexanoic acid and was prepared to contain 0.02 mg./ml. of the acid.

The plasma sample plus internal standard solution was adjusted to pH 9 to 10 by adding 0.15 M. NaOH solution. Final pH was determined using pH paper. Protein was removed by adding 40 ml. of isopropyl alcohol and centrifuging at 3500 r.p.m. for 30 minutes. The supernatant liquid was decanted into a 500 ml. round bottom flask. This flask was attached to a rotary film evaporator and the contents evaporated until approximately 1-2 ml. of solution remained. This remaining solution was transferred to a 12 x 75 mm. tube and evaporated to dryness in a drying oven at 98°C. Shortly before analysis, tubes containing dried samples were sealed with a layer of parafilm. A syringe was used to inject 200 ul. of freshly mixed 6+3+1 acetone-ether-formic acid through the film. This resulting hole was immediately sealed with another layer of parafilm. Tube contents were mixed for one minute with a mechanical mixer then allowed to stand for 15 minutes prior to injection onto the gas chromatograph column.

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[m]Varian Aerograph Series 2400, 611 Hansen Way, Palo Alto, California 94303

A dual column gas chromatograph[m], fitted with flame ionization detectors was used. Separations were carried out using a column that was 6 feet long by .125 inch (outside diameter), packed with 20% neopentyl glycol succinate supported on 60-80 mesh firebrick. The following conditions were used: column temperature 145°C., detector temperature 200°C., nitrogen flow rate 120 ml./min., and air flow rate 250 ml./min.

Four samples were prepared with each batch of acetone-ether-formic acid mixture. These samples were refrigerated to reduce evaporation until injection onto the column. A 4 µl. sample was injected on the column and the attenuation factor was set at 128X. A calibration graph was prepared by taking a solution containing known amounts of the volatile fatty acids plus hexanoic acid through the analysis procedure. The mass of each acid recovered was plotted relative to the mass of hexanoic acid recovered. A calibration factor equal to the slope of the line plotted in the calibration graph was determined. The mass of each acid present in a sample was calculated by the following formula:

$$\text{Mass of acid} = \frac{\text{Peak area of acid x mass of n-hexanoic acid}}{\text{Peak area of n-hexanoic acid x calibration factor}}$$

Peak area was estimated using peak height x width at half-height. The amount of each acid recovered was determined. Values for all acids in each sample were then added to give total mass of volatile fatty acids present in each sample. No attempt was made to determine ratios between the amounts of individual acids present in samples.

RESULTS

Two of the seven sheep in the principal group did not die during the experimental period. Data values for these two animals did not correspond with values obtained for the other principal animals nor with those obtained for control animals. Inspection of BAN values showed that ammonia levels were elevated in these animals but not nearly as much as in the other principal group animals. Changes occurring in each of the seven principal sheep and the control group sheep for each parameter measured are shown in fig. 1 to 8 and in appendix tables 3 to 10.

Interpretation of the data obtained for only the five principal sheep which died during the experimental period would give a clearer picture of the effects of acute ammonia toxicity on the parameters tested. Average values for each parameter at each of the sampling times are shown in table 1 for control animals and for the five principal animals which died during the experimental period. It is evident based on BAN values listed in this table that ammonia levels rose until the time of death in principal animals while little change occurred in the control animals. Blood pH decreased in the principal group but varied little from pretreatment values in the control group. There was little change in pO_2 levels in either principal or control animals for the first four samples taken. A decrease in pO_2 levels was noted in the terminal samples for principal group sheep. The pCO_2 levels increased slightly in principal animals as toxicity progressed while no such trend occurred in the control group animals. Terminal pCO_2 levels in principal

sheep were higher than those in the control sheep. Glucose levels rose throughout the experimental period in the principal group sheep. The glucose levels in control group sheep remained near pretreatment levels. Triglyceride levels in principal animals were higher than those in control animals for the 90-minute and terminal samples. The FFA values for principal sheep were decreased in the 90-minute and terminal samples as compared to controls. The VFA levels in principal animals increased as ammonia intoxication became more evident. This trend was not noted in the control animals.

Analysis of variance was performed on raw data for all eleven animals used in the experiment. Regression lines were determined, based on time prior to collection of terminal samples. Lines of regression with time revealed highly significant changes in BAN, blood glucose, pH, pO_2, pCO_2, and triglyceride levels. Significant changes with time were found for VFA and FFA levels. Comparison of regression equations showed that differences between principal and control sheep were highly significant for BAN levels. Significant differences between the two groups were revealed for blood glucose, pO_2, pCO_2, and triglyceride levels. Analysis of variance results are summarized in the appendix (table 2).

Table 1.-Average blood values for principal* and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	BAN (µg./ml.)	pH	pO_2 (mm. Hg.)	pCO_2 (mm. Hg)	Glucose (mg./dl.)	Triglyceride (mg./dl.)	FFA (mg./dl.)	VFA mg./6 ml. plasma
Control								
pretreatment	1.85	7.43	43.4	43.8	77	10.0	25.3	.193
30 Minutes	2.06	7.44	42.0	44.0	77	9.9	25.5	.196
60 Minutes	1.83	7.43	43.8	42.4	74	10.1	25.0	.189
90 Minutes	1.89	7.44	43.6	43.3	74	9.4	25.4	.187
Terminal**	1.78	7.43	43.6	42.6	77	9.6	25.5	.211
Principal								
pretreatment	2.25	7.43	41.3	42.4	73	9.3	26.5	.149
30 Minutes	4.75	7.44	42.7	42.2	79	9.7	26.2	.141
60 Minutes	7.23	7.42	43.1	41.5	98	9.6	25.2	.160
90 Minutes	11.28	7.37	42.0	44.5	147	10.9	23.6	.198
Terminal**	23.71	7.12	32.2	51.0	206	18.8	21.5	.355

* Seven ewes were drenched with urea solution, but only five died within the experimental period. Average blood values were calculated using data collected for these five principal animals only.

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

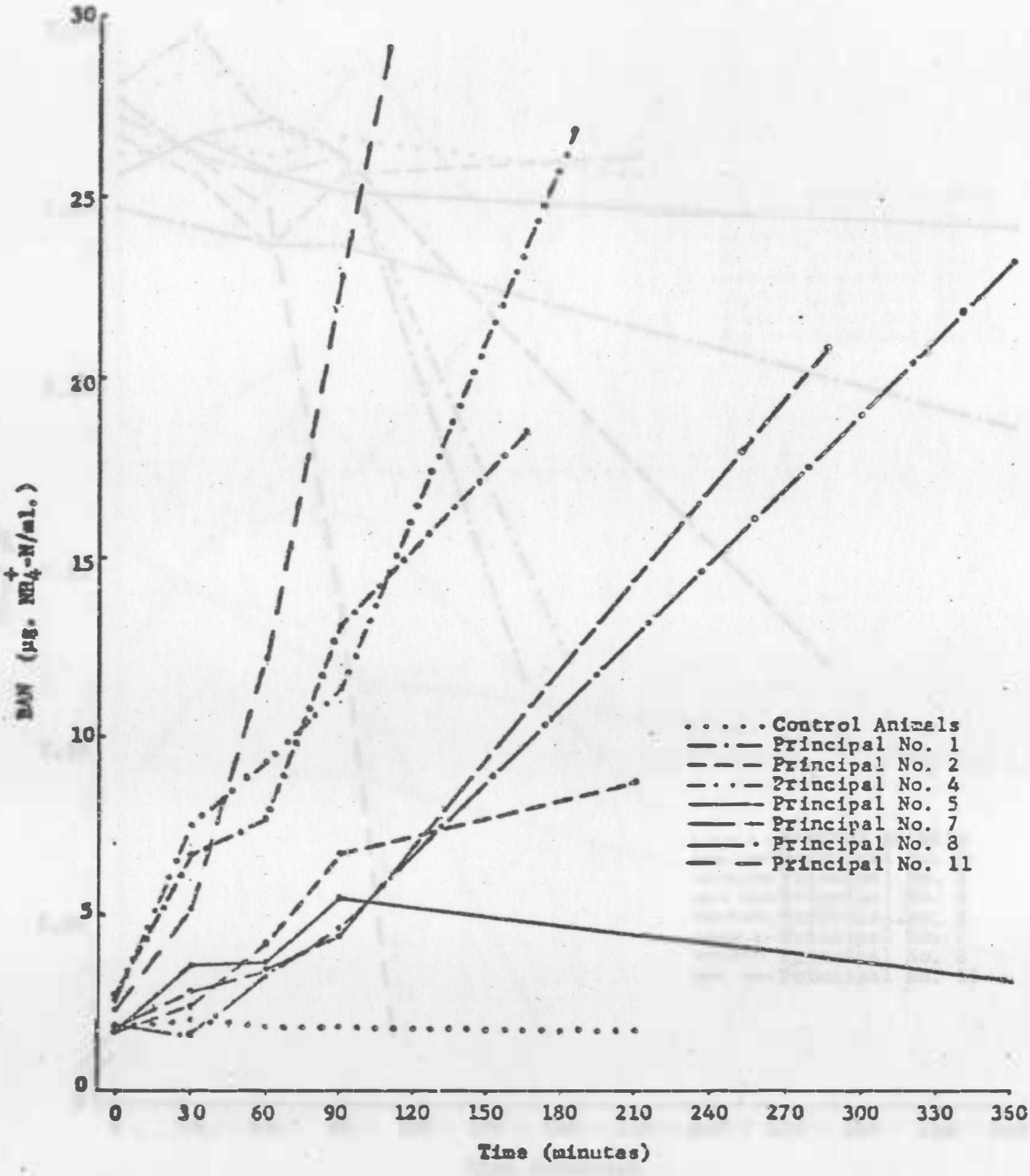

Figure 1. Graphic comparison of blood ammonium nitrogen levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

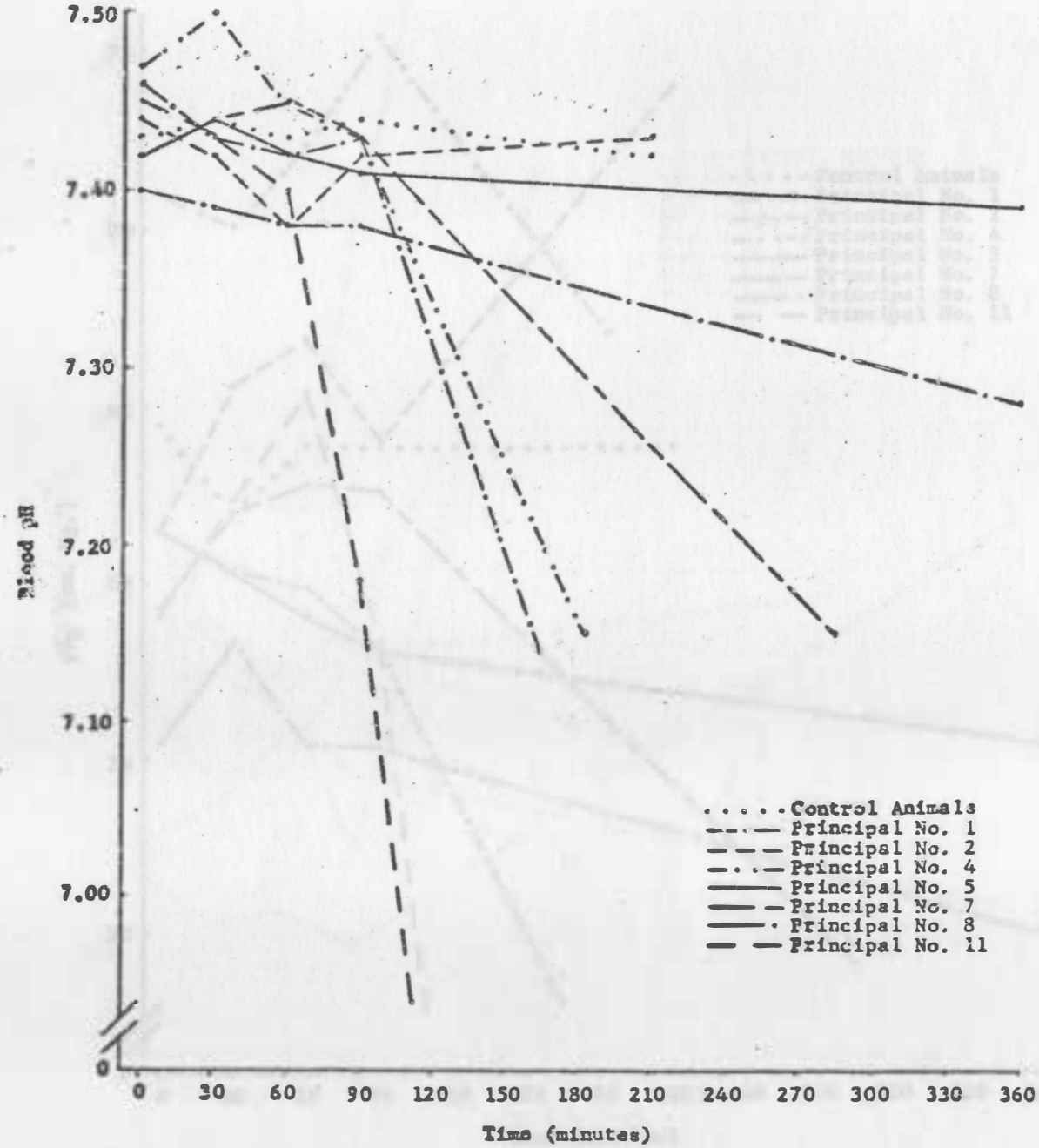

Figure 2. Graphic comparison of blood pH levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

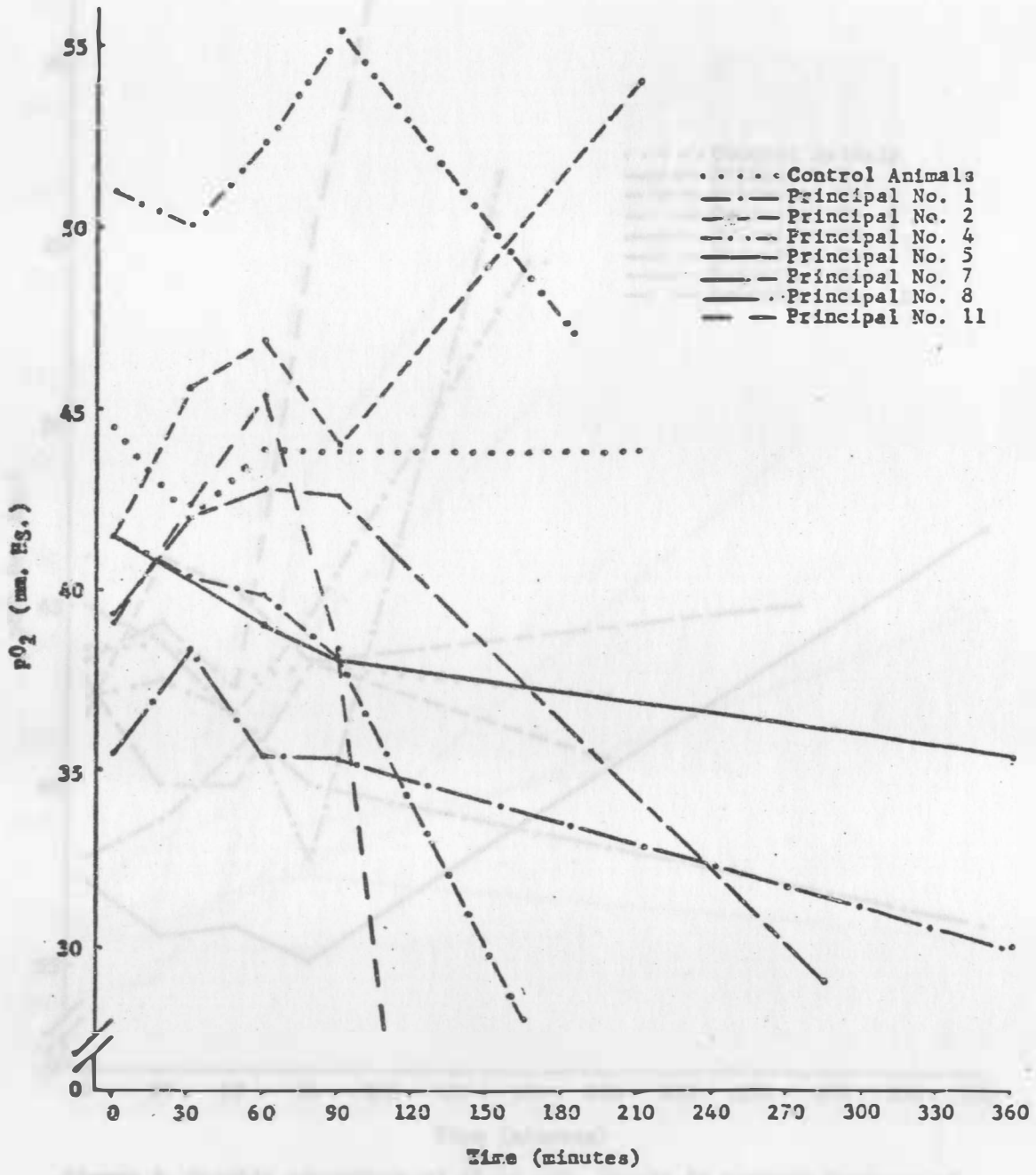

Figure 3. Graphic comparison of blood pO_2 levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

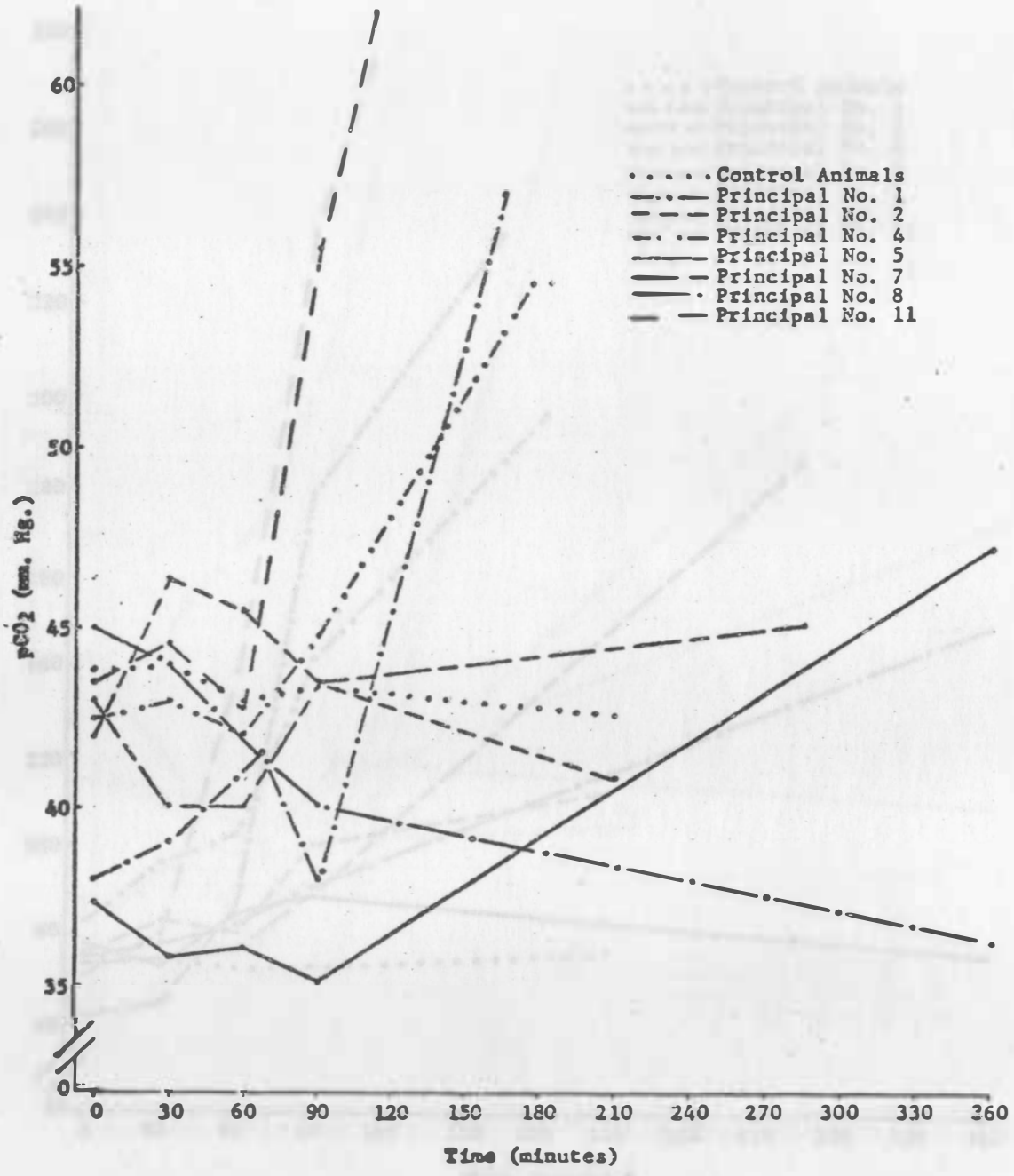

Figure 4. Graphic comparison of blood pCO_2 levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

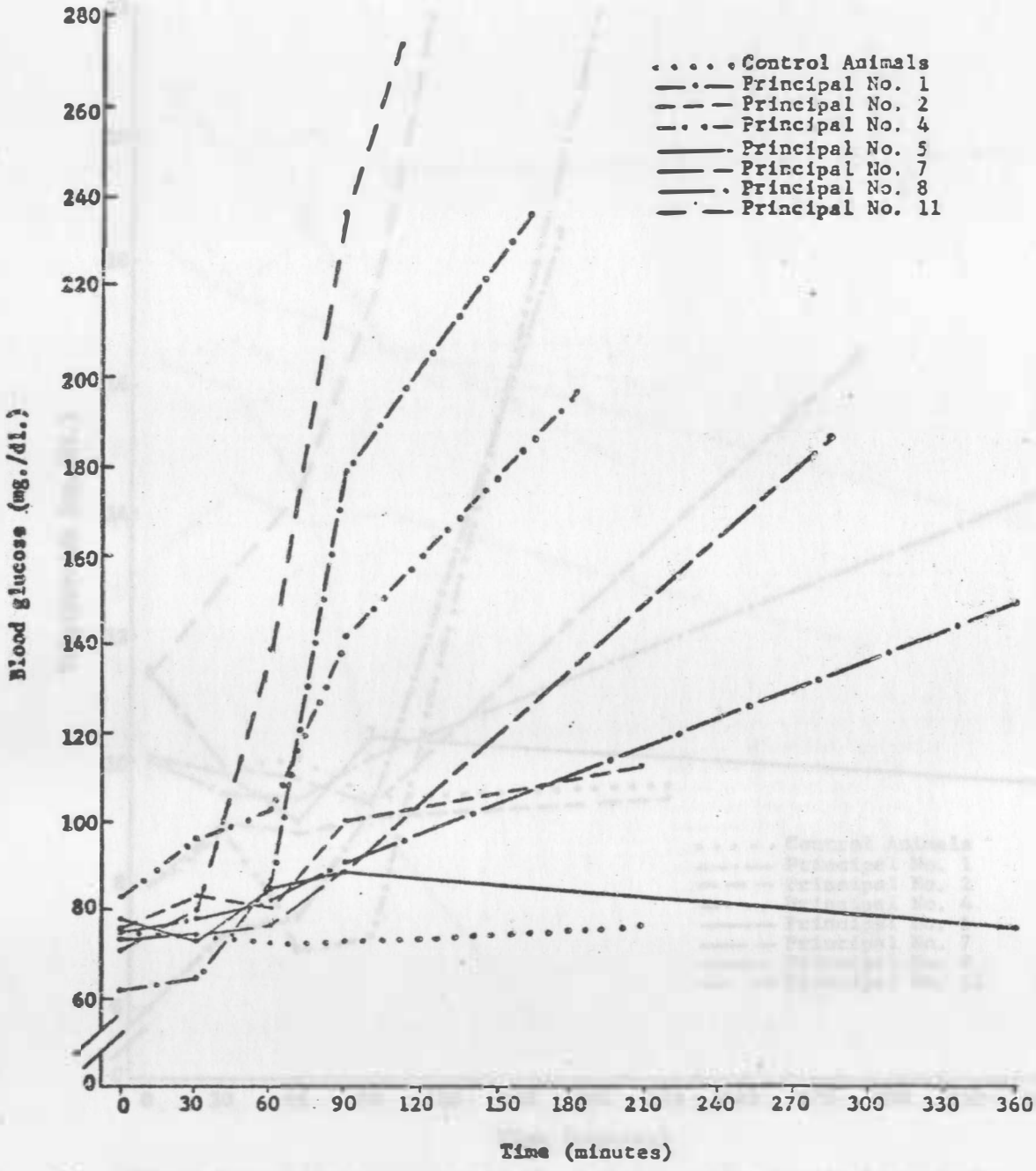

Figure 5. Graphic comparison of blood glucose levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

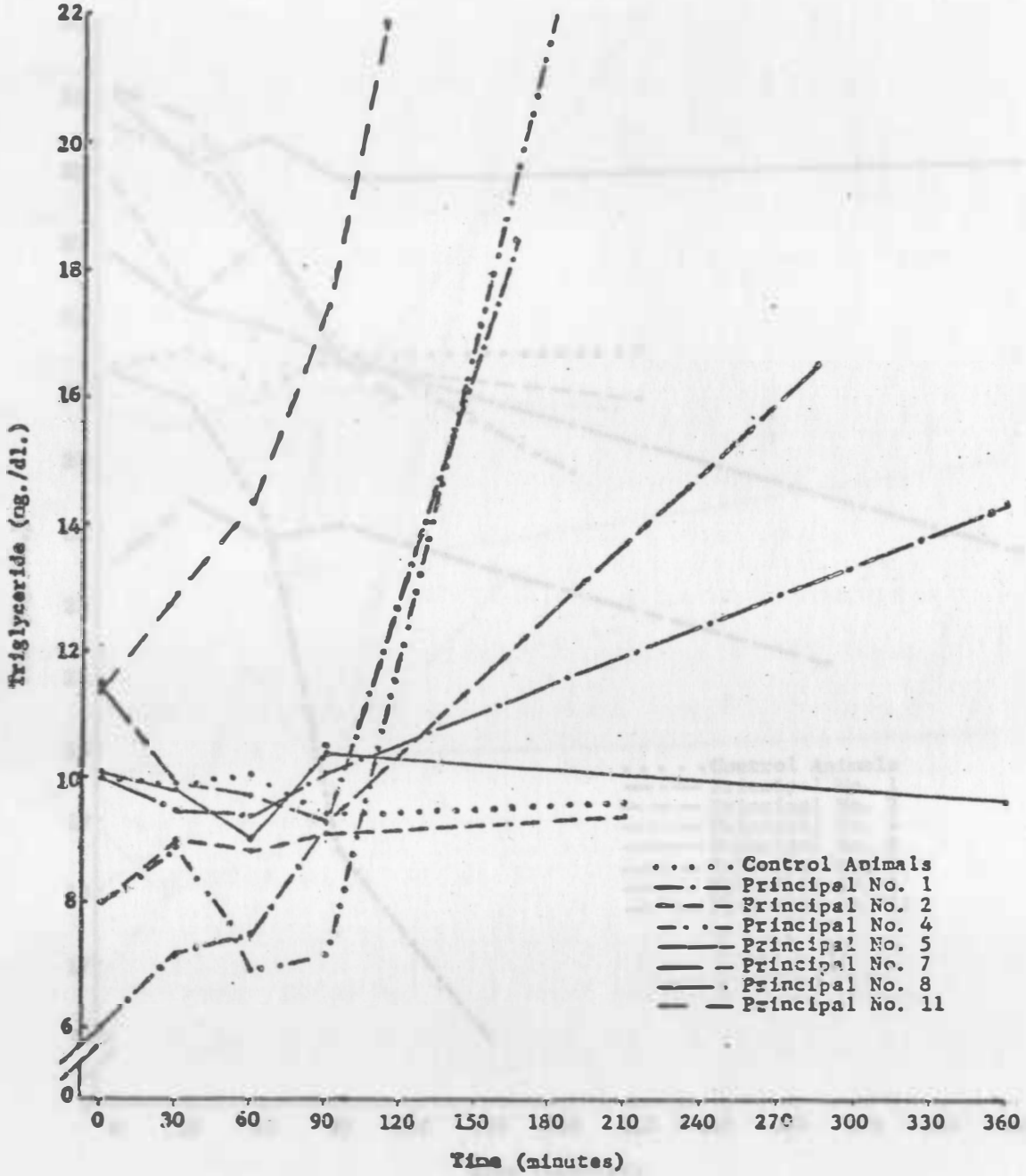

Figure 6. Graphic comparison of blood triglyceride levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

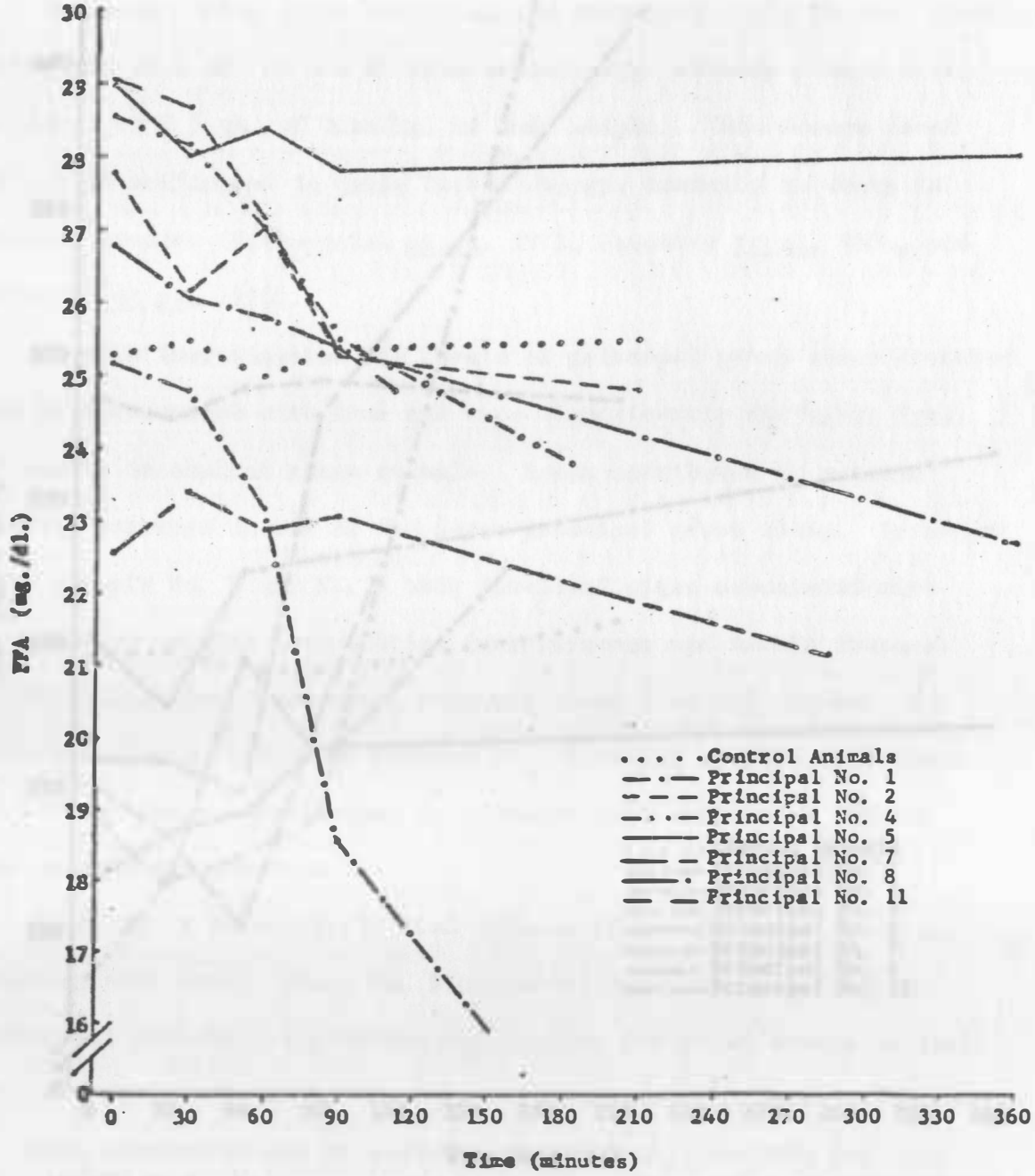

Figure 7. Graphic comparison of blood free fatty acid levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

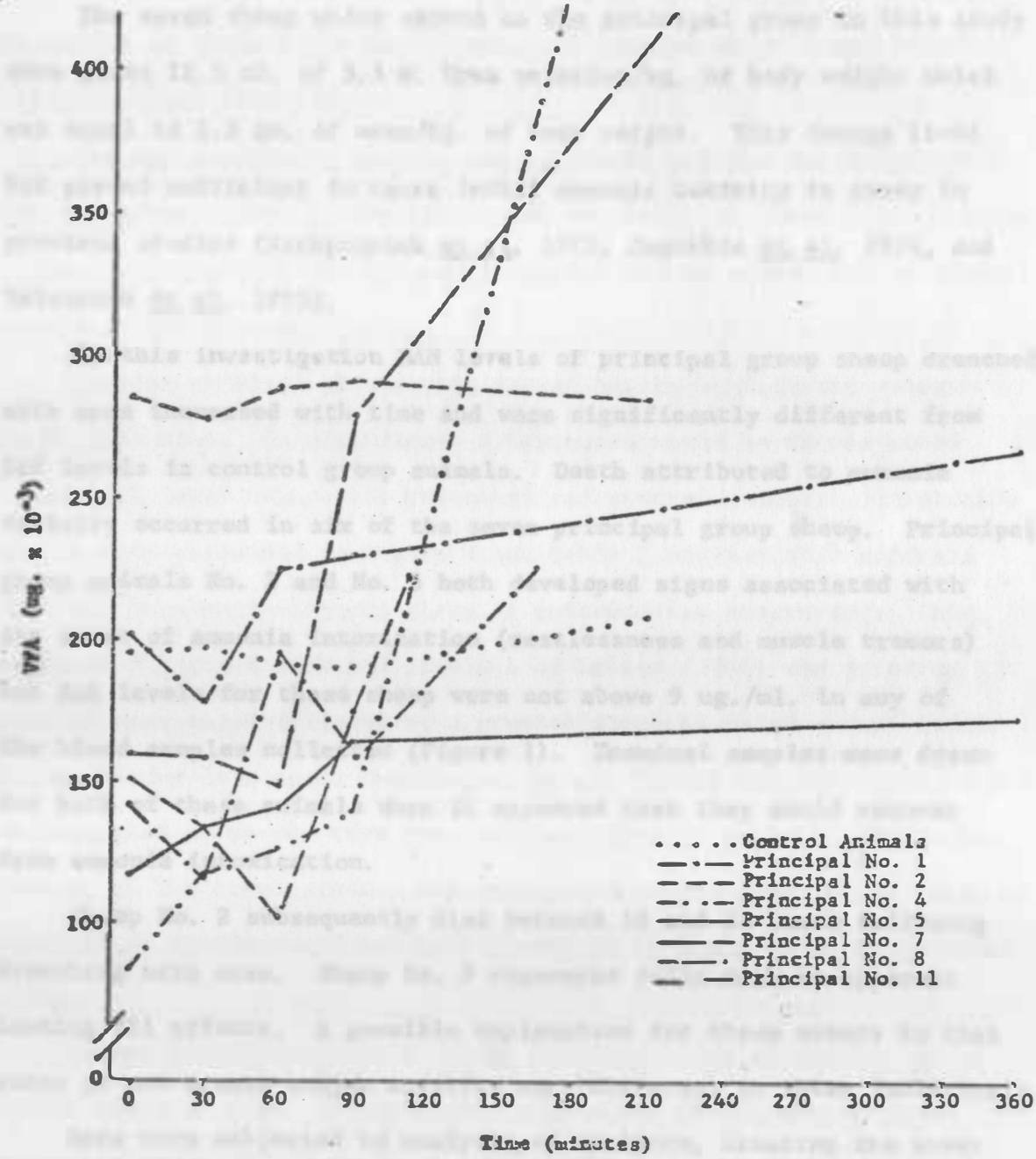

Figure 8. Graphic comparison of blood volatile fatty acid levels in control (average of four animals) and principal sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively. The mg. units represent the total amount of volatile fatty acids recovered from 6 ml. of plasma.

DISCUSSION

The seven sheep which served as the principal group in this study were given 12.5 ml. of 3.3 M. Urea solution/kg. of body weight which was equal to 2.5 gm. of urea/kg. of body weight. This dosage level had proved sufficient to cause lethal ammonia toxicity in sheep in previous studies (Kirkpatrick et al. 1973, Jaquette et al. 1974, and Yelverton et al. 1975).

In this investigation BAN levels of principal group sheep drenched with urea increased with time and were significantly different from BAN levels in control group animals. Death attributed to ammonia toxicity occurred in six of the seven principal group sheep. Principal group animals No. 2 and No. 5 both developed signs associated with the onset of ammonia intoxication (restlessness and muscle tremors) but BAN levels for these sheep were not above 9 ug./ml. in any of the blood samples collected (Figure 1). Terminal samples were drawn for both of these animals when it appeared that they would recover from ammonia intoxication.

Sheep No. 2 subsequently died between 10 and 24 hours following drenching with urea. Sheep No. 5 recovered fully with no apparent lasting ill effects. A possible explanation for these events is that rumen pH and urease enzyme activity were different in these individuals.

Data were subjected to analysis of variance, treating the seven principal sheep as one group. Evaluation of results must, therefore, take into consideration the fact that BAN in two of the principal animals did not reach lethal levels during the experimental period. Terminal

samples for these two animals were not drawn at death. The effect of including data for these animals in the principal group may have been to reduce or obscure the significance of changes which occurred in the parameters measured.

Average values for control and principal animals omitting data for sheep Nos. 2 and 5 were calculated for table 1. This table presents a clearer picture of the changes occurring in the parameters measured due to lethal ammonia levels.

Results of blood pH analysis showed highly significant changes in pH with time. No significant differences could be demonstrated between pH level changes in principal and control animals. Inspection of the data presented in figure 2 and table 1 suggest that acidosis occurred in principal group sheep as intoxication progressed. This response disagrees with the findings of Roller (1966) who noted an initial rise in pH followed by a greater decrease below normal levels before tetany developed. Davidovich et al. (1977) reported that blood pH increased up to the time when initial signs of toxicity developed. Neither of the above studies involved administration of lethal doses of urea and pH trends could not be followed until death as in the present study.

Apland (1969) observed a steady decrease in pH in rabbits given lethal doses of urea. Lloyd (1970) also noted a decrease in pH in intoxication resulting in death. He attributed part of the pH decrease to an increase in lactic acid levels. Findings in the above studies suggest that changes in blood pH may vary depending on the severity of ammonia intoxication. Accumulation of metabolic acids due to the

disruption of normal metabolism could account in part for the decrease in pH. The degree to which normal metabolism is altered could in turn be linked to the severity of ammonia intoxication.

The responses of blood gases to ammonia intoxication were highly significant. Significant differences were also shown to occur between blood gas levels of principal and control groups. General trends seen in figures 3 and 4 and in table 1 suggest that pO_2 decreased while pCO_2 increased in principal animals as death neared. Such trends agree with the findings of Rash *et al.* (1968) and Lloyd (1970) both of whom noted similar results in sheep given lethal doses of urea.

Davidovich *et al.* (1977) observed no change in blood pO_2 and pCO_2 in cattle given sublethal doses of urea. Apparently the increase in pCO_2 and decrease in pO_2 occur close to death from ammonia toxicity. These changes probably result from respiratory difficulty occuring shortly before death. Respiratory problems were noted in this study shortly before death and have been reported by others (Rash *et al.* 1968 and Lloyd 1970).

Blood glucose changes were highly significant relative to time in principal animals. Significant differences in glucose levels were also demonstrated to occur between principal and control animals. Figure 5 and table 1 indicate that glucose levels increased in principal animals as intoxication progressed. This response agrees with the findings of Lloyd (1970) and Singer (1969) who observed high glucose levels in experiments involving acute ammonia toxicity. Chalupa and Opliger (1969) noted increased blood glucose levels in sheep given sublethal doses of urea.

Increased glucose levels noted in this and other studies suggest that high ammonia levels have a dramatic effect on body metabolism. One theory suggests that ammonia toxicity decreases glucose utilization by disrupting the TCA cycle. The TCA cycle is affected by overloading of the urea cycle during ammonia intoxication. Stress caused by ammonia intoxication may also be responsible for altering metabolic pathways. Hepatic glycogenolysis caused by adrenalin release has been cited as a factor which may contribute to high glucose levels. Accumulation of glucose in the blood also suggests that glycolysis is being interrupted.

Triglyceride levels showed highly significant changes relative to time in principal sheep. Differences occurring between principal and control group triglyceride levels were also significant. Figure 6 and table 1 show a rise in triglyceride levels in response to progressing ammonia intoxication. These results are opposite those found by Reideman (1977) who observed an initial decrease and continued depression of triglyceride levels as intoxication progressed in sheep given lethal doses of urea. Stress and the corresponding release of adrenalin might explain an increase in triglyceride levels. Increased triglyceride levels, as in the case of glucose, could indicate disruption of some metabolic pathway. The TCA is common to the utilization of lipids as well as glucose and its disruption could lead to accumulation of many metabolic intermediates.

No significant differences could be demonstrated between free fatty acid levels in principal and control sheep. A significant response was noted with time for FFA levels. Figure 7 and table 1

show the response of principal and control animals to ammonia toxicity. The increase in blood glucose levels associated with ammonia intoxication was earlier suggested to be a possible result of disruption of glucose metabolism. If the necessary metabolic pathways remained intact, it would be logical to suspect that the animal would then convert to lipid metabolism as its primary energy source. This conversion would be reflected by an increase in the FFA levels found in the blood. This hypothesis was not supported by findings of this study, however. The FFA levels in principal animals did not increase and in fact, appear to have decreased slightly. Any decrease in FFA levels would agree with the findings of Chalupa and Opliger (1969) who observed a decrease in FFA levels in sheep given a sublethal dose of urea. A decrease in FFA's would be expected in response to high glucose levels. Insulin release stimulated by the high glucose levels would be the basis for this decrease. Adrenalin release caused by stress would be expected to increase blood FFA levels. Disruption of metabolic pathways, in this case the liver hydrolysis of triglycerides, could again be suggested as a possible explanation for FFA level changes. If blood triglycerides are serving as a primary source of precursors for FFA's an increase in triglyceride levels might be expected to correspond to decreased FFA levels.

Blood VFA levels showed significant changes with time in principal animals. No significant response could be demonstrated when principal and control groups were compared. Figure 8 and table 1 suggest that VFA levels may increase near death in principal animals. Studies by

Chalupa and Opliger (1969) showed that acetic acid levels increased in sheep given sublethal doses of urea. An increase in VFA levels lends further support to the hypothesis that metabolism via the TCA cycle is being blocked.

SUMMARY

The effects of acute ammonia toxicity on certain blood parameters in sheep were investigated. Eleven Southdown sheep were divided into two groups. Seven animals in the principal group were given 12.5 ml. of 3.3 M. urea solution/kg. of body weight. Four control sheep received 12.5 ml. of water/kg. of body weight.

Five blood samples for analysis were drawn from each animal by jugular venipuncture during this study. One sample was drawn prior to dosing, one sample was drawn at 30, 60 and 90 minutes following dosing and a terminal sample was collected at death. Samples were analyzed for blood ammonium nitrogen (BAN), blood pH, pO_2, pCO_2, triglyceride, glucose, free fatty acid (FFA), and volatile fatty acid (VFA).

Blood ammonium nitrogen, triglyceride, glucose, and VFA levels increased while blood pH decreased in principal sheep as toxicity progressed. Levels for these parameters remained near pretreatment levels in control sheep. The pO_2 and FFA levels were lower in terminal samples for principal animals than in those for controls. Terminal sample pCO_2 levels were higher for the principal group sheep than for the control group sheep.

Analysis of variance revealed highly significant changes with time for BAN, blood pH, pO_2, pCO_2, triglyceride, and glucose levels. Significant changes were shown with time for VFA and FFA levels.

Analysis of lines of regression for principal and control groups demonstrated highly significant differences for BAN levels. Significant differences between principal and control groups were shown for blood glucose, triglyceride, pO_2 and pCO_2 levels.

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APPENDIX

Title. Determination of statistically valid equations for lines of regression.

The sum of squares of the quadratic equation was subtracted from the sum of squares of the cubic equation. The difference was subjected to an "F"-test by dividing it by the error mean squares to see if the difference was statistically significant. If not, no validity would be lost by using the quadratic equation. The difference between sums of squares of linear and quadratic equations was treated in the same manner to determine whether validity was lost by using the linear equation in place of the quadratic. If not, the sum of squares of the linear equation was divided by the appropriate error mean squares to determine whether the linear equation was valid.

Table 2.-Analysis of variance for lines of regression of tested parameters with time. Results of "F"-test to determine whether a significant difference existed between control and principal lines of regression are listed in the extreme right-hand column. Principal and control sheep were drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

Basis	Source	Degree of Polynomial tested[+]	d.f.	S.S.	M.S.	F	F‡
BAN							
Principal	X	2	2	324120.94	162060.44	21.32**	
	Residual		32	243213.06	7600.40		20.48**
Control	X	2	2	15342.35	7671.17	1.11	
	Residual		17	117137.63	6890.45		
pH							
Principal	X		1	157305.31	157305.31	12.66**	
	Residual	1	33	410028.69	12425.11		3.14
Control	X		1	624.60	624.60	.085	
	Residual	1	18	131855.38	7325.30		
PCO_2							
Principal	X	1	1	169727.00	169727.00	14.09**	
	Residual		33	397607.00	12048.70		5.86*
Control	X	1	1	19325.52	19325.52	3.07	
	Residual		18	113154.44	6286.36		

Table 2 continued

Basis	Source	Degree of Polynomial tested†	d.f.	S.S.	M.S.	F	F‡
pO_2							
Principal	X	2	2	149479.50	74739.75	5.72**	
	Residual		32	417854.50	13057.95		4.06*
Control	X	2	2	12745.48	6372.74	0.90	
	Residual		17	119734.50	7043.20		
Glucose							
Principal	X	1	1	226378.50	226378.50	21.91**	
	Residual		33	340955.50	10331.98		6.30*
Control	X	1	1	4854.50	4854.50	0.68	
	Residual		18	127625.50	7090.30		
Triglyceride							
Principal	X	3	3	228985.56	76328.50	6.99**	
	Residual		31	338348.44	10914.46		6.91*
Control	X	3	3	13006.86	4335.62	0.58	
	Residual		16	119473.13	7467.07		
FFA							
Principal	X	1	1	89365.00	89365.00	6.17*	
	Residual		33	477969.00	14483.91		2.02
Control	X	1	1	1155.88	1155.88	0.16	
	Residual		18	131324.13	7295.78		

Table 2 continued

Basis	Source	Degree of Polynomial tested+	d.f.	S.S.	M.S.	F	F‡
VFA							
Principal	X	1	1	66828.25	66828.25	4.41*	
	Residual		33	50050.75	15166.84		3.29
Control	X	1	1	7782.98	7782.98	1.12	
	Residual		18	124697.00	6927.61		

* $P < 0.05$
** $P < 0.01$
+ Method for determining statistically valid equations for lines of regression is outlined in the appendix.
‡ Test for homogeneity of regression coefficients for principal and control lines of regression.

Table 3 .-Whole blood ammonium nitrogen levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	μg. NH_4^+-N/ml.										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	2.22	2.00	1.86	1.32	2.75	1.72	2.60	1.64	1.82	1.68	2.37
30 Minutes	2.45	1.77	2.17	1.85	6.70	2.41	7.58	3.59	2.82	1.51	5.14
60 Minutes	1.97	2.13	1.65	1.55	7.72	4.26	9.36	3.68	3.50	3.32	12.23
90 Minutes	1.85	1.96	1.77	1.96	13.16	6.77	11.43	5.50	4.41	4.59	22.82
Terminal*	1.91	1.85	1.68	1.68	18.52	8.76	26.90	3.18	20.82	23.14	29.18

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 4 .-Blood pH values for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	7.44	7.42	7.42	7.43	7.46	7.44	7.47	7.42	7.42	7.40	7.43
30 Minutes	7.42	7.43	7.46	7.46	7.43	7.42	7.50	7.44	7.44	7.39	7.43
60 Minutes	7.44	7.41	7.42	7.44	7.42	7.38	7.45	7.42	7.45	7.38	7.40
90 Minutes	7.44	7.43	7.43	7.44	7.43	7.42	7.43	7.41	7.43	7.38	7.18
Terminal*	7.43	7.42	7.43	7.42	7.14	7.43	7.15	7.39	7.15	7.24	6.94

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 5.-Blood pO_2 levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	mm. Hg.										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	43.0	41.6	41.7	47.2	41.5	41.5	51.0	41.5	39.4	35.5	39.1
30 Minutes	42.4	39.2	42.2	45.3	40.3	45.6	50.1	40.4	42.3	38.3	42.3
60 Minutes	45.1	39.4	44.8	46.0	39.8	46.9	52.3	39.0	42.8	35.4	45.2
90 Minutes	44.5	38.3	43.5	48.1	38.2	44.0	55.4	38.1	42.6	35.3	38.3
Terminal*	45.0	40.7	42.0	46.7	28.0	54.0	47.1	35.3	29.1	30.1	26.8

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 6.-Blood pCO_2 levels for principal and control sheep dranched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

mm. Hg.

	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	40.6	46.0	45.3	43.4	38.0	42.0	42.5	37.4	43.0	45.0	43.5
30 Minutes	43.7	45.1	43.8	43.5	39.1	46.4	43.0	35.8	40.1	44.0	44.6
60 Minutes	39.2	45.0	43.6	41.8	41.6	45.5	42.1	36.1	40.1	40.8	42.8
90 Minutes	42.1	46.2	44.0	40.8	38.0	43.5	44.8	35.1	44.0	40.1	55.5
Terminal*	42.5	44.9	42.1	40.9	57.0	40.8	54.6	47.2	45.1	36.2	62.0

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 7.-Blood glucose levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	mg./dl.										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	77	71	81	80	62	76	83	78	74	76	71
30 Minutes	74	75	78	81	65	83	97	74	75	78	80
60 Minutes	71	73	76	76	87	81	103	85	77	82	139
90 Minutes	71	69	80	75	179	101	142	89	87	91	237
Terminal*	77	72	82	79	236	113	197	76	186	149	274

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 8.-Triglyceride levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	mg./dl.										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	9.5	9.9	8.4	12.0	6.0	8.0	8.0	10.1	11.3	10.0	11.4
30 Minutes	10.3	9.1	8.5	11.7	7.2	9.0	8.9	9.8	9.9	9.5	12.9
60 Minutes	11.0	8.4	3.7	12.3	7.5	8.8	6.9	9.5	9.7	9.4	14.3
90 Minutes	9.0	8.4	9.0	11.0	9.3	9.1	7.2	10.3	10.5	10.1	17.3
Terminal*	8.0	9.3	10.0	10.9	16.5	9.3	22.9	9.7	16.6	14.3	21.8

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 9.-Free fatty acid levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	mg./dl.										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	23.0	24.1	28.1	26.1	25.2	27.8	28.6	29.0	22.6	26.8	29.1
30 Minutes	24.1	25.0	27.5	25.4	24.8	26.2	28.2	28.1	23.4	26.1	28.7
60 Minutes	23.3	24.8	27.1	24.9	23.2	27.1	27.1	28.4	22.9	25.8	27.2
90 Minutes	22.9	25.3	27.9	25.6	18.6	25.3	25.5	27.8	23.1	25.4	25.3
Terminal*	24.0	26.0	27.0	25.1	15.3	24.8	23.8	28.0	20.9	22.7	24.8

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.

Table 10.-Volatile fatty acid levels for principal and control sheep drenched with 12.5 ml. of 3.3 M. urea solution and 12.5 ml. of water/kg. of body weight, respectively.

	mg./6 ml. plasma										
	Sheep Number										
	Control				Principal						
	3	6	9	10	1	2	4	5	7	8	11
Pretreatment	.312	.112	.104	.241	.142	.185	.084	.118	.160	.206	.152
30 Minutes	.281	.132	.154	.215	.116	.177	.117	.134	.159	.179	.133
60 Minutes	.238	.113	.142	.258	.196	.188	.126	.141	.150	.225	.103
90 Minutes	.171	.189	.172	.215	.161	.191	.140	.164	.279	.230	.175
Terminal*	.217	.202	.158	.266	.226	.184	.489	.173	.574	.267	.221

* Terminal samples for control animals were collected at a time which approximated the average time of death of principal animals. Principals numbered 2 and 5 did not intoxicate to the point of death during the experimental period.