UNIVERSITY OF MANITOBA

EFFECTS OF NITROGEN LEVEL AND INDUCED METABOLIC ACIDOSIS ON RENAL FUNCTIONS OF SHEEP

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

by

KATHERINE ANNA MUC

DEPARTMENT OF ANIMAL SCIENCE

WINNIPEG, MANITOBA

1981

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KATHERINE ANNA MUC

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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Effects of Nitrogen Level and Induced Metabolic Acidosis on Renal Functions of Sheep

Katherine Anna Muc

ABSTRACT

Experiments were conducted to study the effects of dietary nitrogen level and imposed metabolic acidosis on renal functions of sheep. Sheep were maintained on low and high nitrogen diets containing 0.78% and 1.62% nitrogen, respectively. In the first experiment, sheep were infused continuously with 0.05N hydrochloric acid intra-ruminally at a rate of 2.10 $ml.min^{-1}$ for four days. In a second experiment, a loading dose of 100 mEq hydrochloric acid was given within two hours while sheep were continuously infused intra-ruminally.

Plasma pH, bicarbonate concentration, and base excess were lower for sheep on the low nitrogen diet after acid infusion. Urinary acid excretion increased and urine pH decreased more quickly for sheep on the low nitrogen diet. Cumulative excretion of net acid could account for 37% and 20% of the total amount of acid infused for low and high treatments, respectively. Excretion of titratable acid reached the level of about 0.020 $mEq.h^{-1}.kgBW^{-1}$ for both treatments. Ammonia excretion increased thirty-fold for low and seven-fold for high nitrogen sheep after acid infusion, representing about 26% and 10% of the rate of infusion into the rumen. Total-N, urea-N, and ammonia-N excreted were lower for sheep on the low nitrogen diet before acid infusion. After infusion, ammonia-N excreted increased and other-N decreased for low nitrogen sheep.

For high nitrogen sheep, urea-N excreted decreased, ammonia-N and other-N excreted increased. Before acid infusion, plasma urea-N was lower, while urea and creatinine U/P ratios, and fraction filtered urea reabsorbed were higher for low nitrogen sheep. No significant effect of acid infusion was found on these parameters.

Creatinine clearance was affected by dietary nitrogen, but not acid infusion. In the second experiment, PAH clearance was not affected by nitrogen level or acid infusion.

It can be concluded that sheep on a low nitrogen diet have a decreased buffering ability in the body fluids. Thus, the greater increase in H^+ concentration for these sheep would stimulate greater excretion of acid by the tubular cells. For the low nitrogen sheep, nitrogen utilized for increased ammonia excretion was from nitrogen normally excreted as other-N. For high nitrogen sheep, urea-N excreted decreased and some nitrogen was rerouted to ammonia production. Dietary nitrogen has some effect on filtration and reabsorption of urea and perhaps on the plasma colloid osmotic pressure. As plasma ammonia-N and glutamine concentration, and PAH clearance did not change significantly during acid infusion, an increased extraction rate of glutamine must occur.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS i

LIST OF TABLES ii

LIST OF FIGURES iii

LIST OF APPENDIX TABLES iv

INTRODUCTION 1

LITERATURE REVIEW

I ACID-BASE BALANCE 2

A. Introduction 2

B. Respiratory vs. metabolic acidosis 3

C. Causes of metabolic acidosis 3

D. General signs of metabolic acidosis 5

II MECHANISMS FOR BUFFERING STRONG ACIDS 5

A. Chemical buffers 5

B. Respiratory compensation 7

C. Renal correction 8

1. Tubular secretion of hydrogen ions 8

2. Titratable acid excretion 11

3. Ammonia secretion 14

Increase in acidosis 14

Precursors of urinary ammonia 15

Pathways of ammonia production 16

Control of ammonia production and its adaptive increase 17

Theories of control 24

Control of ammonia excretion 27

Sites of production of glutamine 30

TABLE OF CONTENTS - cont'd

III NITROGEN METABOLISM IN RUMINANT ANIMALS 31

A. Introduction 31

B. Effects of nitrogen level 32

C. Effect of acidosis 34

EXPERIMENTAL OBJECTIVES. 37

MATERIALS AND METHODS

I EXPERIMENT I 38

A. Animal maintenance 38

1. Animals 38

2. Housing 38

3. Feeding 38

B. Animal preparation 40

1. Vein catheterization 40

2. Urinary bladder catheterization 40

C. Sampling schedule. 41

D. Sampling technique 41

1. Blood sampling 41

2. Urine sampling 42

E. Analytical methods 42

1. Plasma pH, pCO_2, HCO_3^-, base excess 42

2. Urine pH. 43

3. Urine titratable acid and net acid 43

4. Plasma and urine ammonia-N, urea-N, and creatinine 43

5. Total-N 44

F. Statistical analysis 44

TABLE OF CONTENTS - cont'd

II EXPERIMENT 2 45

A. Animal maintenance 45

1. Animals 45

2. Housing 45

3. Feeding 45

B. Animal preparation 46

1. Vein catheterization 46

2. Arterial catheterization 46

3. Urinary bladder catheterization 46

C. Sampling schedule 46

D. Sampling technique 48

E. Analytical methods 48

1. Plasma and urine PAH 48

2. Plasma glutamine 49

F. Statistical analysis 49

RESULTS

I EXPERIMENT I 50

A. Blood acid-base parameters 50

B. Urinary acid excretion 54

C. Plasma urea-N and ammonia-N 62

D. Urinary excretion of nitrogenous constituents . . . 62

E. Urine flow rate, creatinine and urea clearances, U/P creatinine and urea ratios, and filtered urea reabsorbed 64

II EXPERIMENT II 68

A. Blood acid-base parameters 68

B. Urine acid-base parameters 70

C. Arterial plasma ammonia-N and glutamine concentration 72

TABLE OF CONTENTS - cont'd

D. Urine flow rate, creatinine and PAH clearances, and filtration fraction 72

DISCUSSION 76

SUMMARY AND CONCLUSIONS 94

REFERENCES 97

APPENDIX 107

ACKNOWLEDGEMENTS

The author expresses appreciation to Dr. M. E. Seale, previous head of the Department of Animal Science, for the use of the department facilities.

Sincere gratitude is extended to Dr. G. D. Phillips, Professor of Physiology, and present Department head, for his patience, advice, and guidance throughout this study. The assistance of Dr. A. D. Graham during the latter stages of this study is gratefully acknowledged.

Appreciation is extended to Mr. H. Deneer, Mrs. D. Talbot, and Mr. P. Mills for their technical assistance and to my aunt Mrs. J. Kosowan for her typing of this thesis.

Financial support for this study was provided by NSERC grant No. 1440 awarded to Dr. G. D. Phillips.

LIST OF TABLES

TABLE

1.	Metabolic pathways of ammonia formation	18
2.	Composition of diets	39
3.	Blood acid-base parameters before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid	51
4.	Urine acid-base parameters before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid	55
5.	Plasma concentrations of urea-N and ammonia-N before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid	63
6.	Urinary excretion rates of nitrogenous constituents before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid	65
7.	Urine flow rates, creatinine and urea clearances, creatinine and urea U/P concentration ratios, and fraction filtered urea reabsorbed before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid.	66
8.	Blood acid-base parameters taken at 1030 after a saline loading dose, after an acid loading dose and three days later after infusion of a total of 568.75 mEq of acid	69
9.	Urine pH and urinary titratable acid and ammonia excreted before and after acid infusions	71
10.	Arterial plasma ammonia-N and glutamine concentration before and after acid infusions	73
11.	Urine flow rate, creatinine and PAH clearances, and filtration fraction before and after acid infusions	75

LIST OF FIGURES

FIGURE

1. The transient effects of intra-ruminal infusion of hydrochloric acid on plasma pH, bicarbonate concentration and base excess 53

2. The effects of intra-ruminal infusion of hydrochloric acid on urine pH and excretion of acid as net acid in ammonia 58

3. Regression analysis of the relationships between cumulative acid infused and cumulative net acid, titratable acid, and ammonia excreted 61

4. Hypothetical model for the relationship between the cumulative amount of acid infused and the cumulative amount of acid excreted 85

LIST OF APPENDIX TABLES

TABLES

1 - 6	Data on urinary flow rate, pH, titratable acid, ammonia and nitrogenous constituents excreted, for Experiment I	108
7 - 12	Data on blood acid-base parameters, plasma ammonia-N and urea-N concentrations, and creatinine clearances, for Experiment I.	115
13	Data on acid-base parameters of venous blood samples taken at 1030 and on plasma ammonia-N and glutamine of arterial blood samples taken at 1330, for Experiment 2.	122
14	Data on urinary pH, titratable acid and ammonia excreted, urine flow rate, creatinine and PAH clearances, and filtration fraction for the interval 1130 - 1530, for Experiment 2	123

INTRODUCTION

Many biological activities and metabolic processes are significantly influenced by the hydrogen ion concentration or potential in the body fluids. The maintenance of body pH has been placed third on the list of homeostatic priorities, just behind oxygen need and heat dissipation. Therefore, the body is aptly equipped with the body fluid buffer system, the respiratory system, and the excretion of acid or base by the urinary system, which all participate in the regulation of acid-base homeostasis.

Ruminant animals were chosen for the present study because of their added susceptibility to the overloading of organic acids. Clinical conditions, such as acute D-lactic acidosis have been well recognized, but many milder conditions may have a greater economic importance. Metabolic acidosis of a chronic nature can often go undetected in ruminants and can seriously affect their productive efficiency.

The focus of the present research was on the renal functions of sheep during an imposed acid load of several days duration. Two levels of dietary nitrogen were fed to obtain information on the urinary excretion of nitrogenous compounds. Blood plasma samples were analyzed for creatinine, ammonia-N, urea-N, and glutamine. Clearances of creatinine, urea, and para-aminohippurate were also studied.

LITERATURE REVIEW

I ACID-BASE BALANCE

A. Introduction

The hydrogen ion concentration of the extracellular fluids is one of the most vigorously regulated variables of the body. A relatively constant pH is maintained by a balance between acids and bases. According to the Lowry-Bronsted scheme, an acid is a compound that tends to donate a hydrogen ion (proton) to a base. A base is a substance that will accept and bind a hydrogen ion. Body fluids contain a variety of buffers capable of partially neutralizing acids and bases, and transiently preventing wide fluctuations in pH (Rector, 1973). Thus measurement of both pH and buffer capacity is necessary to access the acid-base status of the body fluids.

Under normal conditions the pH of arterial blood plasma of mammals is regulated within a range of pH 7.35 to 7.45, despite continual addition of acids and bases from the diet and from metabolism. A depression of blood pH below the normal range is known as acidemia, while an increase above the normal pH range is called alkalemia (Houpt, 1977). In general, the terms "acidosis" and "alkalosis" are used to describe these deviations from normal pH.

Acid-base balance is attained through the operation of chemical buffering and physiological correcting mechanisms. The chemical buffers of plasma, interstitial fluid, tissues, and bone absorb the immediate shock of liberation of acid or base in the body. The physiological correcting mechanisms which include the respiratory and urinary systems,

then come into effect. The respiratory system by eliminating carbon dioxide, regulates the partial pressure of CO_2 and therefore determines the concentration of carbonic acid in the body fluids. The kidneys correct hydrogen ion imbalances by excreting excess acid or base.

B. Respiratory vs. metabolic acidosis

The present study is concerned with the condition of "metabolic" acidosis. To avoid confusion, the distinction between "metabolic" and "respiratory" acidosis will be briefly outlined.

Ganong (1977) states that respiratory acidosis is due to increase in the carbon dioxide content of the body, caused by, for example, decreased ventilation. It is characterized by a rise in pCO_2 representing a rise in carbonic acid, and by a decrease in pH.

The term metabolic acidosis describes the condition in which the gain of strong acid to or the loss of base from the extracellular fluid occurs (Houpt, 1977). Bicarbonate concentration falls and pH is decreased. Metabolic acidosis refers to acidosis caused by any factor other than excess CO_2 in the body fluids.

Guyton (1976) used the following distinction: carbonic acid resulting from dissolved CO_2 is called a "respiratory acid", while any acid formed by metabolism or by ingestion is a "metabolic acid".

C. Causes of metabolic acidosis

The formation of acids in the body is generally the result of metabolic reactions involving substances taken into the body through the alimentary tract. Most organic compounds are catabolized forming water, and CO_2 which is expired. If this process does not go to completion,

organic acids such as lactate, pyruvate and aceto-acetate accumulate in the body fluids (Elkington, 1962). Other sources of hydrogen ions from foods are the oxidation of sulfur-containing amino acids and the oxidation and hydrolysis of iso-electric phosphoprotein residues. McCance and Widdowson (1942) observed that foods rich in protein and hence sulfur (meat, fish, eggs, cereals) are acid-forming, whereas foods relatively low in protein (fruits, vegetables) are base-forming. Thus, foods consumed may have a predisposing effect towards acidosis. This is especially evident in the case of ruminant animals with the complex fermentation system in the rumeno-reticulum. The tendency towards metabolic acidosis is low in ruminants consuming pasture forages which are relatively slowly fermented and contain abundant alkaline materials (Kronfeld, 1976). However, an abrupt change in the diet from hay to concentrates rich in readily fermentable carbohydrates (sugars and starches), results in a condition known as lactic acidosis (Uhart and Carroll, 1967). Lactic acidosis is characterized by depressed blood and rumen pH which are consequences of the excessive levels of lactic acid produced in the rumen (Elam, 1976).

Guyton (1976) discussed a number of disease conditions which can also cause metabolic acidosis. Diarrhea results in excessive loss of sodium bicarbonate added to digesta by gastrointestinal secretions. Duodenal vomiting has a similar effect. Diabetes mellitus and ketosis, conditions in which essentially no carbohydrates are metabolized, results in fat being utilized for energy and β-hydroxy-butyric and acetoacetic acids are formed in large quantities. This causes depletion of bicarbonate stores and metabolic acidosis. Morris (1979) reviewed studies done on the condition of renal tubular acidosis, a clinical syndrome of

disordered renal acidification when even normal amounts of formed acids are not disposed of by the kidneys.

D. General signs of metabolic acidosis

Slight decreases in blood pH over a long period are characterized by anorexia, nausea, diarrhea, and dullness (MacKenzie, 1967). Respiration may be deep and sighing, or forced and distressed (dyspnea). Kronfeld (1976) cited more severe signs such as lethargy, and a fall in cardiac output; eventually convulsions, coma, and death.

II MECHANISMS FOR BUFFERING STRONG ACIDS

A. Chemical buffers

Harvey (1974) defined a buffer as a substance which in solution acts to minimize a change in pH if strong acid (or alkali) is added. A buffer system consists of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid and is related to pH by the Henderson-Hasselbalch equation shown below:

$$pH = pK + \log\frac{[base]}{[acid]}$$

Buffer systems are most effective at a pH equal to the pK of its weak acid, and a range of ± 1 pH unit from this pK value (Houpt, 1977). The protective function of a buffer system is limited by the amount of base available to be used up in reaction with increasing amounts of hydrogen ions.

Van Slyke and Cullen (1917) reported that in a dog infused with

one normal sulfuric acid, whole blood bicarbonate buffered one-sixth of the load of acid. Subsequently, the responses of blood to acid-base imbalances have been well worked out both qualitatively and quantitatively. Schwartz et al. (1954)showed that if acid is infused intermittently for 45 minutes and a period of recovery of two hours is permitted before the next acid load is administered, buffering by extracellular bicarbonate is predominant during the infusion, whereas cellular buffering partially restores the plasma bicarbonate level during each recovery interval. Swan and Pitts (1955) infused 180 mEq of hydrochloric acid to nephrectomized dogs, and studied the mechanisms of buffering. Plasma pH decreased from 7.40 to 7.10. One percent of the acid load was buffered by plasma proteins according to the following equation:

$$Na^+, Pr^- + H^+, Cl^- \longrightarrow Na^+, Cl^- + H^+, Pr^-$$

Extracellular bicarbonate, including plasma and interstitial fluid moieties buffered 42% of the acid as follows:

$$Na^+, HCO_3^- + H^+, Cl^- \longrightarrow Na^+, Cl^- + H_2CO_3$$

Cellular buffering accounted for the remaining 57% of the acid load. Of this 57%, six percent of the infused acid was buffered within cells by hemoglobin or by the exchange of extracellular chloride for intracellular bicarbonate. Exchange of cellular potassium (15%) and sodium (36%) for hydrogen ions across the cell membranes of all tissues accounted for the remaining cellular buffering. In this way large stores of intracellular protein and organic phosphate complexes become available for the buffering of extracellular hydrogen ions. Tobin (1956) infused nephrectomized cats with hydrochloric acid in loads of from 3.5 - 9.6 $mEq.kg^{-1}$ body weight. Extracellular moderation of the acidosis was

proportioned as follows: 35% by Na and 5% by K entering the extracellular space, and 20% by Cl and 24% by CO_2 leaving the extracellular space. Direct muscle analysis showed a fall in intracellular Na in response to extracellular acidosis. Yoshimura et al. (1961) carried out similar studies on dogs with normal renal function and observed changes in the sites of acid buffering with the passage of time following acid administration. On the day of administration, the acid was buffered essentially as found by Swan and Pitts (1955). One day later, about one quarter of the acid had been excreted in the urine, the rest being buffered within cells or by bone. Over the succeeding 2 - 6 days, this acid was gradually returned to the extracellular fluid and excreted in the urine. Lemann and Lennon (1972) reviewed the role of bone in acid-base homeostasis. They found that thinning of the bones and a loss of bone carbonate along with negative calcium balance occurs during metabolic acidosis in animals.

In summary, from the research of Van Slyke and Cullen (1917), Swan and Pitts (1955), Schwartz et al. (1954) and Tobin (1956), the blood buffered only one-fifth of an imposed acid load. The buffers in the interstitial fluid, and buffers of cells and bone rapidly assumed most of the load.

B. Respiratory compensation

Guyton (1976) discussed the role of the respiratory system in the buffering of strong acids. Carbon dioxide formed in the reaction of hydrogen ions with bicarbonate ions as shown below:

$$H^+ + HCO_3^- \longrightarrow H_2CO_3 \longrightarrow CO_2 + H_2O$$

is expired. Upon addition of acid, the $HCO_3^-/s \cdot pCO_2$ ratio decreases

(where $s \cdot pCO_2$ equals the concentration of dissolved CO_2 which is proportional to true H_2CO_3) as more H_2CO_3 is formed and pH decreases. However, the increase in CO_2 and decrease in pH stimulate the respiratory center in the medulla oblongata, causing an increase in the rate of pulmonary ventilation. This increase of as much as five times the normal rate causes expiration of the extra CO_2 formed. Over a period of hours, the ratio of $HCO_3/s \cdot pCO_2$ is nearly normal, but the amounts of each component will remain subnormal, until the kidneys correct the problem.

C. Renal correction

1. Tubular secretion of hydrogen ions

Hypobasemia caused by metabolic acidosis requires renal corrective action by the excretion of H^+ and the restoration of depleted bicarbonate reserves. Early micropuncture studies on kidneys indicated there was some spatial separation of the various processes involved in the acidification of urine (Rector, 1973). Most filtered bicarbonate appeared to be absorbed in the proximal tubule by an isohydric process, whereas less was absorbed in the distal tubule of amphibia (Montgomery and Pierce, 1937). More recent micropuncture experiments (Glabman et al., 1963; Vieira and Malnic, 1968; Malnic and Vieira, 1972) however, have demonstrated that a hydrogen ion gradient exists from blood to tubular lumen under practically all experimental conditions. All segments of the nephron appear to reabsorb bicarbonate, lower tubular fluid pH, generate titratable acid, and add ammonia. The detailed mechanisms whereby this proton secretion is accomplished have remained controversial.

There were three early theories to account for acidification of

the urine. According to the phosphate reabsorption theory proposed by Peters and Van Slyke (1932) an ultrafiltrate of plasma containing Na_2HPO_4 and NaH_2PO_4 in a ratio of 4:1 is formed at the glomeruli. The dibasic phosphate is presumed to be reabsorbed by the renal tubules, whereas monobasic phosphate is excreted and constitutes the titratable acid of the urine. The carbonic acid filtration theory (Sendroy et al., (1934) adds that the glomerular ultrafiltrate contains $NaHCO_3$ and H_2CO_3 in a ratio of 20:1, as well as phosphate. Sendroy et al. (1934) assumed that renal tubules are completely impermeable to carbonic acid and the following reaction:

$$Na_2HPO_4 + H_2CO_3 \longrightarrow NaHCO_3 + NaH_2PO_4$$

is forced to the right by the reabsorption of $NaHCO_3$. The tubular ionic exchange theory (Smith, 1937) differs from the previous ones in that a tubular secretory mechanism is responsible for the elimination of acid, rather than a filtration-reabsorption mechanism. The significant constituents of the glomerular filtrate are mono-and dibasic phosphate. In the passage of filtrate down the tubules, H^+ ions derived from H_2CO_3 produced in tubular cells, are exchanged for Na^+ ions, converting dibasic phosphate to the monobasic form, and base is returned to the blood. Pitts and Alexander (1945) demonstrated in support of this theory that acidotic dogs infused with large amounts of phosphate excreted as much as 0.380 $mEq.min^{-1}$ of titratable acid. Because the quantity of acid excreted far exceeded the quantity of acid filtered through the glomeruli, acid must have been added to the filtrate by a cellular mechanism. The tubular ionic exchange theory supported by many investigations (Berliner, 1952; Rector et al., 1960, 1965; Vieira and Malnic, 1968; Rector, 1973) is

generally accepted as the mechanism for acidifying the urine.

The cellular mechanisms involved in hydrogen ion secretion have also been studied extensively. Malnic and Giebisch (1972) reviewed the available literature and concluded that a hydrogen ion pump-leak system represents the tubular transfer mechanism of hydrogen ions. The hydrogen ion transfer system was set by a balance between 1) a secretory hydrogen pump whose rate, governed by the intracellular hydrogen ion concentration, remained constant in a given experimental condition, and 2) a passive leak component from the lumen which was proportional to the luminal pH. The H^+ pump is tentatively thought to be located at the luminal membrane (Rector, 1973). Pumping of H^+ may be coupled directly or indirectly to Na reabsorption, however in a review by Al-Awqati (1978), the evidence for Na:H exchange was not compelling. Al-Awqati (1978) suggested that H^+ secretion in the proximal tubule is a passive exchange dependent on both the Na^+ and H^+ concentration gradient. Studies by Al-Awqati et al. (1975) and Sebastian et al. (1977) indicated that aldosterone has a direct stimulatory effect on H^+ transport. Malnic (1974) suggested that the hydrogen ion exchange mechanism was fed by a H^+ pool, which appears to be replenished by several processes working in parallel: catalyzed and uncatalyzed hydration of CO_2, back-diffusion of carbonic acid from the lumen, and uptake of H^+ from the peritubular space.

Hydrogen ion secretion can be limited by insufficient delivery of H^+ to the cellular pool, or by the transport capacity of the H^+ pump or by reduction of peritubular H^+ concentration (Malnic, 1974).

Quantitatively, about 84% of all the hydrogen ions are secreted in the proximal tubules (Guyton, 1976). This high secretory rate

appears to be due to the presence of carbonic anhydrase in the brush border of proximal tubule cells which dissipates excess H_2CO_3 and prevents the development of limiting pH gradients (Warnock and Rector, 1978). Thus, the proximal tubule secretes large quantities of hydrogen ions against a gradient of about 0.4 - 0.5 pH units, while the collecting ducts secrete hydrogen ions in lesser amounts against a gradient of about three pH units (Pitts, 1976).

Pitts et al. (1949) demonstrated that human subjects under conditions of moderately severe acidosis (HCO_3^- = 13.4 $mM.l^{-1}$; blood pH = 7.27) reabsorbed over 99.9% of the filtered bicarbonate. This reabsorption appeared to be complete at plasma bicarbonate levels below 24 $mM.l^{-1}$, while above 28 $mM.l^{-1}$ gross excretion occurred. Similarly, Schloeder and Stinebaugh (1977) found in fasting humans receiving 160 $mEq.day^{-1}$ of NH_4Cl, that maximum net acid values occurred when serum bicarbonate reached its lowest state, 10.3 - 15.2 $mEq.l^{-1}$. Scott (1972) found that excretion of bicarbonate in the urine of calves fed roughage ranged from 48 - 163 $mEq.day^{-1}$. After intra-ruminal infusion of 0.15 - 0.20 M acid, bicarbonate in urine fell rapidly to values of 1 - 2 $mEq.day^{-1}$. The relationship between urine pH and bicarbonate excreted appeared to be identical to that reported for sheep (Scott, 1969), dog (Berliner, 1952), and rat (Rector et al., 1960, 1965).

2. Titratable acid excretion

The principal buffer in the tubular fluid besides bicarbonate, is the phosphate buffer. Excess hydrogen ions entering the tubules combine with Na_2HPO_4, forming NaH_2PO_4, which passes into the urine. The sodium ion released is reabsorbed and combines with the bicarbonate ion formed

inside the cell to release sodium bicarbonate into the extracellular fluid (Guyton, 1976).

Pitts (1945) discussed factors which determine the rate of titratable acid excretion. These factors are: (1) the rate of excretion of buffer, (2) the acid strength of the buffer (pK'), and (3) the degree of acidosis as reflected by the bicarbonate content of the plasma. Pitts et al. (1948) found that the induction of acidosis (HCO_3^- = 14.2 mM.l^{-1}) by the infusion of phosphate or creatinine greatly increased the rate of excretion of titratable acid. Since the reaction of the urine is limited to an acidity no greater than pH 4.5, it follows that the stronger the buffer acid (pK' < 4.5), the less completely can the renal tubules exchange hydrogen ions for ions of fixed base bound by that buffer in tubular urine. Schiess et al. (1948) demonstrated that secondary phosphate (pK'= 6.8), the weakest acid buffer yields the greatest proportion of base in exchange for hydrogen ions; creatinine (pK'= 4.97), beta-hydroxybutyrate (pK' = 4.70), and p-aminohippurate (pK' = 3.83), stronger acids, yield the least proportion of base. At any given rate of excretion of phosphate, the more severe the acidosis, the greater is the excretion of titratable acid, and the more nearly the kidney approaches the theoretical maximum rate of excretion of acid, (Pitts, 1945). Sartorius et al. (1948) found that the excretion of phosphate increased from initial levels of 41 mM.day^{-1} to 60 mM.day^{-1} on the third day for humans ingesting 15 g of $NH_4Cl.day^{-1}$. Pollak et al. (1965) subjected dogs to a constant acid load of 112 mEq $NH_4Cl.day^{-1}$. The excretion of titratable acid increased from an average of 3.6 to 17.4 mM.day^{-1}. In sheep the infusion of HCl intravenously over two hour periods has been reported to have only marginal effects on phosphorous excretion

(Clark et al., 1968). Scott (1969) found that in sheep excretion of phosphorous, as $H_2PO_4^-$ contributed less than 5 mmole.day^{-1} toward acid excretion, although intra-ruminal infusion rates varied from 70 to 200 mmoles.day^{-1}. In a later study Scott et al. (1971) studied calves consuming either a roughage or concentrate diet and found that phosphorous excretion was related to the type of diet when intra-ruminal acid was infused. Calves fed on roughage were able to increase excretion of phosphorous from 6% of the daily phosphorous intake to 13% during acidosis, so that it contributed up to 25% of the acid excreted. However for calves fed on concentrates, the urinary excretion of phosphorous amounted to about 27% of the total phosphorous intake and infusion of acid did not further increase this value. Scott (1972) found that sheep similarly excreted large amounts of phosphorous in the urine, while concentrate diets were fed regardless of whether acidosis existed. Scott (1972) suggested that the difference between the amounts of phosphorous excreted on roughage and concentrate diets was not due to phosphorous intake, acid excretion, or salivation rate, but rather was due to differences in phosphorous reabsorption by the kidney, related in some way to the observation that more is absorbed from the gut when concentrates are fed. In general it appears that phosphorous excretion is not a major route of acid excretion in sheep during acidosis. Phillips (1968) determined the rate of excretion of titratable acid in sheep to be 0.02 - 0.03 mEq.min^{-1} even though the acid infused intravenously increased by 1.6 mEq.min^{-1}. Williams and Pickering (1980) induced mild acidosis in sheep (HCO_3^- = 19.4 mmoles.l^{-1}) and found an increase in phosphate excreted from three to eight μmol.min^{-1}. In summary, excretion of titratable acid is largely as monobasic phosphate and excretion

rate is largely limited by the phosphate content of the diet but in acid stress moderate amounts of phosphate may be withdrawn from stores in bones and cells (Pitts, 1976). However, ruminant animals do not appear to excrete large amounts of phosphorous and its regulation is different from that in monogastrics (Young et al., 1966; Scott, 1972).

3. Ammonia secretion

Increase in acidosis. Ammonia is a buffer that is potentially available in much larger amounts than phosphate, theoretically equivalent to that of the nitrogen derived from protein metabolism (Pitts, 1976). Under normal conditions, most unused dietary nitrogen is excreted as urea, however in acidosis, the nitrogen from amino acids deaminated in the liver can be converted in increased amounts to ammonia precursors rather than to urea (Pitts, 1976). Pitts (1964) estimated that in normal humans, ammonia excretion accounted for 2/3 - 3/4 of the hydrogen ions excreted, while only 1/3 - 1/4 were eliminated as titratable acid. Scott et al., (1971) found this to be true of calves fed roughage and infused with acid intraruminally. Within a few days of the start of acid infusion, the excretion of acid phosphate contributed up to 25% of the acid excreted, and ammonium ions the remainder. Scott (1969) continuously infused solutions of 0.15M HCl intra-ruminally to sheep for periods of up to 12 days at rates varying from 70 to 200 m moles.day^{-1} and found that over 90% of the net acid excreted was as ammonium ions. Net acid excretion per day rose until it about equalled the acid infused per day. This type of response was also shown in dogs (Pollak et al., 1965). Dogs were given 112 mEq of NH_4Cl daily for seven days. Dogs excreted an average of 20 mmoles of ammonia per day

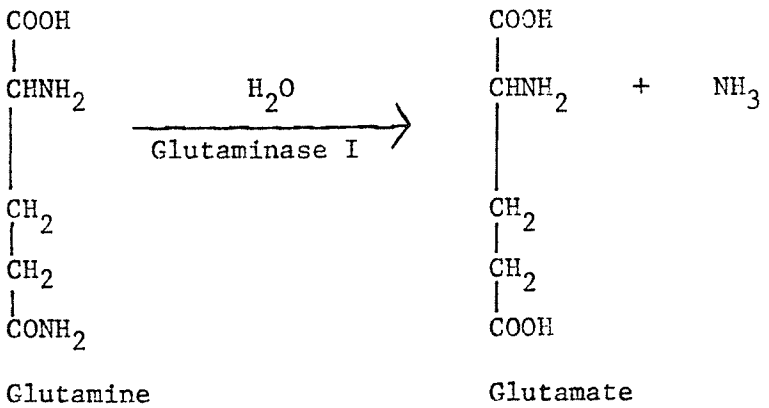
under normal metabolic conditions; the excretion increased to 78 mmoles. day^{-1} in metabolic acidosis. Net H^+ excretion reached 93.5 mmoles.day^{-1}, almost equal to the amount of acid given daily. The importance of ammonia secretion in long term acidosis is evident.

Precursors of urinary ammonia. Nash and Benedict (1921) demonstrated that the renal tubular cells synthesized ammonia and secreted it into the tubular urine. They observed that the concentration of blood ammonia was low, and remained unchanged during acidosis although the excretion of ammonia was much increased. The quantity of ammonia excreted exceeded the quantity filtered in the glomeruli. They concluded that ammonia must be formed in the kidney from some precursor in the blood. Van Slyke et al. (1943) determined that the precursor was plasma glutamine. The amide nitrogen of circulating plasma glutamine was removed in much greater amounts than appeared in the urine. This extraction could account for all of the ammonia removed from the kidney by the renal vein and for two-thirds or more excreted in the urine. A study on acidotic dogs by Shalhoub et al. (1963) corroborated this point. Samples of arterial and renal venous plasma were collected simultaneously from 20 dogs made acidotic by ingesting 10 g NH_4Cl for three days and the concentrations of 23 free amino acids were measured following chromatographic separation. It was found that the extraction of glutamine plus asparagine from renal blood plasma in metabolic acidosis greatly exceeded the extraction of other α-amino acids. Pitts (1976) summarized the data obtained from 36 experiments using amino acids labelled with isotopic nitrogen to determining from which amino acids ammonia was formed. The major proportion of the total ammonia pool (43.3%) was derived from the amide nitrogen of glutamine and an additional 18% was derived from the amino nitrogen. Alanine,

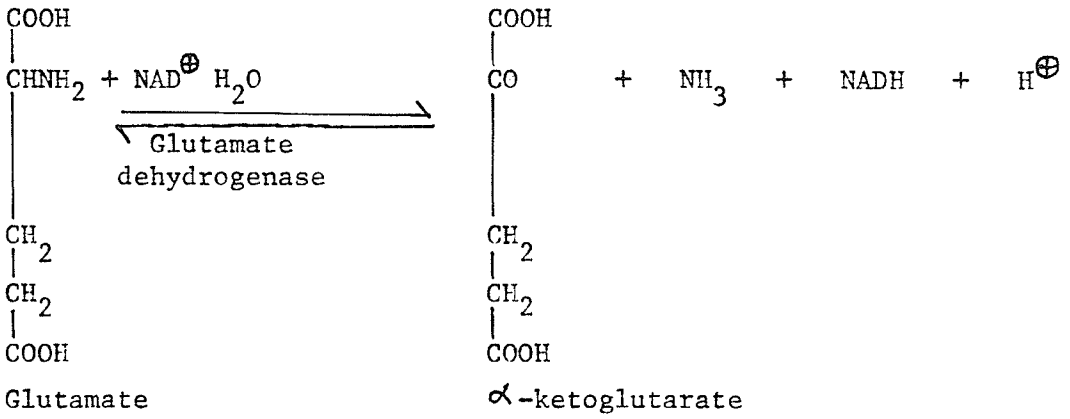
glycine, and glutamic acid accounted for 12% of the ammonia; the remaining 35% was derived from preformed arterial ammonia.

Pathways of ammonia production. Glutamine is an important nitrogen and carbon carrier released into the circulation from muscle and liver, and used by the liver and kidneys for ureogenesis, ammoniagenesis, and gluconeogenesis, depending on the physiological state of the animal, (Heitman and Bergman, 1978). Enhanced renal uptake of glutamine accounted for the increase in ammonia formation in metabolic acidosis (Addae and Lotspeich, 1968). The pathways involved in ammonia formation as shown in Table 1, will be outlined briefly. For a more complete understanding, the reviews by Hems (1974) and Tannen (1978) are recommended.

The major pathway of degradation in the kidney involves the deamidation of glutamine to glutamate (glutaminase I reaction), followed by the formation of α-ketoglutarate (glutamate dehydrogenase reaction) as shown below:



Glutamine Glutamate



Both these reactions occur within the mitochondria and glutamine must first be transported across the mitochondrial membrane. α-Ketoglutarate can be completely degraded via the Krebs cycle giving the following overall reaction:

$$\text{Glutamine} + 4\ 1/2\ O_2 \longrightarrow 2\ \text{ammonia} + CO_2 + 2\ H_2O$$

There are two isoenzymes of glutaminase I: phosphate- dependent glutaminase (PDG) and phosphate-independent glutaminase (PIG), PIG is entirely extramitochondrial and also reflects the glutaminase activity of γ-glutamyl transpeptidase (γ-GT). Glutamine can also be metabolized by the glutaminase II pathway in the cytosol, which involves transamination and deamidation of glutamine and α-ketoglutarate. The kidneys of the rat, rabbit, guinea pig, and sheep, but not those of the dog, man, pig, or cat contain glutamine synthetase which converts glutamate to glutamine (Pitts, 1973).

Control of ammonia production and its adaptive increase. Ammonia production and glutamine utilization are considerably increased in chronic metabolic acidosis, an adaptive phenomenon for which no adequate explanation is available. This area has been the subject of intensive investigation.

Table 1. Metabolic pathways of ammonia formation.

Substrate	Enzyme	Intracellular location	Reaction
Glutamine	Phosphate-dependent glutaminase I (PDG)	Mitochondria	Glutamine $\xrightarrow[\text{PDG}]{NH_3}$ Glutamate
	Phosphate-independent glutaminase I (PIG) and γ-glutamyl transpeptidase (γ-GT)	Cytosol	Glutamine $\xrightarrow[\text{PIG}]{NH_3}$ Glutamate Glutamine $\xrightarrow[\gamma\text{-GT}]{NH_3}$ Glutamylpeptide
	Glutaminase II [Glutamine keto-acid amino transferase (GKA) and ω-amidase]	Cytosol	Glutamine $\xrightarrow[\text{GKA}]{\text{keto acid}\ \rightarrow\ \text{amino acid}}$ α-ketoglutaramate α-ketoglutaramate $\xrightarrow[\omega\text{-amidase}]{NH_3}$ α-ketoglutarate
Glutamate	Glutamine synthetase (GS)	Cytosol	Glutamate $\xrightarrow[\text{GS}]{NH_3,\ ATP}$ Glutamine
	Glutamate dehydrogenase (GDH)	Mitochondria	Glutamate $\xrightarrow[\text{GDH}]{NH_3}$ α-ketoglutarate

Most researchers have believed that the cellular production of ammonia was the major determinant of its rate of excretion. Researchers investigated the possibility that the functional activity of the ammonia producing enzymes was increased as a consequence of some alteration in the intracellular milieu. Davies and Yudkin (1952) ascribed the adaptive increase in ammonia production during chronic acidosis in rats to an increase in the renal content of the enzyme, glutaminase I. Rector et al., (1955) found that the administration of a constant NH_4Cl load resulted in a progressive, stepwise increase to a plateau in the activity of renal glutaminase, from 650 to 3000 units.g^{-1}, which was closely paralleled by a similar increase in ammonia excretion. The adaptation of renal glutaminase was proportional to the administered acid load, but was independent of the concentration of serum bicarbonate. Seyama et al. (1971) also demonstrated increased activity of phosphate-dependent glutaminase in the kidneys of rats. Others (Rector and Orloff, 1959; Pollak et al., 1965) could not demonstrate an increase in renal glutaminase activity in acidotic dogs. Goldstein (1965) gave Actinomycin D to acidotic rats and prevented enzyme adaptation but did not prevent increased ammonia production. Thus, it appeared that an increased glutaminase content was not obligatory to increased production of ammonia. The role of glutamate dehydrogenase in the adaptive increase of ammonia production was reviewed by Schoolwerth et al. (1977). Under normal conditions, mitochondrial ammonia production occurred exclusively by glutamine deamidation and glutamate dehydrogenase was relatively inactive as judged by a negligible flux through GDH. During acidosis, GDH flux increased markedly and accounted for 50% of the increased ammonia

produced. Schoolwerth et al., (1978) showed that the increased flux was due to provision of more glutamate from glutamine deamidation, new enzyme formation, and by some change in the mitochondrial environment.

The cellular contents of various reaction products were examined as possible regulations of ammonia production. The renal cellular concentration of ammonia could exert some control over ammonia production. Canessa-Fischer et al. (1963) infused ammonium lactate into one renal artery of anesthetized dogs in acidosis. This infusion resulted in a prompt increase in ammonia excretion restricted to the side infused. The pNH_3 of the renal arterial blood must have been greatly elevated causing an increase in the diffusion gradient from peritubular blood to urine. Thus 50% of the infused ammonia appeared as additional ammonia in the urine (Canessa-Fischer et al., 1963). These findings differed from those of Pilkington et al., (1965), who found that increased blood and cellular pHN_3 due to infusion of ammonium chloride decreased cellular production of ammonia. Sayre and Roberts (1958) found that ammonia inhibited the glutaminase I reaction competitively with glutamine in vitro. However Goldstein and Schooler (1967) later determined that the concentrations Sayre and Roberts (1958) used were not compatible with effects observed in vivo.

Tannen (1978) reviewed the literature on ammonia metabolism and maintained that glutamate metabolism was a focal point in the regulation of ammoniagenesis, since glutamate is an end-product inhibitor of PDG. Renal tissue concentrations of glutamate were diminished in both acute and chronic acidosis suggesting an accelerated rate of glutamate disposal

(Hems and Brosnan, 1971; Goldstein, 1975). Goldstein and Schooler (1967) found that metabolic acidosis produced a rapid decrease in renal glutamate concentration in the rat kidney, a decrease of 25% within four hours of administration of NH_4Cl. This fall paralleled, inversely, the rise in renal ammonia excretion during the initial phases of metabolic acidosis. There was a further 10% decrease in renal glutamate concentration over the next 18 hours of acidosis, but the concentration of total glutamate did not decrease after that, even though ammonia excretion continued to rise for the next few days. Goldstein (1975) suggested that the acid-induced drop in glutamate concentration might play a role in the initial phase of adaptation by partially releasing glutaminase from end-product inhibition.

Balagura-Baruch et al. (1970) studied the substrate α-ketoglutarate as a controlling factor in ammonia production. They found that the concentration of α-ketoglutarate was low in acidosis in comparison to that found under normal acid-base conditions. Infusion of α-ketoglutarate intravenously into dogs in chronic metabolic acidosis decreased renal ammonia output and increased renal concentrations of α-ketoglutarate and glutamate. Balagura-Baruch et al. (1970) supported the postulate that acidosis increased renal ammonia production because of increased utilization of α-KG, lowered renal level of α-KG and glutamate, and deinhibited glutaminase I activity. Goldstein et al. (1977) found that α-ketoglutarate was a potent inhibitor of mitochondrial glutamine transport and could therefore decrease its rate of utilization.

Yablon and Relman (1977) discussed the role plasma levels of

glutamine might play in the adaptive increase in ammonia production. Yablon and Relman (1977) imposed chronic acidosis (9 days of NH_4Cl feeding) in rats and found that ammonia production increased 5 - 10 fold. Plasma glutamine concentration decreased by a third and cortical glutamine by more than two thirds. Elevation of plasma glutamine levels by infusion of glutamine caused a further doubling of ammonia production. However, there was an enhancement of ammonia production during glutamine infusion in normal rats. Yablon and Relman (1977) concluded that plasma glutamine levels are important in regulating ammonia production, and that in chronic acidosis, glutamine supply may become limiting for ammonia production. Bennett et al. (1979) also studied the relationship of plasma glutamine to ammonia production in rats. Rats were made acutely acidotic with NH_4Cl and HCl given intragastrically. Plasma glutamine increased significantly as a response to acidosis. Bennett et al. (1979) determined that plasma glutamine alone was not the mediator for adaptive changes which occur in the kidney since persistent elevation of plasma glutamine at two levels (for 24 hours) did not enhance ammoniagenesis. Preincubation of cortical slices in glutamine similarly depressed ammoniagenesis. In experiments with dogs, Fine et al. (1978) showed that acute acidosis, induced by HCl or NH_4Cl, increased the level of plasma glutamine. Plasma glutamine level rose during acute metabolic acidosis induced with sulfuric acid in dogs (Addae and Lotspeich, 1968). However, it is doubtful if the absolute amounts of glutamine presented to the kidney can be of great physiological significance as plasma glutamine concentrations remained unaltered in both chronic acidosis and alkalosis in dogs, in spite of large differences in ammonia production (Pitts et al., 1972). Fine et al. (1977) induced

chronic acidosis in humans (200 $mM.day^{-1}$ oral HCl) and found that plasma glutamine also remained unaltered. However, Heitman and Bergman (1975, 1978) found that the mean arterial glutamine level decreased in sheep with chronic NH_4Cl acidosis.

Changes in hydrogen ion concentration could provide the signal initiating alterations in ammonia metabolism. But in recent findings when renal tissue from normal rats was incubated with glutamine in an acid medium, ammonia production was either unchanged or diminished, and isolated mitochondria bathed in a low pH environment demonstrated an unequivocal decrease in ammonia production (Relman and Nairns, 1975; Tannen and Kunin, 1976). Tannen and Kunin (1976) suggested that the diminution in ammonia production was due to altered activity of PDG, since the activity of the isolated enzyme was also decreased when the pH was decreased below 7.4. When glutamate was employed as the substrate, a decrease in pH increased ammonia production by both renal tissue *in vitro* and isolated mitochondria (Relman and Nairns, 1975). This acute reduction in pH accelerated the formation of malate and facilitated its transport from the mitochondrial matrix to the cytosol, however the significance of this response was unclear (Relman and Nairns, 1975). Tannen and Kunin (1981) found that mitochondria from normal rats incubated at a low pH stimulated the metabolism of α-ketoglutarate to succinate. These results suggested that adaptation was not an immediate, direct result of an alteration in pH.

Tannen (1977) reviewed studies done on potassium depletion and suggested that potassium homeostasis regulates renal ammonia production, which in turn influences both urinary potassium and hydrogen ion secretion. Burnell *et al.* (1974) removed potassium from the diet of dogs

and caused a prompt decrease in distal H^+ secretion but because of a resulting positive H^+ balance, ammonia excretion subsequently increased. St. Omer and Roberts (1967) found that urinary ammonia excretion increased when heifers were fed a low potassium diet.

Theories of control. The theory of control that has received the most attention and support in recent years is based on an increase in renal gluconeogenesis and an increase in the renal content of phosphoenolpyruvate carboxykinase (PEPCK). PEPCK converts oxaloacetate to phosphoenolpyruvate. Goodman et al. (1966) observed that gluconeogenesis by slices of rat renal cortex from such substrates as glutamine, glutamate, α-ketoglutarate (α-KG), and oxaloacetate was stimulated by prior induction of metabolic acidosis. Thus, the tissue concentrations of Krebs cycle intermediates from oxalacetate back through α-KG were reduced. The decrease in α-KG deinhibited GDH which resulted in increased conversion of glutamate to α-KG and ammonia. Reduction of glutamate then increased ammonia production by the glutaminase I reaction. According to this theory all ammonia produced from glutamine, must be ascribed to an adaptive increase in PEPCK. Alleyne and Schullard (1969) found that accelerated gluconeogenesis during metabolic acidosis was accompanied by increased PEPCK activity in whole kidneys as well as the kidney cortex of rats. The increase in PEPCK activity in kidney cortex varied with the degree of acidosis and there was a close correlation between cortical PEPCK activity and urinary ammonia. Similarly, Seyama et al. (1971) found that gluconeogenesis was increased in renal tissue from diabetic (acidotic) rats. However, it would appear that net glucose metabolism cannot be clearly demonstrated in the intact kidney. Roxe (1972) studied net glucose metabolism of the kidney of the intact dog and could not demonstrate any consistant

addition of glucose to renal venous blood in acidosis when ammonia production was increased. Renal gluconeogenesis did not appear to be stimulated in sheep during metabolic acidosis, (Kaufman and Bergman, 1971). Pitts et al. (1972) infused tracer amounts of glutamine-^{14}C (uniformly labelled) into intact dogs and found that, in alkalosis, approximately 10% of the glutamine extracted by the kidneys was converted to glucose, the remainder appearing mostly as CO_2. In acidotic dogs, glutamine extraction and conversion to CO_2 increased threefold, although total production of CO_2 was unchanged. Conversion to glucose increased by more than fivefold, but glucose carbons still only represented 20% of the utilized glutamine. The authors concluded that gluconeogenesis could not be rate limiting for ammonia production. McIntosh et al. (1973) found that the daily net output of glucose by the sheep kidney was less than 2% of the animal's requirement. During acidosis, the contribution by the kidneys to the overall glucose requirement was no greater than 6%.

Welbourne (1975) offered a new hypothesis, which explained the adaptation phenomena in terms of a single event, which takes place at the mitochondrial membrane, and is mediated by adrenocorticoids. This "unitary hypothesis" had three basic tenets: dual cytoplasmic and mitochondrial glutaminases, glutamine diffusion to these enzyme sites, and glucocorticoid control of glutaminase I activity. In acid-base balance, renal glutamine utilization occurred predominantly by the cytoplasmic γ-glutamyltransferase pathway. In metabolic acidosis, the threefold increase in glutamine extraction is coupled to mitochondrial glutaminase I-GDH pathway activation which exhibited a 15-fold increase in activity (Welbourne, 1977). Phenix and Welbourne (1975) demonstrated

that bilateral adrenalectomy produced a 30% fall in daily NH_4^+ excretion which became apparent within 24 hours and persisted despite the animals becoming acidotic. Administering a glucocorticoid markedly stimulated ammonia excretion in adrenalectomized rats regardless of the acid-base status. Hughey et al. (1980) observed also that adrenalectomized rats exhibited decreased blood glutamine and an impaired ability to increase arterial levels in resonse to acute acidosis. Welbourne (1975) suggested that the adrenocorticoids played a key role in the adaptive response in ammonia production in chronic acidosis. The mechanism of their action was by affecting the permeability to glutamine of the inner mitochondrial membrane (Welbourne, 1975).

In the light of the above research, the focus of attention is turning toward the possibility that entry of glutamine into the mitochondrial matrix may be the rate-limiting step governing the adaptation of ammoniagenesis to acidosis. The increased ammonia production and glutamine utilization in chronic acidosis are a consequence of an adaptive increase in mitochondrial glutamine transport (Adam and Simpson, 1973). Kovačević (1977) proposed that glutamine crossed (possibly carrier-mediated)the mitochondrial membrane as an electroneutral species driven by a concentration gradient. A proton gradient and membrane potential generated by the energization of kidney mitochondria stimulated the influx of glutamine. Adam and Simpson (1973) found that mitochondria from the renal cortex of rats with prolonged (>5 days) acidosis took up four times as much ^{14}C-glutamine into the matrix as those from control animals. Tannen and Kunin (1976) studied ammonia production by renal cortical mitochondria isolated from rats with metabolic acidosis and determined that increased glutamine entry and/or phosphate-dependent

glutaminase accounted for increased ammoniagenesis.

Control of ammonia excretion. The mechanism of release of ammonia from tubular cells is one of nonionic diffusion (Pitts, 1971). Accordingly, not only the cellular ammonia production, but the concentration gradients of hydrogen ions between cells and tubular urine, and between cells and peritubular blood, as well as the rates of flow of these two liquid phases determine the distribution of ammonia (Pitts, 1973).

Pitts (1948) demonstrated that the rate of ammonia production was inversely proportional to the urine pH. This relationship depended upon the mechanism of ammonia transport across the luminal membrane of tubular cells. The preponderant form of ammonia in tissue is as NH_4^+ (Hems, 1974). Only one of each 100 molecules existed as the free base NH_3, however as rapidly as that one molecule diffused from the cell, it bound a hydrogen ion and was trapped as a relatively nondiffusable ammonium ion (Pitts, 1971). At any given urine pH, Pitts (1948) found that the rate of ammonium excretion was higher when a dog was made chronically acidotic, than when it was in its normal state. Balagura and Pitts (1962) made dogs chronically acidotic by administering ammonium chloride (10 g) for three days, after which a saline solution containing creatinine and ammonium acetate was rapidly injected into the left renal artery. The injected ammonia appeared in the urine before the creatinine, indicating that ammonia passed from peritubular blood into the tubular urine downstream from the glomerulus. When the acidosis was corrected by giving sodium bicarbonate and alkaline urine resulted, the time course of excretion of creatinine was the same, but no ammonia appeared in the urine. These findings support the view that the mechanism of ammonia

secretion is one of diffusion trapping and that ammonia diffuses passively in both directions across the tubular epithelium. Tannen and Ross (1979) suggested that trapping of ammonia in the urine may be a critical component for stimulating ammonia production in acute acidosis. Studies were carried out using isolated kidneys of rats perfused with an albumin Krebs-Henseleit medium. Kidneys were made "non-urinating" by increasing the albumin concentration of the perfusate to 10 $g.dl^{-1}$ and lowering the perfusion pressure to 90/70 mmHg. Under these conditions acute acidification of the perfusate resulted in a small, but significant, increase in ammonia production. This increase in ammonia production was not significantly less than the response to acute acidosis in all the studies with filtering kidneys, however it was significantly less in comparison with those experiments in which urine pH declined to less than 6.0. Since an effect on ammonia production mediated by urinary acidification was only apparent with this degree of urine acidification, the lesser stimulation with non-filtering kidneys was consistant with the thesis that ammonia trapping in the urine mediated by urine pH was required for a maximal response to acute acidosis (Tannen and Ross, 1979). Schloeder and Stinebaugh (1977) interpreted their results on chronically acidotic humans to indicate that urinary ammonium concentration determines urinary pH, and not vice-versa as appeared to be the case for acutely acidotic rats. Schloeder and Stinebaugh (1977) showed that urinary ammonium concentration varied directly with the severity of the systemic acidosis and that, in turn, urinary ammonium concentration correlated directly with urinary pH. Urine pH rose after the second to sixth day of acidosis, and the rise in urine pH was greatest in subjects with the most profound acidosis.

The effect of urine flow rate was examined in connection with studies of urine pH and ammonia excretion (Orloff and Berliner, 1956; Tannen, 1969). Orloff and Berliner (1956) found in the dog, that at high urinary H^+ concentrations, the rate of ammonia excretion remained constant and independent of urine flow. This implied that the bulk of the ammonia which was destined for excretion had entered the tubular urine prior to the final process of osmotic concentration. If the urine was weakly acidic, the low rates of ammonia excretion were more definitely flow dependent. There was a greater effect of back-diffusion at lower urine flow rates which decreased the rate of ammonia excretion (Orloff and Berliner, 1956).

The effects of renal blood flow (RBF) and glomerular filtration rate (GFR) on ammonia excretion have been studied. Pilkington et al. (1970) determined that in acidosis, the amount of glutamine extracted from the blood perfusing the kidney and converted to ammonia exceeded the amount filtered and reabsorbed. Some of this glutamine was absorbed across the peritubular membrane from the blood and hence could be influenced by renal blood flow. Pitts (1972) made a comparison between chronic acidotic and alkalotic dogs, and found that GFR, renal arterial blood inflow, and arterial concentration of glutamine did not differ significantly between the two groups. Mahnensmith et al. (1979) studied acute acidosis in humans. Subjects were given 0.1 $g.KgBW^{-1}$ NH_4Cl over 80 minutes, orally. No change was found in GFR and renal plasma flow after NH_4Cl loading. Studies with ruminant animals appear more conflicting. Huber (1969) studied the effects of acute lactic acidosis in sheep and found that renal blood flow was significantly reduced when the blood pH was 7.30 or less. However GFR was not decreased significantly until the blood pH

was less than 7.14. At the time that the decrease in GFR was observed RBF had decreased to approximately 44% of the control flow. Huber (1969) concluded that this reduction in RBF seriously compromised the ability of the kidney to excrete hydrogen ions and reabsorb bicarbonate. McIntosh et al. (1973) induced metabolic acidosis in sheep by drenching with 20 g NH_4Cl daily or by constantly infusing NH_4Cl into the rumen for 3 - 11 days. Renal blood flow decreased, but GFR did not change. These authors suggested that the decrease in RBF during acidosis was a physiological response to the altered acid-base status, in that there may have been a redistribution of blood flow, leading to a greater percentage of blood perfusing the active zone of the renal cortex. Phillips (1968) infused hydrochloric acid into sheep at a rate of 1.6 $mEq.min^{-1}$ for 2 hours and GFR was unaltered. Kaufman and Bergman (1971) measured renal blood flow in acidotic sheep and although it tended to decrease, the values were not significantly different from controls. The acidosis was produced and maintained by the daily administration of NH_4Cl via a stomach tube into the rumen. A priming dose of 0.5 - 0.7 $g.kg\ BW^{-1}$ of NH_4Cl was followed by daily maintenance doses of 0.2 - 0.4 $g.kg\ BW^{-1}$. Williams and Pickering (1980) infused hydrochloric acid intravenously (150 $\mu mol.min^{-1}$ for three days) to eight ewes. No changes in GFR or renal plasma flow were detected as blood and urine pH decreased.

Sites of production of glutamine. Increased extraction and use of glutamine by the kidney in acidosis implies increased production or release of glutamine from other organs. The metabolic response to acidosis must be coordinated so that glutamine production is regulated to meet its changing rates of utilization. According to Addae and Lotspeich (1968) the liver is the major site of increased glutamine production in the acidotic dog. Glutamine was extracted in increased amounts from the splanchnic arterial blood and was converted to ammonia by the gastrointestinal

tract (GIT). The authors suggested that this increased ammonia release by the GIT stimulated increased hepatic glutamine synthesis. Heitman and Bergman (1975) found a dramatic shift from net renal glutamine release in normal fed sheep to a net uptake by kidneys in acidotic sheep. During acidosis, the plasma glutamine concentration decreased and net peripheral release of glutamine tended to increase. Prior to acid administration, glutamine was removed by the liver, but in acidosis net hepatic glutamine removal was decreased (Heitman and Bergman, 1978).

III NITROGEN METABOLISM IN RUMINANT ANIMALS

A. Introduction

The first step in the digestion of dietary protein by ruminants is microbial proteolysis in the reticulo-rumen. Amino acids so produced are mainly deaminated to form volatile fatty acids, CO_2, ammonia, and a variety of organic compounds. The ammonia produced can be utilized by microbes to form microbial protein, which is the main form of nitrogen reaching the duodenum (Wolfe et al., 1972). The concentration of ammonia in the rumen is influenced by the quantity and solubility of the dietary protein, the quantity of urea that enters the rumen in saliva, the diffusion of urea through the rumen wall, and the rate at which ammonia is absorbed from the rumen (Phillipson, 1977). Ammonia absorbed from the rumen is detoxified in the liver by incorporation into urea. Urea can then be returned to the rumen across the rumen wall (Houpt, 1970), or in saliva (Somers, 1961; Lee and Cross, 1969). A possible mechanism whereby the kidneys decrease the amount of urea excreted and thereby facilitate recycling of urea-N through microbial protein synthesis in

the reticulo-rumen is the subject of much research. (Schmidt-Nielsen et al., 1958; McIntyre and Williams, 1970; Scott and Mason, 1970; Ergene and Pickering, 1978a).

B. Effects of nitrogen level

Feeding sheep on a high-protein diet resulted in increased plasma urea concentration, and increased urea excretion in urine (Schmidt-Nielsen et al., 1958; Thornton, 1970; Rabinowitz et al., 1973; Ergene and Pickering, 1978a). Severe curtailment of protein intake resulted in decreased pNH_3 of rumen gas, decreased plasma urea concentration, and increased diffusion of urea from plasma into the rumen, where it was hydrolyzed to form CO_2 and ammonia (Phillipson, 1977). If there was sufficient carbohydrate available, the ammonia resulting from the hydrolysis of urea was incorporated into bacterial or protozoal protein, thus becoming available to the host.

The role of the kidneys in the conservation of urea has been examined by Schmidt-Nielsen and his co-workers. Schmidt-Nielsen and and Osaki (1958) and Schmidt-Nielsen et al. (1958) fed diets containing either 7.5% or 1.9% digestible crude protein to sheep. With the 1.9% protein diet, the fraction of filtered urea appearing in the urine was very low. This low urea excretion was characterized by a urea urine/plasma concentration ratio not exceeding 5 - 7 even at low urine flows. It was also observed that when the low nitrogen diet was fed, an increase in the inulin urine/plasma concentration ratio (indicative of increased water reabsorption by the kidney) from 10 to 200 was accompanied by an increase in the percentage of filtered urea reabsorbed from 40 to 90%. For the normal protein diet, the reabsorption of filtered urea was

steady at between 35 - 55% over the same range of inulin urine/plasma ratios. Schmidt-Nielsen et al. (1958) concluded that regulation of urea was at the tubular level since it was independent of GFR, plasma urea concentration and osmotic load. These authors suggested that the excretion of urea is brought about through a regulated active transport of urea, accentuated by a countercurrent multiplier system in Henle s loop. Gans and Mercer (1962) fed sheep on either high (high quality hay supplement with grain) or low nitrogen (poor quality hay and straw) diets and determined that glomerular filtration rate and renal plasma flow were lower for sheep given the low nitrogen diet, a result contrary to that of Schmidt-Nielsen et al. (1958). Scott and Mason (1970) studied renal tubular absorption of urea in sheep fed diets containing either 2.7% or 0.88% nitrogen. As the amount of urea filtered at the glomeruli increased through the range 54 - 1347 μmole.min^{-1} the amount reabsorbed by the tubules increased proportionately, over a wide range of urine flow rates. For the low nitrogen diet, under conditions of low urine flow rate there was an appreciable increase in the percentage of urea reabsorbed. Scott and Mason (1970) suggested that this effect appeared to be independent of the amount of urea filtered but was by some unknown mechanism sensitive to nitrogen intake. Phillips et al. (1975) reduced the dietary nitrogen intake of four heifers and found that this resulted in a fall in the concentration of urea nitrogen in plasma and in its rate of excretion and tubular reabsorption. However, the fraction of filtered urea reabsorbed was increased from about 45 to 80%, despite the associated reduction in the concentration gradient between urine and plasma. Ergene and Pickering (1978a) found similar results for sheep maintained on diets supplying 18.5 - 20.5 g.day^{-1} of nitrogen.

The fall in plasma urea nitrogen and urea excretion rate was accompanied by a significant reduction in GFR and urine osmolality. Similar to Phillips et al. (1975) the fraction of filtered urea reabsorbed increased despite a reduction in the urea urine/plasma concentration ratio. Their findings support the suggestion (Schmidt-Nielsen et al., 1958) that tubular reabsorption of urea has an active component.

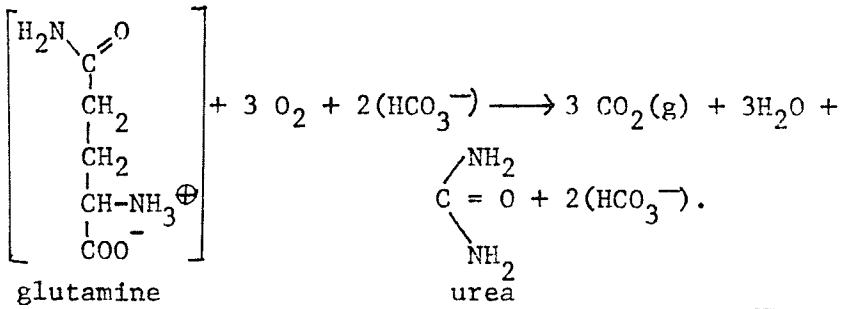
C. Effect of acidosis.

The urinary nitrogen of man is partitioned among urea (80 - 90%); ammonia (5 - 10%); and creatinine, uric acid, and hippuric acid (5 - 10%), Pitts, 1973). For sheep the usual distribution of urinary nitrogen is as follows: urea (82%); ammonia (0 - 7%); and creatinine (4%), (Morris and Ray, 1939). In both species, nitrogen excretion as ammonia is minimal. Steenbock et al. (1914) maintained that the increased excretion of ammonia in acidosis was at the expense of urea excretion, the two varying reciprocally under conditions of constant protein intake. Oliver and Bourke (1975) found that hydrochloric acid acidosis significantly reduced urea excretion in the rat, with an equimolar increase in ammonia excretion and therefore no change in their total sum. In man (Fine et al., 1977) hydrochloric acid acidosis reduced blood and urinary urea levels with a concomitant rise in ammonium excretion, however these changes were not equimolar as in the rat. Telle and Preston (1971) imposed lactic acidosis on sheep and found that ammonia nitrogen excretion increased steadily while urea nitrogen excretion showed no trend over a ten-hour experimental period after an acute acid load. The long term effects of a chronic acid load on urea excretion in ruminants has not been studied.

In some species it is evident that metabolic acidosis results in

the diversion of waste nitrogen, normally excreted predominatly as urea, to elimination as ammonia. Oliver and Bourke (1975) discussed the significance of this shift in terms of the urea cycle producing H^+ and contributing to acidosis. At physiological pH production of urea from ammonium-or amino-nitrogen utilizes two bicarbonate ions. In the liver the ammonium ion derived from the amide group of glutamine is incorporated into carbamyl phosphate, which is then reacted with ornithine to form citrulline. Two bicarbonate ions are used in these first two steps. However metabolic use of the carbon skeleton of glutamine regenerates the two bicarbonate ions. There is therefore, no net gain or loss of base. In acidosis, when renal glutamine use is increased, the amino groups of glutamine are eliminated in urine as NH_4^+ and no loss of bicarbonate occurs. As in urea production, the glutamine carbon skeleton remains and can generate two bicarbonates. The equations below illustrate this point.

<u>Urea excretion</u>



glutamine

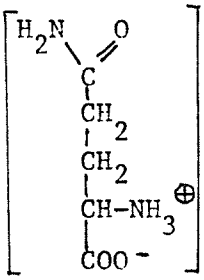
$$+ 3\ O_2 + 2(HCO_3^-) \longrightarrow 3\ CO_2(g) + 3H_2O +$$

urea

$$+ 2(HCO_3^-).$$

No net gain or loss of HCO_3^-

<u>Ammonia excretion</u>



glutamine

$$+ 3\ O_2 \longrightarrow 2\ O_2(g) + 2\ NH_4^{\oplus} + 2(HCO_3^-)$$

Net gain of 2 HCO_3^-

Ammonia excretion in effect counteracts acidosis by resulting in a net gain of two bicarbonate ions. The diversion of nitrogen from urea synthesis in the liver to increased use of nitrogen (i.e. glutamine) in the kidney is advantageous to the maintenance of hydrogen ion homeostasis.

The following experiments were carried out in an attempt to reexamine the effects of two dietary nitrogen levels on the amounts of urea and ammonia excreted and whether acidosis affects this distribution. The effect of acidosis on renal plasma flow and glomerular filtration rate were also studied to determine if the kidney's synthesis of ammonia was seriously impaired under conditions of limiting nitrogen intake.

EXPERIMENTAL OBJECTIVES

In several species metabolic acidosis leads to an increase in the renal production and excretion of ammonia. The tubule cells may be supplied with increased amounts of the precursor, glutamine, by an increased concentration of glutamine in the renal blood, increased extraction rate, increased blood flow, or a combination of these factors.

In ruminant animals, urea is conserved when a diet low in nitrogen is fed. This nitrogen as urea, is recycled into the rumen for microbial protein synthesis. An acidotic ruminant animal on a low nitrogen diet would have two problems - first, to conserve urea to maintain rumen protein synthesis, second, to increase glutamine production to maintain ammonia excretion.

Sheep maintained on high or low nitrogen diets and infused intraruminally with hydrochloric acid were compared to test the following hypotheses:

1. That urinary ammonia-N excretion is at the expense of urea-N excretion, with no change in total-N excretion. Nitrogen normally synthesized into urea in the liver is being utilized for glutamine production and hence renal ammonia.

2. That in acidosis increased supply of glutamine to the kidney occurs because of increased plasma concentration of glutamine and/or increased plasma flow.

MATERIALS AND METHODS

I EXPERIMENT I

A. Animal maintenance

1. Animals

Six 2-year old Dorset x Western Whiteface ewes weighing between 43 and 60 Kg were randomly allotted to two treatment groups. Several months previously the ewes had been fitted with permanent plastic rumen cannulae (1 cm diameter).

2. Housing

The sheep were maintained tethered in individual plexiglass-sided adjoining pens. Each had an individual water nipple and feed bucket. The holding room was well-ventilated and lighting was of medium intensity.

3. Feeding

Two diets were formulated to meet the NRC (1975) recommendations for maintenance of non-lactating ewes, as shown in Table 2. Similar ingredients were used, but corn and corn starch were substituted for part of the soybean meal to give the low nitrogen diet. For the purpose of comparison, the diets are referred to as "high nitrogen" (HN) and "low nitrogen" (LN) diets. A potassium supplement (mixture of equal parts of K acetate, K bicarbonate, and K_3 citrate) was included daily bringing the potassium content of both diets to 1.14%, to ensure that before the induction of acidosis, blood and urine were alkaline.

Table 2. Composition of diets.[1]

Ingredient	Diet	
	High nitrogen (%)	Low nitrogen (%)
Wheat straw (ground)	33.96	33.92
Corn	19.41	26.65
Corn starch	19.41	26.65
Oats	9.70	9.69
Soybean meal (44%)	14.56	-
Limestone	0.34	-
Rock phosphate	0.19	0.68
Trace mineralized salt	0.49	0.49
Soybean oil	1.94	1.94
	100	100

1. Both diets were pelleted and had calculated metabolizable energy of 11.3 $MJ.Kg^{-1}$. The nitrogen content was analyzed to be 1.62% and 0.78% for the high and low nitrogen diets, respectively.

Water and cobalt-iodized salt licks were provided to sheep ad libitum at all times. Animals were fed continuously by an automatic feeder which trickled the daily feed allotment into the feed bucket on a 24-hour basis. This continuous feeding was designed to allow relatively constant rumen fermentation and absorption of nutrients thereby avoiding large fluctuations in plasma constituents. Feed refusals were removed and weighed at 0900 daily.

B. Animal preparation

1. Vein catherization

One day before samples were collected, the saphenous vein was catherized according to the technique described by Phillips (1968). While the sheep was held prone, an 18-Gauge needle was inserted in the vein. A selfex guide wire (0.635 mmd) was gently inserted into the vein through the needle to a depth of 15 cm. The needle was then removed and a polyethylene catheter (OD=1.27 mm; ID=0.86 mm) was threaded over the guide wire about 20 cm into the vein. After withdrawing the guide wire, a blunt 1 cm long 20-Gauge needle was inserted in the catheter end. The catheter was taped in position where it emerged from the skin, and the entire area of the leg was wrapped with an elastisized bandage to prevent the catheter from kinking.

2. Urinary bladder catheterization

On the same day, the urethra and vulva were anesthetized with "xylocaine jelly" (Lignocaine hydrochloride 20 $mg.ml^{-1}$), and the urinary bladder was catheterized with a 14-FG Foley balloon catheter. A wire stillette was first put into the catheter and with a finger in the vulva

the tip of the catheter was guided into the urethra. After withdrawing the wire, 5 ml of sterile saline were injected into the balloon to retain the catheter in place. To enable anaerobic urine sampling, a 3-way stop-cock was attached to the drainage tube from the catheter to the collection vessel.

All sheep were given 3 cc of penicillin intramuscularly prior to and for three days following catheterization to reduce the chance of bladder infection.

C. Sampling schedule

A week prior to collection of data, a continuous infusion of water, three liters per day was begun through the rumen cannula. An 8-channel Masterflex pump (Model 7568) was set at 2.10 $ml.min^{-1}$. The purpose of this infusion was to allow urine flows to stabilize. Measurements were started on the last day of water infusion into the rumen. Urine was collected for six consecutive 4-hour periods, and venous blood was sampled at the midpoint of each period. After twenty-four hours, the infusion into the rumen was changed to three liters of 0.05N hydrochloric acid per day (i.e. 150 $mEqH^{+} \cdot day^{-1}$). This was continued for four days. Urine was collected for sixteen hours following the change to acid infusion followed by eight urine and blood sampling periods each of 4 hours. The next two days consisted of a 16-hour period of urine collection, followed by two 4-hour periods of urine and blood collection. The sampling schedule is shown in Appendix tables 1 - 12.

D. Sampling technique

1. Blood sampling

A 5 ml plastic syringe was filled to three-quarters with heparinized saline and used to withdraw blood from the catheter. Then blood was drawn into a 5 ml sample syringe containing a heparin solution (1000 USP units/ml) in its dead space. The catheter was then flushed with heparinized saline syringe. The small bubble of air from the air space in the sample syringe was ejected and the end of the syringe was sealed with a small plastic cap. The blood samples were kept on ice until blood-gas analysis, usually within 1-2 hours. Afterwards the samples were centrifuged at 2000 rpm for 20 minutes in a cold room and the plasma was separated. The plasma samples were stored at −20° C until analyzed.

2. Urine sampling

An anaerobic urine sample was taken at the midpoint of each collection period. A 10 ml plastic syringe was attached to the 3-way stop-cock connected to the drainage tube. The diameters of the catheter and drainage tubes were such that they were always filled with urine. The flow to the collecting bottle was shut off and 10 ml of urine was withdrawn from the bladder into the syringe. The small bubble of air from the dead space was removed and the end was capped. The urine sample was kept on ice until pH was determined.

Total urine produced was collected into bottles containing 5 ml of 4.5 N sulfuric acid to acidify the urine and keep the ammonia in solution. The volume collected in each period was measured and after thorough mixing, a 30 ml sample was frozen at −20°C until analyzed.

E. Analytical methods

1. Plasma pH, pCO_2, HCO_3^-, base excess

Measurements of plasma pH, pCO_2, bicarbonate (HCO_3^-) and base excess (BE) were made on whole blood using a Corning Model 165/2 pH Blood-Gas Analyzer.

2. Urine pH.

Urine pH was measured on a Radiometer pH Meter 26.

3. Urine titratable acid and net acid

Duplicate 10 ml samples of urine were titrated with 0.05 N sodium hydroxide to an endpoint which corresponded to the pH of the blood sample taken in the respective collection period. Since the urine was previously acidified with H_2SO_4, the titratable acid was calculated as the number of mEq of NaOH used in the titration minus the number of mEq of acid added. If the amount of acid initially added was greater than the amount of base, titratable base was present in the urine and was designated in the tables as a negative titratable acid value. Urine net acid was calculated according to the following formulae:

$$\text{Urine net acid} = H^+_{NH_4^+} + H^+_{\text{Tit. Acid}}$$

$$\text{Urine net acid} = H^+_{NH_4^+} - H^+_{\text{Tit. Base}}$$

where $H^+NH_4^+$ equals the milliequivalents of H^+ excreted combined with ammonia.

4. Plasma and urine ammonia-N, urea-N, and creatinine

Measurements of ammonia-N, urea-N, and creatinine concentrations were made on a Technicon Autoanalyzer II (Model 7-70-140A). Clearances of endogenous true creatinine (ETC) was used as a measure of glomerular filtration rate (GFR), as it had been shown with sheep that the ETC clearance approximates values obtained with inulin (Schmidt-Nielsen et al., 1958).

The rate of formation of creatinine and its concentration in plasma remain relatively constant. Creatinine and urea clearances were determined according to the following equation:

$$\text{Clearance of x } (\text{ml.min}^{-1}) = \frac{\text{Urine concentration of x } (\text{mg.ml}^{-1}) \times \text{Urine volume } (\text{ml.min}^{-1})}{\text{Plasma concentration of x } (\text{mg.ml}^{-1})}$$

To determine the percentage of filtered urea-N reabsorbed, the following equations were used:

$$\underset{(\text{mg.min}^{-1})}{\text{Urea-N filtered}} = \underset{(\text{mg.ml}^{-1})}{\text{Plasma conc. urea-N}} \times \underset{(\text{ml.min}^{-1})}{\text{GFR}}$$

$$\text{Urea-N reabsorbed} = \text{Urea-N filtered} - \text{Urea-N excreted}$$

$$\%\text{ of filtered urea-N reabsorbed} = \frac{\text{Urea-N reabsorbed}}{\text{Urea-N filtered}} \times 100$$

5. Urine total-N

Urine samples were digested using Kjeldahl reagents and a Technicon BD-20 Block Heater. Urine total nitrogen concentration in digested samples was determined by using the Technicon Auto-Analyzer II (Industrial method no. 329-74A). This is a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium hypochlorite in a buffered alkaline medium at a pH of 12.8 - 13.0. The ammonia-salicylate complex is read at 600 nm.

F. Statistical analysis

Means and standard errors were calculated. Treatment means were compared before acid infusion and after infusion of 550 - 600 mEq of acid using an unpaired t-test. Within treatments, means for before and after infusion were also compared. Correlations and linear or curvilinear regression analysis was done to determine the relationship between various parameters during acid infusion. The level of probability accepted as being significant was $P < 0.05$.

II EXPERIMENT 2

A. Animal maintenance

1. Animals

Three Dorset x Western Whiteface ewes, three years of age, weighing between 48 and 62 kg were used. They had previously been fitted with permanent rumen cannulae. A month before the experiment began, the sheep were operated on to exteriorize the carotid artery in a skin loop using the technique described by Bone et al. (1962) and Linzell (1963).

2. Housing

The sheep were housed as described in Experiment I. They were restrained with halters to allow access to jugular veins.

3. Feeding

As before sheep were fed continuously over 24 hours. The diet was altered slightly from that fed in the previous experiment. Sheep #299 and # 275 of the low nitrogen group in Experiment I refused feed after acid infusion was begun. Sheep # 299 decreased its feed intake by 50% on the second day of acid infusion and by 80% on the fourth. Sheep # 275 decreased its feed intake by 80% on the third day of acid infusion. Because of these decreased feed consumptions in Experiment I, sugar beet molasses (3% of the total diet) was added to the diets as formulated in Table 2, to try to improve palatability of the low nitrogen diet. The nitrogen thus added to both diets was calculated to be 0.04%. The same three sheep were tested on each diet with a two-week adjustment period for the changeover in diet. The sheep began consuming all feed given them within two days of the change in diet. However, on the day of intravenous infusion of acid, feed consumed decreased to about half

of the allotted amount for both dietary treatments. Intake increased again on the following days. Sheep #254 was an exception and consumed all feed at all times.

B. Animal preparation

1. Vein catheterization

The day before samples were collected, both jugular veins were catheterized using the previously described technique for insertion of the catheters. As the catheter was of a wider diameter (OD=1.57 mm; ID=1.14 mm) a guide wire of 0.81 mm diameter and a 16-Gauge needle was used. The catheter was inserted about 15 cm on the left side, which was used for blood collection and about 25 cm on the right side, which was used for infusions. The greater length was allowed so that the catheter reached near the heart to decrease the problem of hemolysis which can be caused by fast acid infusions. Using "Tuohy" adapters, the catheters were connected to 3-way stop-cocks and were tied to tufts of wool on the sheep's back.

2. Arterial catheterization

The carotid artery was catheterized using the same technique and materials as was used for the saphenous vein in Experiment 1.

3. Urinary bladder catheterization

Technique was the same as previously described.

C. Sampling schedule

The sampling schedule of blood and urine collections was as follows:

Day 1 - 0830 - 1030 - 600 ml of normal saline were infused at a rate of 5 $ml.min^{-1}$ into the right jugular vein

- 1030 - venous blood sample taken for blood-gas analysis.

- 1030 - 1130 - PAH loading dose was injected into the right jugular, followed by a continuous infusion of PAH at a rate of 1 $ml.min^{-1}$.

- 1130 - 1530 - four 1-hour clearance periods of urine collection with venous blood taken on the hour.

- 1330 - arterial blood sample taken

Day 2 - 0830 - infusion of 0.05 N HCl into rumen (3 $l.day^{-1}$) begun, at rate of 2.10 $ml.min^{-1}$; continued for 4 days

- 0830 - 1030 - 600 ml of 0.178 N HCl was infused at a rate of 5 $ml.min^{-1}$ into the right jugular vein.
- 1030 - venous blood sample taken for blood-gas analysis

- 1030 - 1130 - same as previous day

- 1330 - arterial blood sample taken

Day 3 - continued intra-ruminal infusion

Day 4 - continued intra-ruminal infusion

Day 5 - 1030 - venous blood sample taken

- 1030 - 1130 - same as Day 1

- 1130 - 1530 - same as Day 1

- 1330 - arterial blood sample taken

- 1530 - end of experiment

The sampling schedule is shown in Appendix tables 13 - 14. The concentrations of para-amino hippuric acid (PAH) used were 1 gm % PAH (0.4 g PAH in 40 ml saline) and 0.25 gm % PAH in saline, for the injection and infusion, respectively. An hour was allowed for equilibration of PAH in the bloodstream once the infusion began. A Harvard peristaltic pump (Model 1201) infused the PAH at a rate of 1 $ml.min^{-1}$.

The infusion of 600 ml of normal saline on Day 1 served as a control for the infusion of 600 ml of 0.178 N HCl on Day 2. The Harvard peristaltic pump was used for these infusions. The dose of acid given

intravenously within two hours acted as a priming dose in that it caused blood and urine pH to decrease and eliminated the sixteen hours in Experiment 1 allowed for these changes to occur. The dose used for this fast infusion of acid was decided upon from preliminary trials. The sheep did not appear to suffer discomfort at this level of acid infusion. In the preliminary trials it was observed that a faster rate and/or greater concentration of HCl caused listlessness, hemoglobinuria, intermittent panting, and loss of appetite. As in the previous experiment, the Masterflex pump was used for continuous intra-ruminal infusion of 0.05 N HCl.

D. Sampling technique

A 3 ml blood sample was withdrawn from the left jugular vein and a 5 ml carotid arterial blood sample were taken in the same manner as described in Experiment 1.

Urine was collected during the 4-one hour clearance periods on Days 1, 2, and 5, and volume was measured. A 30-ml aliquot was removed and the remining urine was pooled and was again subsampled. These aliquots were acidified with a few drops of 1.0 N H_2SO_4 and kept frozen for future analysis.

E. Analytical techniques

1. Plasma and urine PAH

Para-amino hippuric acid was analyzed by using the technique of Varley (1969). Plasma proteins were first precipitated with cadmium sulfate and sodium hydroxide. The supernatant was diazotized and then coupled with N-(1-napthyl)-ethelene dihydrochloride to yield a pink

color. Absorbance was read using a Bauch and Lomb Spectronic 20. Urines were first diluted and the same procedure was followed for color development. Calculation of PAH clearance was made using the clearance equation given in Experiment 1 under analytical techniques. When the PAH clearance is high and is independent of plasma concentration over a range of 1 to 6 mg.100ml^{-1}, the clearance of PAH may be a measure of renal plasma flow (Pitts, 1976). By also simultaneously measuring the creatinine clearance (i.e. GFR), the fraction of plasma filtered through the glomeruli was calculated as:

$$\text{Filtration Fraction} = \frac{\text{Glomerular filtration rate}}{\text{Renal plasma flow}}$$

2. Plasma glutamine

Immediately after collection of arterial blood samples on ice, blood was centrifuged and plasma was separated, in a cold room. Plasma was then deproteinized, adding an equal volume of 10% sulfosalicylic acid. This was centrifuged at 8000 rpm at -4 °C. The protein-free supernatant was frozen until analyzed on a Beckman Automatic Amino Acid Analyzer.

F. Statistical analysis

Means and standard errors were calculated. Factorial analysis was done on the data collected on Days 1, 2, and 5. Tests of the significance of the differences between means were made using the Student-Newman-Keuls test. The level of probability accepted as being significant was $P < 0.05$.

RESULTS

I. EXPERIMENT I

A. Blood acid-base parameters

Before imposition of metabolic acidosis, there was no significant difference between the high and low nitrogen treatments in the acid-base status of the blood (Table 3). Significant differences in plasma pH, HCO_3^-, and base excess were found when treatment means were compared during acid infusion after 550 to 600 milliequivalents of acid had been given. The LN sheep thus had a significantly lower plasma pH than HN sheep. For the LN sheep, mean pH decreased significantly from the pre-infusion value of 7.42, to 7.28 but for the HN sheep, the mean decrease from 7.43 to 7.39 was not significant.

Values for plasma bicarbonate concentration for LN sheep were significantly different before and after acid infusion, as were bicarbonate values for HN sheep. The LN sheep had a significantly lower mean HCO_3^- concentration of 15.6 $mEq.l^{-1}$ compared to 21.5 $mEq.l^{-1}$ for the HN sheep.

Within treatments, values for mean pCO_2 did not change significantly before and after acid infusion, although mean pCO_2 was lower during acid infusion for both treatments. There was no difference in mean pCO_2 between the treatments after acid infusion.

Mean blood base excess values were significantly lower after acid infusion for both dietary treatments. Base excess decreased to -8.97 $mEq.l^{-1}$ and -1.85 $mEq.l^{-1}$ for LN and HN sheep, respectively. These means were also significantly different.

Table 3. Blood acid-base parameters before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid.[1]

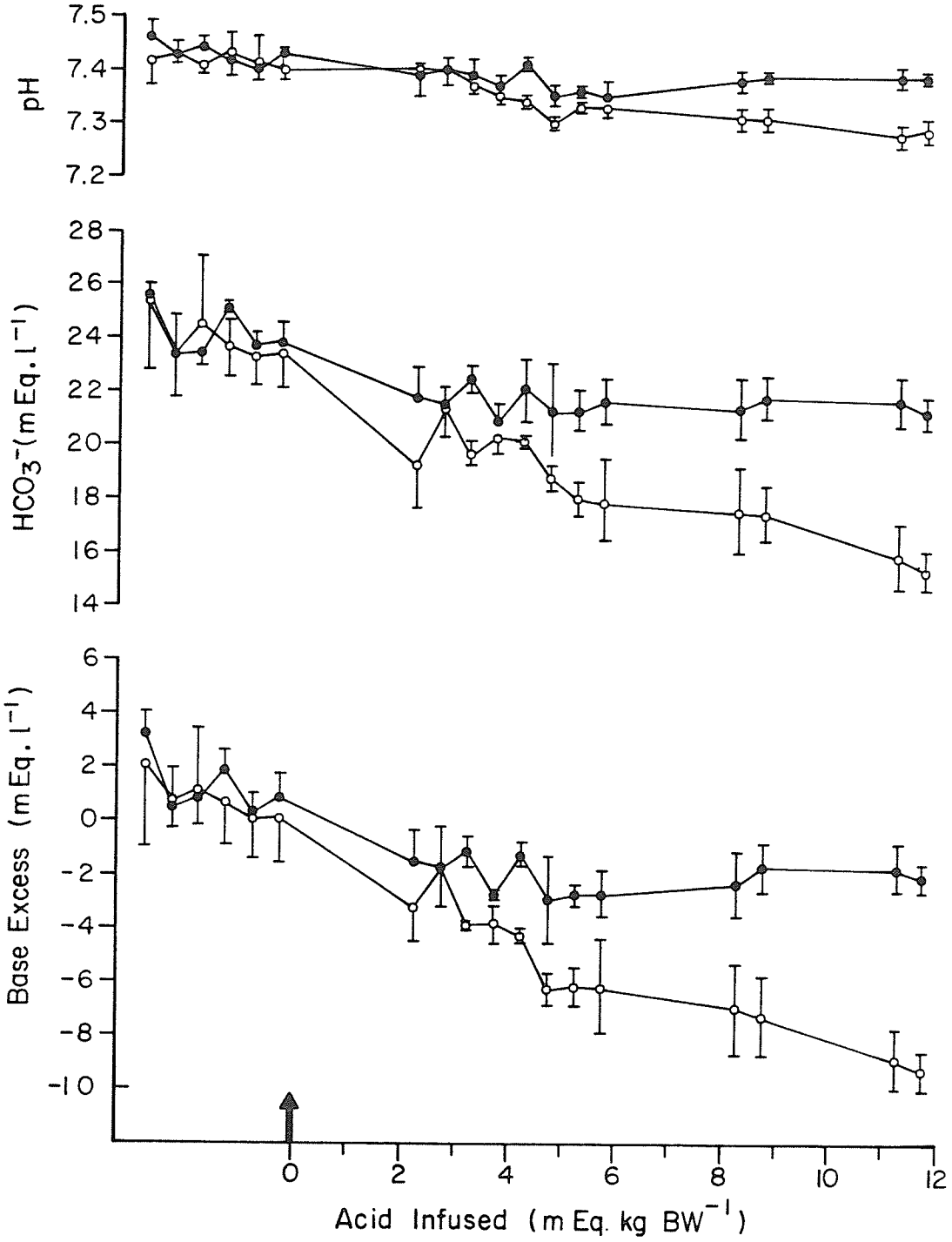
Treatment	pH	HCO_3^- ($mEq.l^{-1}$)	pCO_2 (mmHg)	Base excess ($mEq.l^{-1}$)
Before acid infusion:				
Low N diet	7.42±0.027^{a}[2]	23.8±1.17^{ab}	37.3±1.87	0.79±1.400^{ab}
High N diet	7.43±0.018^{a}	24.2±0.22^{a}	38.0±1.76	1.30±0.379^{a}
After acid infusion				
Low N diet	7.28±0.013^{b}	15.6±0.96^{c}	34.2±1.29	-8.97±0.883^{c}
High N diet	7.39±0.009^{a}	21.5±0.62^{b}	36.5±1.67	-1.85±0.362^{b}

1. Mean ± S.E.
2. Means within a column with a common superscript are not significantly different.

Figure 1. The transient effects of intra-ruminal infusion of hydrochloric acid on plasma pH, bicarbonate concentration and base excess. Values are means ± S.E. Arrow indicates the time of starting the acid infusion.

Open circles - Low N diet

Closed circles - High N diet


pH
7.5
7.4
7.3
7.2
HCO_3^- (m Eq. l^{-1})
28
26
24
22
20
18
16
14
Base Excess (m Eq. l^{-1})
6
4
2
0
-2
-4
-6
-8
-10
0
2
4
6
8
10
12
Acid Infused (m Eq. kg BW^{-1})

The transient effects of intra-ruminal infusion of hydrochloric acid on plasma pH, HCO_3^-, and base excess are illustrated in Fig. 1. In general, sheep maintained on the low nitrogen diet had a steady progressive decrease in these parameters. Sheep maintained on the high nitrogen diet had major decreases in these parameters during infusion of the 3 mEq.kg BW^{-1} of acid and thereafter, values tended to plateau. The relationship of these parameters to the amount of acid infused per kilogram of body weight was determined by linear and curvilinear regression analysis. Significant linear relationships and high negative correlations (r values) were found between plasma pH, HCO_3^-, base excess (dependent variables) and the amount of acid infused (independent variable) for LN sheep as follows:

pH	Acid infused (mEq.kg BW^{-1})	r= -.82	Y= 7.407-0.011 X
HCO_3^- (mEq.l^{-1})	"	r= -.81	Y= 22.29-0.573 X
Base excess (mEq.l^{-1})	"	r= -.85	Y= 0.880-0.719 X

The results for the HN sheep indicated that there was no significant relationship for plasma pH and HCO_3^- with acid infused, while the relationship between base excess and acid infused was curvilinear described by the following equation:

Base excess (mEq.l^{-1}) Acid infused (mEq.kg BW^{-1}) $Y= 0.674-0.931\ X + 0.063\ X^2$

B. Urinary acid excretion

Initially, urine of both groups was alkaline (Table 4). The urine pH of LN sheep ($\bar{X}$=7.54) was not significantly different from HN sheep ($\bar{X}$= 7.27). For both treatments, mean urine pH had decreased significantly after the acid infusion but differences between the dietary treatments was not significant. After infusion of the 3 mEq.kg BW^{-1} of acid into LN

Table 4. Urine acid-base parameters before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid.[1]

Treatment	pH	Net acid excreted	Titratable acid excreted	Ammonia excreted
		($mEq.h^{-1}.kgBW^{-1}$)		
Before acid infusion:				
Low N diet	7.54 ± 0.099[a][4]	-0.093 ± 0.0106[a]	-0.094 ± 0.0105[a]	0.0011 ± 0.0024[a]
High N diet	7.27 ± 0.081[a]	-0.104 ± 0.0120[a]	-0.105 ± 0.0017[a]	0.0021 ± 0.0007[a]
After acid infusion:				
Low N diet	5.56 ± 0.081[b]	0.053 ± 0.0235[b]	0.020 ± 0.0092[b]	0.0328 ± 0.0156[ab]
High N diet	5.84 ± 0.359[b]	0.035 ± 0.0082[b]	0.021 ± 0.0073[b]	0.0143 ± 0.0040[b]

1. Mean ± S.E.
2. Negative values indicate net base was excreted.
3. Negative values indicate titratable base was excreted.
4. Means within a column with a common superscript are not significantly different.

sheep urine pH had fallen sharply to a mean of pH 5.5, but the decrease was less drastic to 6.3 in the HN sheep (Fig. 2). From the linear and curvilinear regression analysis, the relationship of urine pH (dependent variable) to the amount of acid infused (independent variable) was curvilinear for LN sheep and linear for HN sheep. The equations are as follows:

LN: Urine pH Acid infused (mEq.kg BW^{-1}) $Y=7.478-0.69X+0.083X^2-0.031X^3$
HN: " " r= -0.43 $Y=6.698-0.093X$

Before acid infusion, net acid excretion values were negative for both treatment groups indicating the presence of basic substances in the urine (Table 4). The difference between mean acid excretion for the two treatments was not significant. After acid infusion, net acid values become positive and within the treatments means were significantly different from pre-infusion values, but the difference between the dietary treatment means after acid infusion was not significant. As urine pH decreased from pre-infusion levels, net acid excretion increased and values were positive (Fig. 2). For LN sheep, values became positive during infusion of the first 2 mEq.kg BW^{-1} of acid, but for HN sheep, acid excretion became positive after infusion of 2-3 mEq.kg BW^{-1} of acid.

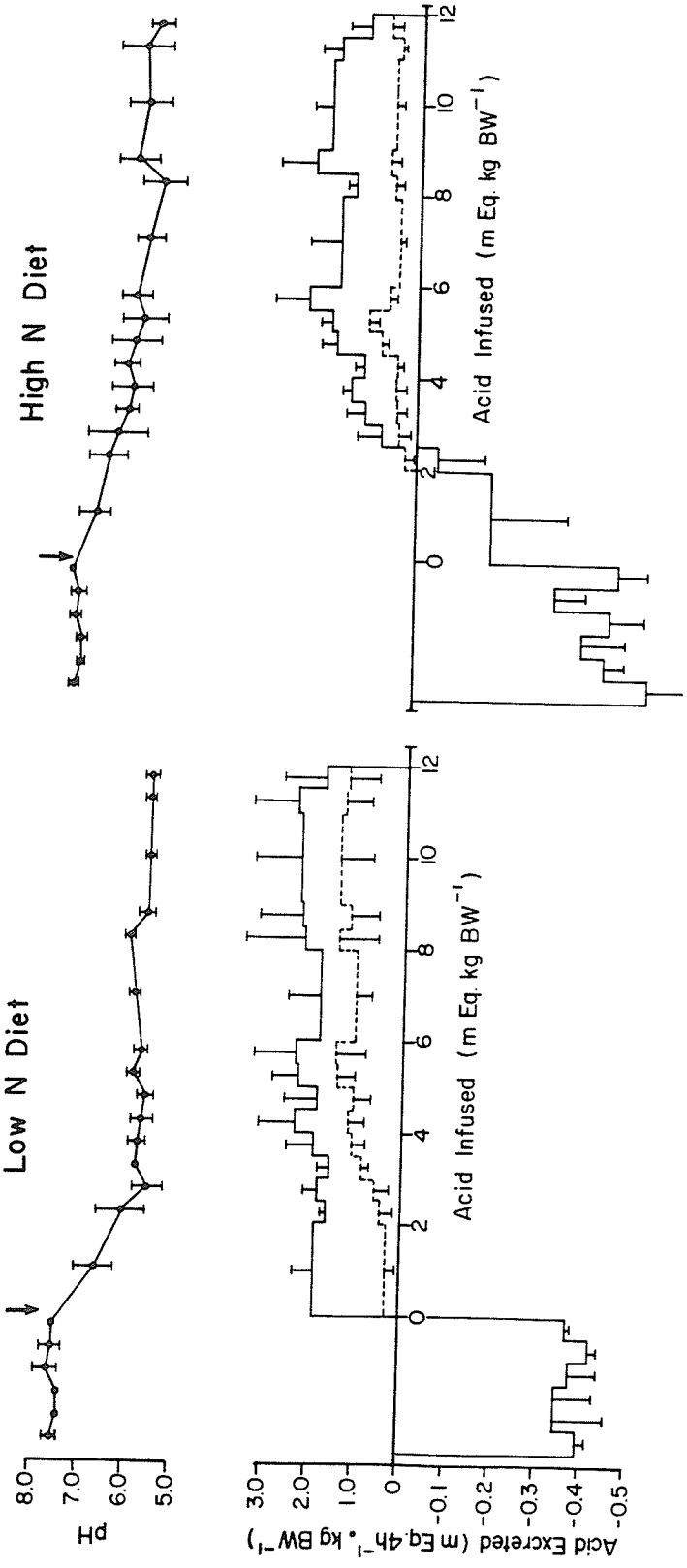
Results of linear and curvilinear regression analysis shown in Fig. 3 indicate that cumulative acid infused and cumulative net acid excreted are linearly related for both dietary treatments.

Before acid infusion, as urine was alkaline (Table 4), titratable base, i.e. negative titratable acid, was excreted in amounts which were not significantly different between the dietary treatments. Acid

Figure 2. The effects of intra-ruminal infusion of hydrochloric acid on urine pH and excretion of acid as net acid and as ammonia. Values for acid excreted are $mEq.4h^{-1}.kg\ BW^{-1}$ ± S.E. Arrow indicates the time of starting acid infusion. Ammonia excretion values for before acid infusion are not shown since for both treatments, these were less than 0.01 $mEq.4h^{-1}.kg\ BW^{-1}$.

Net acid excreted ——

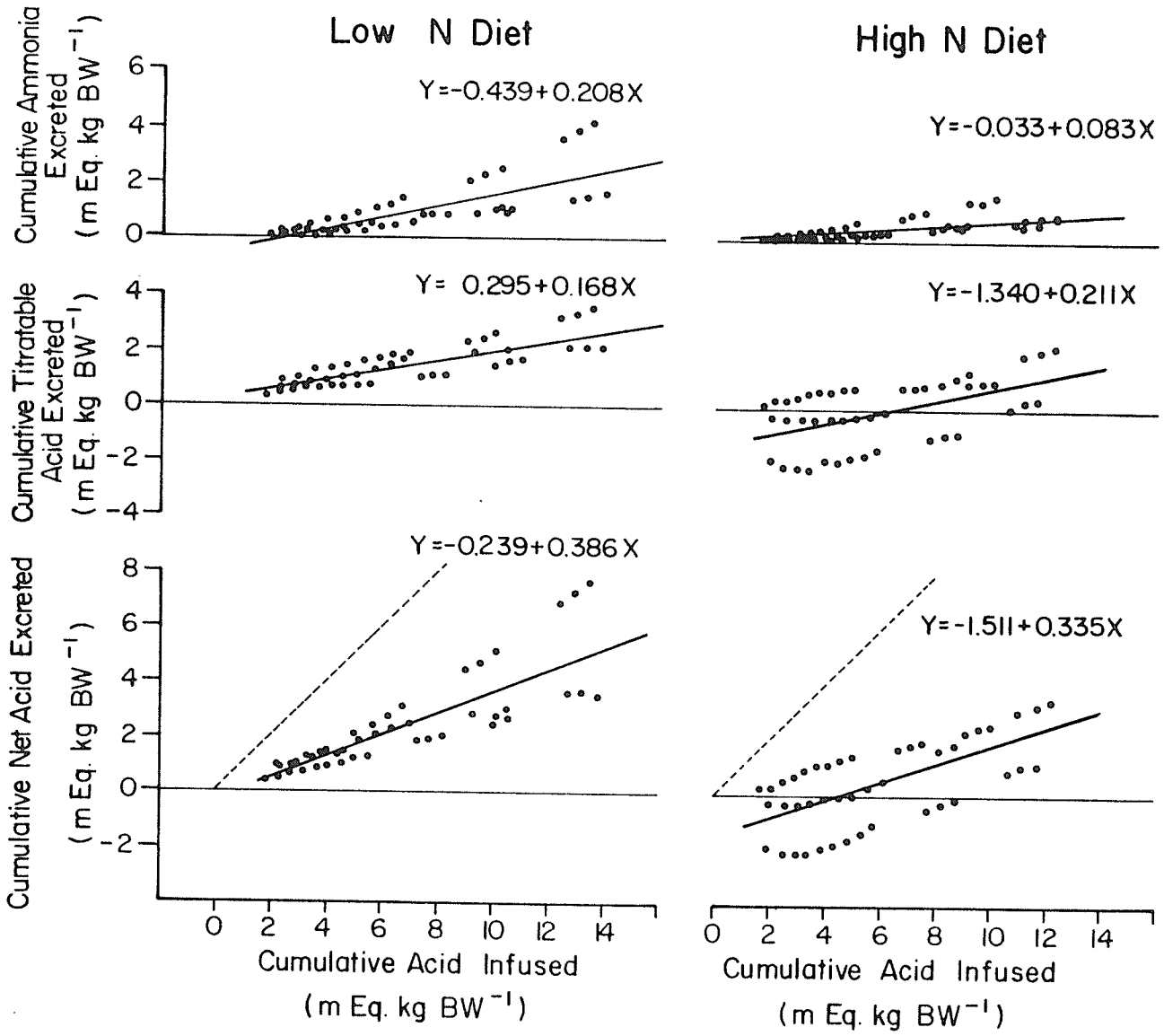
Ammonia excreted ----


High N Diet
Low N Diet
pH
8.0
7.0
6.0
5.0
Acid Excreted (m Eq. 4h⁻¹. kg BW⁻¹)
3.0
2.0
1.0
0
-0.1
-0.2
-0.3
-0.4
-0.5
Acid Infused (m Eq. kg BW⁻¹)
0
2
4
6
8
10
12

infusion caused excretion of titratable acid with the values being significantly different from pre-infusion for both treatments. Mean net acid values after acid infusion were not significantly different between the treatments. After the start of acid infusion, there was an immediate changeover to excretion of titratable acid by the LN group (Figure 2). On this figure, titratable acid is represented by the area between the lines drawn for ammonia excreted and net acid excreted. The HN group only started to excrete titratable acid after 3 mEq.kg BW^{-1} had been infused and there was a continuous increase as the acid dose increased to about 5 mEq.kg BW^{-1}. Again the relationship between cumulative acid infused and cumulative titratable acid excreted was linear (Fig. 3).

The amount of ammonia excreted before acid infusion was not different between the two dietary treatments. Values increased during acid infusion to a mean of 0.0328 mEq.h^{-1}.kg BW^{-1} for the LN sheep, and to 0.0143 mEq.h^{-1}.kg BW^{-1} for HN sheep, an increase of about 30 times and 7 times the pre-infusion values, respectively. Mean ammonia excreted was not significantly different for the LN sheep before and after acid infusion due to a large standard error in the latter case. For the HN sheep, the mean rate of ammonia excretion after acid infusion was significantly greater than for the pre-infusion. There was no difference in the treatment means after acid infusion. There was a continuous gradual increase in ammonia excretion with increasing acid load, for the LN sheep (Fig. 2). Ammonia excretion of the HN group increased also, but plateaued after about 5.5 mEq.kg BW^{-1} had been infused. From regression analysis, the relationship between urine pH (independent variable) and urinary ammonia-N excretion (dependent variable) was found to be linear for LN and HN sheep, but the correlation was higher for HN sheep as shown below:

Figure 3. Regression analysis of the relationships between cumulative acid infused and cumulative net acid, titratable acid, and ammonia excreted. The dashed line represents the theoretical rate of net acid excretion if the amount of acid infused equaled the amount of acid excreted.


Low N Diet
High N Diet
Cumulative Ammonia Excreted (m Eq. kg BW⁻¹)
Y=-0.439+0.208X
Y=-0.033+0.083X
Cumulative Titratable Acid Excreted (m Eq. kg BW⁻¹)
Y= 0.295+0.168X
Y=-1.340+0.211X
Cumulative Net Acid Excreted (m Eq. kg BW⁻¹)
Y=-0.239+0.386X
Y=-1.511+0.335X
Cumulative Acid Infused (m Eq. kg BW⁻¹)
Cumulative Acid Infused (m Eq. kg BW⁻¹)

LN: Ammonia-N excreted ($mg.h^{-1}.kg\ BW^{-1}$) Urine pH r= -.38 Y= 1.212-0.148 X
HN: " " r= -.65 Y= 0.760-0.096 X

The relationship between cumulative acid infused and cumulative ammonia excreted was determined to be linear for both treatments as shown in Fig. 3. Assuming that the average body weight of 50 kg was used and a total of 12 $mEq.kg\ BW^{-1}$ acid infused, it is calculated for LN sheep that 37% of the infused acid had appeared in the urine as net acid i.e. 4.4 $mEq.kg\ BW^{-1}$ (52% as titratable acid and 48% as ammonia). For the HN sheep, about 20% of the infused acid appeared in the urine (50% as titratable acid and 50% as ammonia).

C. Plasma urea-N and ammonia-N

Before acid infusion, mean plasma urea-N for LN sheep of 3.78 $mg.dl^{-1}$ was significantly lower than the mean of 11.23 $mg.dl^{-1}$ for HN sheep (Table 5). Plasma urea levels were not significantly changed during acid infusion, although mean urea-N did increase for LN sheep and decrease for HN sheep. Comparing the two treatments after acid infusion indicated that mean plasma urea-N levels were not significantly different.

Plasma ammonia-N did not differ significantly between the treatment groups either before or after acid infusion. Mean ammonia-N concentrations had increased by 1.44 and 0.49 $mg.l^{-1}$ for LN and HN sheep, respectively, after acid infusion.

D. Urinary excretion of nitrogenous constituents

Before acid infusion, the excretion rates of total-N, urea-N, and ammonia-N, were all significantly higher for the HN sheep. There was no

Table 5. Plasma concentrations of urea-N and ammonia-N before acid infusion and after intra-ruminal infusion of 550 - 600 mEq of hydrochloric acid.[1]

Treatment	Urea-N ($mg.dl^{-1}$)	Ammonia-N ($mg.l^{-1}$)
Before acid infusion:		
Low N diet	3.73 ± 0.348^{a}[2]	5.23 ± 0.595
High N diet	11.23 ± 0.467^{b}	4.31 ± 0.422
After acid infusion:		
Low N diet	5.40 ± 1.677^{ac}	6.81 ± 0.739
High N diet	9.95 ± 1.375^{bc}	4.81 ± 0.231

1. Mean ± S.E.
2. Means within a column with a common superscript are not significantly different.

difference between the treatments in the rate of excretion of unidentified urinary nitrogen (i.e. other-N). After acid infusion mean urinary total-N excreted was still significantly different between the treatments. There was no significant differences in total-N excreted before and after acid infusion for either treatment, although total-N excreted decreased for HN sheep from 8.45 to 6.87 $mg.h^{-1}.kg\ BW^{-1}$. Excretion of other-N was not significantly different between treatments, before or after acid infusion. Urea-N excreted after acid infusion was unchanged for LN sheep, but was significantly decreased for HN sheep, so that the latter was significantly lower after infusion of acid. Ammonia-N excretion increased for both treatments as a response to acid infusion, however the change was not significant for the LN sheep because of the large S.E. caused by the high excretion rate of sheep no. 299.

To determine whether there was addition to or substitution of the nitrogenous constituents of urine during acidosis, the amounts of other-N, urea-N, and ammonia-N, as percentages of total urinary nitrogen excreted were compared (Table 6). For LN sheep, the percentage urea-N remained the same, while the percentage ammonia-N excreted increased by 15.3%, and other-N decreased by 17.2% after acid infusion. For HN sheep, percentage urea-N excreted decreased 16.9%, while ammonia-N increased by 2.3% and, resulting in a fall in their sum of 14.1%. However the excretion of other-N increased by 14.4% after acid infusion.

E. Urine flow rate, creatinine and urea clearances, U/P creatinine and urea ratios, and filtered urea reabsorbed.

Before acid infusion, mean creatinine clearance (i.e. glomerular filtration rate) for LN sheep was 1.64 $ml.min.^{-1}.kg\ BW^{1}$, significantly lower than the mean of 2.01 $ml.min.^{-1}.kg\ BW^{-1}$ for HN sheep (Table 7).

Table 6. Urinary excretion rates of nitrogenous constituents before acid infusion and after intraruminal infusion of 550 - 600 mEq of hydrochloric acid.[1]

Treatment	Total-N	Other-N	Urea-N	Ammonia-N
	($mg.h^{-1}.kg\ BW^{-1}$)			
Before acid infusion:				
Low N diet	2.75 ± 0.456[a][2]	1.38 ± 0.177(50.1)[3]	1.36 ± 0.302[a](49.5)	0.015 ± 0.0054(0.6)[a]
High N diet	8.45 ± 0.445[b]	1.79 ± 0.221(20.5)	6.64 ± 0.345[b](78.6)	0.028 ± 0.0084(0.3)[a]
After acid infusion:				
Low N diet	2.88 ± 1.129[a]	0.95 ± 0.311(33.0)	1.47 ± 0.648[a](51.0)	0.459 ± 0.2180(15.9)[ab]
High N diet	6.87 ± 0.382[b]	2.40 ± 0.537(34.9)	4.24 ± 0.462[c](61.7)	0.176 ± 0.0302(2.6)[b]

1. Mean ± S.E.
2. Means within a column with a common superscript are not significantly different.
3. Value in parenthesis is the percentage of the total urinary N existing in this form.

Table 7. Urine flow rates, creatinine and urea clearances, creatinine and urea U/P concentration ratios, and fraction of filtered urea reabsorbed before acid infusion and after intraruminal infusion of 550 - 600 mEq of hydrochloric acid.[1]

Treatment	Urine flow rate	Creatinine[2] clearance	Urea clearance	U/P Creatinine	U/P Urea	Fraction filtered urea reabsorbed (%)
	($ml.min^{-1}.kg\ BW^{-1}$)					
Before acid infusion:						
Low N diet	0.047 ± 0.0121[a3]	1.62 ± 0.110[a]	0.61 ± 0.089[a]	40.2 ± 9.77[a]	13.9 ± 1.86[a]	62 ± 4.4[a]
High N diet	0.190 ± 0.0178[b]	2.01 ± 0.139[b]	0.99 ± 0.024[b]	11.0 ± 1.55[b]	5.4 ± 0.65[b]	49 ± 3.0[b]
After acid infusion:						
Low N diet	0.042 ± 0.0149[a]	1.43 ± 0.761[ab]	0.52 ± 0.207[ab]	34.7 ± 4.58[a]	12.3 ± 2.00[ab]	65 ± 1.2[a]
High N diet	0.094 ± 0.0404[ab]	2.01 ± 0.108[ab]	0.76 ± 0.167[ab]	43.0 ± 27.13[ab]	13.6 ± 6.64[ab]	63 ± 7.0[ab]

1. Mean ± S.E.
2. Glomerular filtration rate is measured by creatinine clearance.
3. Means with a common superscript within a column are not significantly different.

Within dietary treatments, GFR was not significantly changed by acid infusion. Also, after acid infusion GFR's of LN sheep and HN sheep were not significantly different. Urea clearances followed similar trends. Initially urea clearance was lower for LN than for HN sheep, and after acid infusion, mean urea clearances had decreased for both groups, but these decreases were not significant. Treatment means after acid infusion were not significantly different.

The changes in renal concentrating ability and in fractional reabsorption of urea were examined to determine a possible link to the reduction in urea excretion of HN sheep during acidosis (discussed in the previous section), and also to the reduced GFR seen on the LN diet before acid infusion. Urine flow rate was significantly different between treatments before acidosis and decreased for HN sheep after acid infusion. This change was reflected by the creatinine U/P concentration ratio (Urine/Plasma) which was used as a measure of water reabsorption. Creatinine U/P ratio increased for the HN sheep in response to acid infusion from 11 to 43, although this was not statistically significant because of the high standard error. Before acid infusion, the urea U/P concentration ratio was significantly higher for LN sheep, indicating the existence of a greater urea concentration ratio between urine and plasma for these sheep. Urea U/P ratio increased for HN sheep after acid infusion accompanied by a 14% increase in the fraction of filtered urea reabsorbed, although this latter increase was not significant. Before acid infusion, the percentage of filtered urea reabsorbed was significantly different between the two dietary treatments, 62% for LN and 49% for HN sheep.

II EXPERIMENT 2

A. Blood acid-base parameters

Venous blood samples taken from sheep on high and low nitrogen diets, after an intravenous loading dose of saline (Day 1), after an intravenous loading dose of 100 mEq of acid within two hours (Day 2), and three days later after infusion of a total of 568.75 mEq of acid (i.e. 100 mEq intravenously + 468.75 mEq intra-ruminally) were compared by factorial analysis. There were no differences at any time in plasma pH, HCO_3^-, and base excess related to dietary treatment (Table 8).

Plasma pH after infusion of the acid loading dose significantly decreased about 0.6 pH units for both treatments. After three days of acid infusion pH did not decrease appreciably from values obtained after the loading dose.

Mean plasma bicarbonate after the loading dose of saline was 25.0 $mEq.l^{-1}$ for LN sheep and 20.3 $mEq.l^{-1}$ for HN sheep. Mean HCO_3^- decreased significantly by 5.3 and 4.4 $mEq.l^{-1}$ for LN and HN treatments, respectively, after infusion of the acid loading dose. Bicarbonate values after three days of acid infusion were not significantly different from values after the saline loading dose.

Plasma pCO_2 was significantly affected by both dietary treatment and acid infusion. Mean pCO_2 was consistently higher for sheep on the low nitrogen diet on the three days when infusions were given. Plasma pCO_2 decreased for both treatments after the loading dose of acid had been given but had increased in samples taken three days later.

Before acid infusion, blood base excess was 2.47 $mEq.l^{-1}$ for LN sheep and -2.17 $mEq.l^{-1}$ for HN sheep. Values decreased proportionately after the acid loading dose was given. However, base excess remained at

Table 8. Blood acid-base parameters taken at 1030 after a saline loading dose, after an acid loading, and three days later after infusion of a total of 568.75 mEq of acid.[1]

Treatment	pH	HCO_3^- ($mEq.l^{-1}$)	pCO_2 (mmHg)	Base excess ($mEq.l^{-1}$)
After saline loading dose:				
	a[2]	a	a	a
Low N diet	7.44±0.012	25.0±1.32	37.1±1.71	2.47±1.19
High N diet	7.40±0.027	20.3±1.22	31.3±1.16	-2.17±1.40
After acid loading dose:				
	b	b	b	b
Low N diet	7.38±0.010	19.7±1.52	33.8±1.76	-3.47±1.48
High N diet	7.33±0.031	15.9±1.79	29.8±0.58	-7.40±2.06
After 3 days of acid infusion;				
	b	a	b	ab
Low N diet	7.35±0.015	20.6±0.27	38.0±0.27	-3.47±0.47
High N diet	7.37±0.019	21.4±2.06	36.9±2.17	-2.10±2.05

1. Mean ± S.E.
2. Letter symbols within a column indicate differences between infusion levels for combined treatment means. Infusions with the same letter are not significantly different.

-3.47 $mEq.l^{-1}$ for LN sheep but increased from -7.40 to -2.10 $mEq.l^{-1}$ for HN sheep by the end of acid infusion.

B. Urine acid-base parameters

Urine pH, urinary titratable acid and ammonia excreted on low and high nitrogen diets were analyzed before acid infusion on Day 1, after acid loading by which time each sheep had received a total of 125 - 150 mEq (Day 2), and three days later when each sheep had received a total of 575 - 600 mEq of acid. There were no differences in any of these parameters related to dietary treatment. There were significant effects of acid infusion on urine pH and titratable acid, but not on ammonia excretion.

Initially, urine of the LN sheep was basic ($\bar{X}$=7.35) while that of HN sheep was slightly more acidic ($\bar{X}$=7.17) (Table 9). Sheep no. 268 when on the HN diet had a pH of 5.6 before acid infusion and contributed to the lower mean for this treatment. Urine pH decreased significantly to acidic values by five hours after infusion of 125 - 150 mEq of acid. The decrease in urine pH was greater for the LN sheep. Three days later, after infusion of 575 - 600 mEq of acid, urine pH was still acidic, however for LN sheep, pH decreased to 5.23 but that of the HN sheep had increased to 6.20.

Urinary excretion of titratable acid increased as urine pH decreased (Table 9). Before infusion, titratable base was excreted as indicated by the negative values. After infusion of 125 - 150 mEq of acid, the sheep excreted titratable acid, but the level of excretion did not increase markedly after the additional acid had been infused. Mean values increased for LN sheep but decreased for HN sheep by the end of acid infusion.

Mean ammonia excretion was higher for LN sheep before and during

Table 9. Urine pH and urinary titratable acid and ammonia excreted before and after acid infusions. [1]

Treatment	pH	Titratable acid[2] ($mEq.h^{-1}.kg\ BW^{-1}$)	Ammonia ($mEq.h^{-1}.kg\ BW^{-1}$)
Before acid infusion:	a[3]	a	
Low N diet	7.35 ± 0.445	-0.025 ± 0.0125	0.012 ± 0.0093
High N diet	7.17 ± 0.789	-0.014 ± 0.0148	0.007 ± 0.0093
After infusion of 125-150 mEq:	b	b	
Low N diet	5.47 ± 0.181	0.015 ± 0.0062	0.024 ± 0.0092
High N diet	5.66 ± 0.590	0.020 ± 0.0149	0.018 ± 0.0111
After infusion of 575-600 mEq:	b	b	
Low N diet	5.23 ± 0.066	0.021 ± 0.0040	0.030 ± 0.0033
High N diet	6.20 ± 0.786	0.013 ± 0.0089	0.017 ± 0.0044

1. Mean ± S.E.
2. Negative values indicate titratable base was excreted.
3. Letter symbols within a column indicate differences between infusion levels for combined treatment means. Infusions with the same letters are not significantly different.

acid infusion (Table 9). It increased from 0.024 $mEq.h^{-1}.kg\,BW^{-1}$ after infusion of 125 - 150 mEq to 0.030 $mEq.h^{-1}.kg\ BW^{-1}$ after infusion of 575-600 mEq. Values for HN sheep remained the same at these two infusion levels. As stated earlier, analysis of ammonia excretion data showed no significant differences due to dietary treatment, amount of acid infused, or their interaction.

The finding that ammonia excretion did not increase during acid infusion was probably caused by sheep no. 268 which had high initial levels of ammonia in urine on both dietary treatments.

C. Arterial plasma ammonia-N and glutamine concentration

Statistical analysis of arterial plasma ammonia-N and glutamine concentrations showed that there were no significant differences related to diet treatment or acid infusion or their interaction. However, it appeared that plasma ammonia-N decreased with acid infusion for the LN sheep and increased for the HN sheep (Table 10). Plasma glutamine followed similar patterns decreasing following acid infusion from 187 to 129 $\mu mol.l^{-1}$ for LN sheep, and increasing from 171 to 196 $\mu mol.l^{-1}$ for HN sheep, by the end of acid infusion.

D. Urine flow rate, creatinine and PAH clearances, and filtration fraction

Urine flow rate was not significantly affected by diet or acid infusion. Urine flow rates tended to be higher for sheep on the HN diet after acid infusion (Table 11). PAH clearance was also not affected by dietary treatment or acid infusion. Mean PAH clearance decreased slightly but not significantly for sheep on the HN diet after infusion of 125 -

Table 10. Arterial plasma ammonia-N and glutamine concentration before and after acid infusions.[1]

Treatment	Ammonia-N ($mg.l^{-1}$)	Glutamine ($\mu mol.l^{-1}$)
Before acid infusion:		
Low N diet	6.72 ± 0.758	187 ± 48.6
High N diet	5.67 ± 0.242	171 ± 11.6
After infusion of 125-150 mEq:		
Low N diet	6.21 ± 0.617	169 ± 38.6
High N diet	6.08 ± 1.093	190 ± 28.7
After infusion of 575-600 mEq:		
Low N diet	6.40 ± 0.750	129 ± 23.8
High N diet	6.41 ± 0.651	196 ± 43.9

1. Mean ± S.E.

150 mEq of acid. Creatinine clearance (GFR) and percent filtration fraction were significantly affected by dietary treatment, but not by acid infusion. Sheep on the LN treatment had lower mean creatinine clearances than sheep on HN diets. The percentage of plasma filtered through the glomeruli (FF) was lower for LN sheep, than for HN sheep before acid infusion but the HN value had declined to a similar level to LN by the end of acid infusion.

Table 11. Urine flow rate, creatinine and PAH clearances, and filtration fraction before and after acid infusions.[1]

Treatment	Urine flow rate	Creatinine clearance[2]	PAH clearance	Filtration fraction
		($ml.min^{-1}.kg\ BW^{-1}$)		(%)
Before acid infusion:				
Low N diet	0.057 ± 0.0211	2.33 ± 0.224	11.1 ± 1.33	21 ± 2.2
High N diet	0.049 ± 0.0080	2.55 ± 0.519	10.9 ± 1.82	24 ± 3.3
After infusion of 125-150 mEq:				
Low N diet	0.043 ± 0.0108	1.98 ± 0.102	9.9 ± 0.61	20 ± 2.3
High N diet	0.070 ± 0.327	2.57 ± 0.327	10.6 ± 1.39	25 ± 3.0
After infusion of 575-600 mEq:				
Low N diet	0.061 ± 0.0089	2.27 ± 0.335	11.7 ± 0.78	20 ± 3.6
High N diet	0.080 ± 0.0075	3.31 ± 0.410	11.2 ± 1.76	20 ± 2.3

1. Mean ± S.E.
2. Glomerular filtration rate is measured by creatinine clearance.
3. Renal plasma flow is measured by PAH clearance.

DISCUSSION

Intra-ruminal infusion of hydrochloric acid at the rate of 150 $mEq.day^{-1}$ for four days was found to reduce plasma pH, bicarbonate, and base excess. However, the degree of reduction of these parameters appeared to be dependent on the animal's nitrogen intake. Sheep maintained on the low nitrogen diet showed a linear decrease in these parameters in relation to the amount of acid infused. Sheep on the high nitrogen diet had smaller and insignificant changes in these same parameters as the amount of acid infused increased. The response of low nitrogen sheep to acid infusion was essentially the same as found by Phillips (1968) in sheep, and Swan and Pitts (1955) in dogs, during short-term acid infusion experiments. Other researchers, using ruminant animals used much larger doses of acid to produce a drop in blood acid-base parameters similar to that found in the present experiment for the low nitrogen treatment. Moderate metabolic acidosis was produced by Kaufman and Bergman (1971) by administering NH_4Cl by stomach tube for three to six days. A priming dose of about 560 mEq, followed by a maintenance dose of 280 $mEq.day^{-1}$ resulted in mean blood pH of 7.32, mean blood bicarbonate of 14 $mEq.l^{-1}$, and mean base excess of -11 $mEq.l^{-1}$. Their sheep were maintained on good quality alfalfa hay fed ad libitum. Heitman and Bergman (1978) found a decrease in mean blood pH from 7.52 to 7.35, bicarbonate to 12.4 $mEq.l^{-1}$ and base excess to -11.3 $mEq.l^{-1}$, after drenching sheep for 3 days with 20 - 30 g NH_4Cl per day (approx. 344 - 560 mEq of H^+ per day). These sheep were fed 17.7 g $N.day^{-1}$ in a commercial lamb pellet diet (14%). In these two cases the sheep had relatively high dietary levels of nitrogen and apparently a much

greater dose of acid-producing substance was needed to cause a decrease in blood values over a short period of three days. In the present research sheep consuming the high nitrogen diet (16.2 g $N.day^{-1}$) had a decrease in mean plasma pH from 7.43 to 7.38 and bicarbonate from 24.2 to 21.6 $mEq.l^{-1}$ principally while the first 125 mEq were infused over 20 hours. Thereafter, as infusion continued, these values did not change appreciably and so the dose was presumably not large enough or administered rapidly enough to cause further decreases as it did for the LN sheep.

The changes in blood acid-base parameters were slightly different under the conditions of Experiment 2. Sheep on HN diet had lower initial plasma pH, HCO_3^-, and base excess values. When the loading dose of acid was given, mean values decreased by the same magnitude for both dietary treatments. After prolonged infusion of acid three days later, a difference was seen between the treatments in that LN sheep had the lower blood pH, HCO_3^- and base excess. These values were not as low as those reached in Experiment 1, even though the total amount of acid infused was similar. This difference will be discussed later.

The means by which the level of dietary nitrogen causes the differing response of sheep to the same acid load can be speculated on. Swan and Pitts (1955) after infusing 180 mEq of hydrochloric acid intravenously to dogs determined that 57% of this acid load was buffered by cells via exchange of cellular Na and K for hydrogen ions across cell membranes so that intracellular protein stores were utilized. Sheep on a limiting nitrogen diet would have less amino acids available for intracellular protein synthesis and thus, presumably, less protein stores. Therefore less buffering of hydrogen ions would have occurred by this mechanism in

the LN sheep. By the isohydric principle - if the ratio of the components of any buffer pair is altered (i.e. $Keq\frac{HPr}{Pr^-}$), the ratios of all other buffer pairs are altered in proportion (Pitts, 1976). A greater decrease in the cellular protein buffering ratio for the LN sheep, would therefore cause a decrease in other buffer pairs and a greater change in blood pH.

Urine pH initially was lower for sheep consuming the high nitrogen diet since the higher protein feed when digested forms more acid residues. For low nitrogen sheep, a minimum pH of 5.6 was attained and a minimum of pH 5.8 was found for the high nitrogen treatment in Experiment 1. Phillips (1968) after a short-term intravenous infusion of 150 mEq of acid to sheep found a minimum urine pH of 5.1 Scott (1969) infusing 200 $mmol.day^{-1}$ of hydrochloric acid intra-ruminally to sheep found that urine pH fell during 3 - 4 days from 8.1 - 8.6 to the range 5.7 - 7.0. The dietary nitrogen consumed was 12.2 g/day intermediate between the two levels fed in the present study. Urine pH fell to the low level of 5.6 for LN sheep within one day of the start of acid infusion and to 5.8 within two days for the HN treatment. The delayed decrease (2 - 4 days) in urine pH found by Scott (1969) could be due to the high potassium content of the diet fed (3 x level fed in the present study).

The decrease in urine pH was accompanied by an increase in urinary excretion of net acid. For sheep on a low nitrogen diet, the changeover from a negative net acid (i.e. base) excretion to positive net acid values was seen in the first sixteen hours of acid infusion, but for sheep on a high nitrogen diet, the changeover took twenty-four hours. Hydrogen ion secretion by tubular cells has been shown to be limited by a number of factors such as insufficient delivery of H^+ to the cellular pool or

by an acute reduction of peritubular H^+ concentration (Malnic, 1974). Possibly, sheep on the LN diet, because of the greater decrease in blood pH, had a greater increase in the peritubular H^+ concentration and thus may have had an augmented delivery of H^+ to the tubular cells, and thence a faster response in H^+ secretion than sheep on the HN diet. Scott (1969) found that during the first 2 - 3 days of acid infusion, the concentration of bicarbonate in the urine declined as the pH of the urine fell and thereafter remained practically free of this ion until repair of the acidosis. Phillips (1968) could not detect any HCO_3^- in sheep urine once urine pH had decreased significantly. Since the filtered load of bicarbonate was decreased as plasma bicarbonate was decreased, virtually all the filtered bicarbonate was reabsorbed (Pitts et al., 1949). In the present study, in which plasma HCO_3^- concentration decreased less for HN sheep, possibly not all the bicarbonate filtered was reabsorbed, and thus HCO_3^- in the urine contributed to the higher pH during the initial stages of acid infusion. Schloeder and Stinebaugh (1977) using human subjects found that net acid excretion reached maximum values generally at the time when serum bicarbonate reached its lowest level, however this was not evident in the present study. For LN sheep, plasma bicarbonate was continually decreasing over three days, yet acid excretion appeared to be maximal within the first sixteen hours. Acid excretion became maximal for HN sheep after HCO_3^- had already reached its lowest point.

Hydrogen ion secretion in both proximal and distal tubules is accomplished by either cellular exchange of H^+ for Na^+ in the tubular urine (Pitts and Alexander, 1945; Berliner, 1952; and Rector et al, 1960) or as is currently believed by the passive exchange of H^+ down

Na^+ and H^+ concentration gradients in proximal tubules (Al-Awqati, 1978). The secreted H^+ is believed to react with filtered HCO_3^- to form H_2CO_3 which then dissociates to CO_2 and water, the CO_2 being reabsorbed. The net result is a decrease in urinary bicarbonate excretion and a restoration of depleted blood HCO_3^- (Rector, 1973).

Excess hydrogen ions entering the renal tubules can also combine with phosphate and be excreted as titratable acid. Sheep on the low nitrogen diet had an initially higher level of titratable acid excretion during the first sixteen hours of infusion than sheep on the high nitrogen diet. Titratable acid excretion diminished for LN sheep as the amount of acid infused increased. Titratable acid increased gradually for HN sheep during the developing acidosis, but thereafter remained stable. For both dietary treatments and in both experiments the excretion of titratable acid reached a level of about 0.020 mEq.kg BW^{-1} i.e. about 24 mEq.day^{-1}per 50 kg BW^{-1}. In a study of men ingesting 15 g NH_4Cl for two days, followed by 10 g daily for three days there was an increase in the excretion of phosphate from 41 mM/day to 60 mM/day by the third day of acidosis, (Sartorius et al., 1948). Acidosis in both man and dog is said to result in increased excretion of phosphate (i.e. $H_2PO_4^-$), most of which is derived from bone, resulting in negative phosphorous balance (Christensen, 1965). However, in sheep, the urine does not appear to be a major pathway for phosphorous excretion, and it also appears to be a limited route for excretion of H^+. Scott et al (1971) determined that excretion of phosphorous in the urine averaged only about 6% of the daily phosphorous intake and this rose to about 13% during intra-ruminal infusion of acid. Young et al., (1966) suggested that in sheep major variations in phosphate balance are regulated more by intestinal secretion rather than renal

excretion. Scott (1969) found that for sheep, excretion of phosphorous contributed less than 5 mmole.day^{-1} to the titratable acidity of urine, although the rates of HCl infusion into the rumen varied from 70 - 200 mmole.day^{-1}. Phillips (1968) found rates of excretion of titratable acid varying between 0.02 and 0.03 mEq.min^{-1} which contributed little towards compensating for the acid load which was increasing by about 1.6 mEq.min^{-1}. In the present experiment, the cumulative titratable acid excreted by LN sheep over four days could account for only about 16% of the infused amount of acid and for only about 10% by the HN sheep. According to Pitts (1948) the rate of excretion of titratable acid is determined by the rate of excretion of buffer, the acid strength of the buffer (pK') and the degree of acidosis, as reflected in the bicarbonate content of the plasma. The observation that titratable acid excretion reached a plateau level in this experiment and even decreased slightly as acid infusion continued would suggest that there is a limitation to the amount of acid excreted in this form in sheep. Tubular buffers (i.e. phosphate) must become saturated with hydrions and if little or no extraction occurs from bone stores and if reabsorption of intestinally secreted phosphate is limited by intake, no further increase in excretion of titratable acid can occur.

With the limitations on titratable acid excretion in ruminants, ammonia excretion as a means of buffering secreted H^+ is important. Pitts (1950) induced a moderately severe acidosis in dogs and found that the rate of ammonia excretion was inversely proportional to the urine pH. Schloeder and Stinebaugh (1977) found the correlation of ammonia excreted with urine pH to be -0.75 in the developing stages of acidosis in humans. In the present experiments, urine ammonia-N excreted was negatively

correlated with urine pH, r= -.38 and -.65 for low and high nitrogen treatments, respectively. These correlations are not as high as found by Schloeder and Stinebaugh (1977). Urine pH in the present experiment decreased gradually for HN sheep and therefore ammonia-N excreted increased gradually. For LN sheep, urine pH dropped sharply and remained at the same level as infusion progressed, while ammonia-N excreted increased steadily throughout acid infusion. Tannen and Ross (1979) suggested that trapping of ammonia in acid urine may be a critical component for stimulating ammonia production in acute acidosis. In Experiment 2, after infusion of 150 mEq of HCl within seven hours, mean urine pH dropped to 5.47 and 5.66 for LN and HN treatments, respectively. Ammonia excreted doubled for LN sheep and almost tripled for HN sheep. An increase in the capacity for renal ammonia formation could have developed within that short period and could possibly have been stimulated by ammonia trapping in acid urine. In Experiment 1, the increase in excretion of ammonia was gradual and more likely involved adaptation of cellular production, rather than stimulation by ammonia trapping in acid urine.

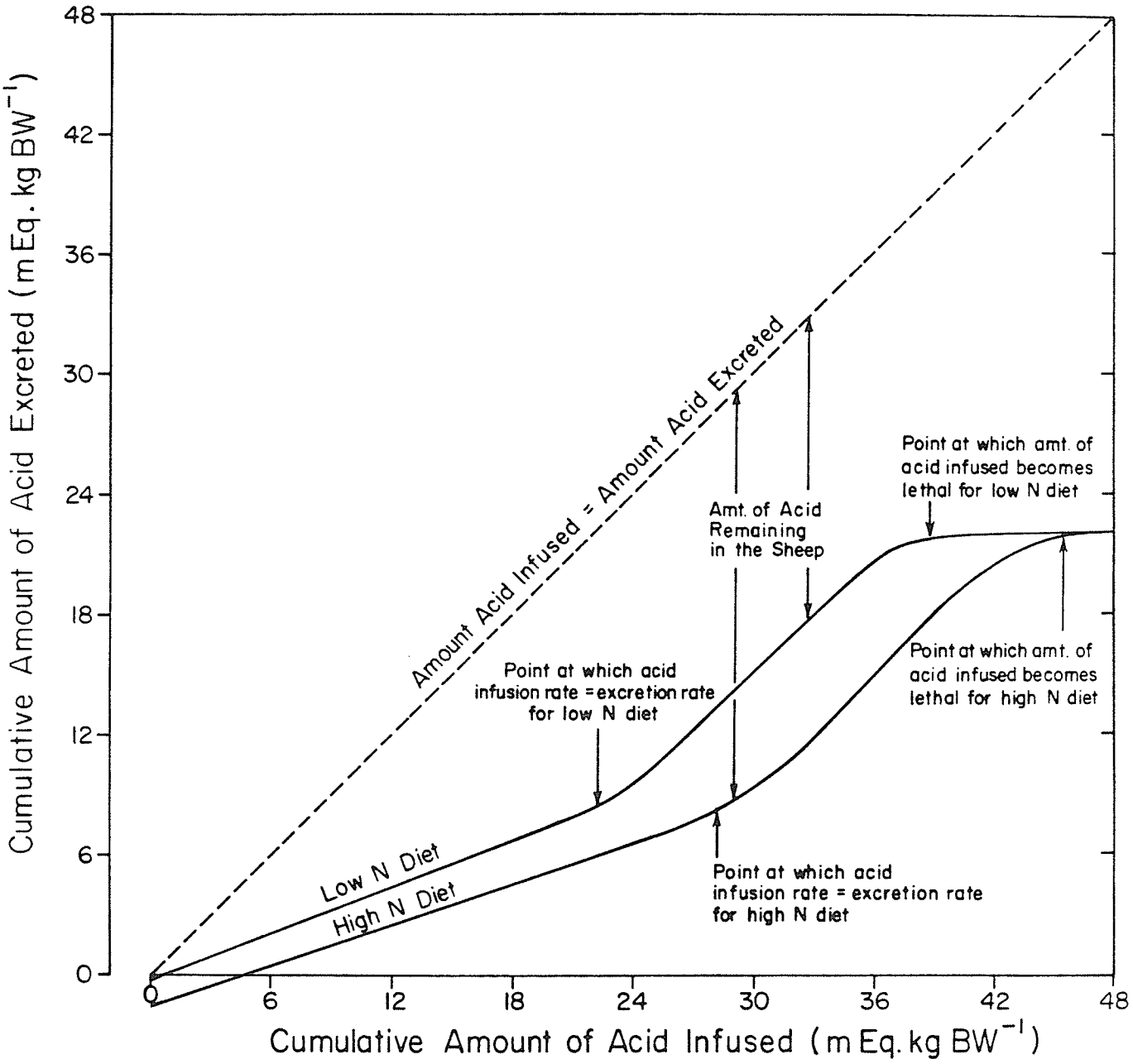
Ammonia-N excretion increased 30-fold for LN sheep and 7-fold for HN sheep during acid infusion but these rates of H^+ excretion represented only 26% and 10% of the rate of acid infusion. Scott (1969) infused a 0.15 M solution of HCl continuously for periods of up to 12 days at rates varying from 70 to 200 mmole.day^{-1}, to sheep maintained on a pelleted grass diet (12.2 g N.day^{-1}). Initial urinary excretion of ammonia was about 20 mEq.day^{-1}, while in the present experiment it was less than 5 mEq.day^{-1} for both treatments. During acid infusion, Scott (1969) found slightly higher rates of ammonia excretion. In one sheep

which he infused at a rate of 150 mEq.day^{-1} of acid, after four days, excretion of ammonia had tripled and its excretion rate represented about 40% of the infusion rate. Two other sheep, infused at a rate of 200 mEq.day^{-1} four days later excreted ammonia at a rate of 50% and 20% of the amount of acid infused. After about nine days of acid infusion Scott (1969) found that the rate of excretion of ammonia reached a level about equal to the rate of infusion of HCl into the rumen. In the present experiment, a longer infusion period may have substantially increased ammonia excretion to rates similar to those found by Scott.

Another possible route for excretion of acid may be by the intestinal tract and fecal excretion. Hydrogen ions may be exchanged with endogenous K^+ and Na^+ or may be combined with endogenous protein and ammonia. Assessment of fecal hydrogen ion concentrations and the forms in which it is present would be necessary to quantify the contribution of fecal H^+excretion to acid-base regulation.

A hypothetical model for the relationship between the cumulative amount of acid infused with the cumulative amount of acid excreted based on the findings shown in Fig. 3 and on Scott (1969) was designed (Fig. 4). Initially cumulative net acid excretion increases linearly for both treatments, however the amount of acid excreted by LN sheep is greater at any amount of acid infused than by HN sheep. The difference between the theoretical line which represents the situation where the amount of acid infused equals the amount of acid excreted and the individual treatments, constitutes the amount of acid remaining in the sheep which must be buffered by the body fluids. More acid remains in the body fluids of the HN sheep and since they were found to show less of a change in blood acid-base status and thus a lesser stimulation of acid

Figure 4. Hypothetical model for the relationship between the cumulative amount of acid infused and the cumulative amount of acid excreted. The dashed line represents the theoretical rate of net acid excretion when the amount of acid infused equals the amount of acid excreted.


Cumulative Amount of Acid Excreted (m Eq. kg BW^{-1})
48
42
36
30
24
18
12
6
0
0
6
12
18
24
30
36
42
48
Cumulative Amount of Acid Infused (m Eq. kg BW^{-1})
Amount Acid Infused = Amount Acid Excreted
Amt. of Acid Remaining in the Sheep
Point at which amt. of acid infused becomes lethal for low N diet
Point at which acid infusion rate = excretion rate for low N diet
Point at which amt. of acid infused becomes lethal for high N diet
Low N Diet
High N Diet
Point at which acid infusion rate = excretion rate for high N diet

excretion mechanisms occurs. A further hypothesis is that if the present experiment had been more prolonged a curvilinear relationship would have developed at some point so that the rate of acid infusion equaled the rate of acid excretion. This point is reached at a lower cumulative acid load for LN sheep because of the greater depletion of blood buffers and also because of a greater stimulation of ammonia production. Finally it is suggested that if acid infusion continued indefinitely the rate of excretion could no longer keep pace with the rate of infusion, possibly due to limitations of nitrogen supply for ammonia production and saturation of kidney transport mechanisms and therefore the dose would become lethal. Again this point is reached at a lower amount of acid infused for LN sheep due to less blood buffering ability and available nitrogen. This model excludes other factors which may affect the amount of acid excreted, such as sheep going "off feed" and therefore starvation conditions.

The relationship between urea and ammonia excretion during acid infusion was not as easily defined for ruminants as for monogastrics. Oliver and Bourke (1975) found that HCl acidosis significantly reduced urea-N excretion in the rat with an equimolar increase in ammonia-N excretion and thus no change in total-N excretion.

In man, HCl acidosis reduced blood and urinary urea excretion with a concomittant rise in ammonia excretion (Fine et al., 1977). However, the fall in urea-N excretion was significantly greater (two times) than the rise in ammonia excretion, resulting in a fall in their sum. The situation in ruminant animals may be more complex because of their ability to conserve urea nitrogen when their diet is inadequate in protein

Schmidt-Nielsen et al., 1957; 1958). In the present experiment, during acid infusion, total-N excretion did not change significantly for LN sheep. Urea-N as a percentage of total-N excreted, remained the same, however ammonia-N as a percentage of total-N excreted increased by 15.3%. The percentage as other-N i.e. undetermined nitrogen consequently decreased during acid infusion by 17% which suggests that nitrogen excreted by that route was redirected to the production of ammonia. Before acid infusion the urinary excretion of other-N was similar, for both dietary treatments suggesting a basal level of nitrogen excretion regardless of the level of dietary intake. Since total-N excreted on the LN diet was lower, other-N made up a larger proportion of the total. Identification of the components comprising other-N is necessary before any speculation can be made as to how this nitrogen becomes available to the kidney, possibly for ammonia production. The high nitrogen sheep showed evidence of diversion of waste nitrogen normally excreted as urea, to excretion of this nitrogen as ammonia. Urine urea-N decreased significantly and ammonia-N increased significantly during acidosis. The percentage urea-N of the total decreased by 16.9%. It is evident that this decrease was not equimolar with the increase in ammonia-N as this is of the magnitude of only 2.3%. Urinary percentage of other-N increased by 14.4%, which suggested that the nitrogen diverted from urea production and not utilized for ammonia production (16.9 - 2.3 = 14.6%) was appearing in the urine as undetermined nitrogen. The fall in plasma urea-N from 11.23 to 9.95 $mg.dl^{-1}$ for the HN sheep tends to confirm the proposition of Fine et al. (1977) that acidosis has some effect on hepatic urea formation.

Sheep on the low nitrogen diet had a significantly lower plasma urea-N as agrees with the findings of McIntyre and Williams (1970),

Scott and Mason (1970), Rabinowitz et al. (1973) and Ergene and Pickering (1978 a). This lower plasma urea-N was accompanied by a lower urea excretion and an increase in the fraction of filtered urea reabsorbed. Sheep on the low N diet also had a significantly higher creatinine and urea-N urine/plasma concentration ratio indicating a greater tendency for urea to diffuse from the tubular lumen to peritubular fluid. During acid infusion, plasma urea-N, urea-N filtered, excreted, and reabsorbed all increased, while urea clearance and the fraction of filtered urea-N reabsorbed was related to the plasma urea-N, but since the fraction of filtered urea-N reabsorbed remained the same, this suggests that there was no change in the ability of the kidney to conserve urea during acidosis on a restricted nitrogen intake.

The high nitrogen sheep reacted differently to acid infusion in terms of urea-N excretion and reabsorption. Urine flow rate decreased during infusion as evidenced by the increased U/P creatinine ratio. The fraction of filtered urea reabsorbed increased along with an increase in the U/P urea ratio. The increased reabsorption of urea-N appeared to be a passive process, whereby urea reabsorption followed water reabsorption.

Glomerular filtration is caused by the hydrostatic pressure inside glomerular capillaries opposed by colloid osmotic pressure in the blood and hydrostatic pressure in Bowman's capsule (Guyton, 1976). Guyton cited the factors which can affect the rate of glomerular filtration as glomerular capillary pressure, plasma colloid osmotic pressure, intracapsular hydrostatic pressure, arterial blood pressure, rate of renal blood flow, and sympathetic stimulation. In both experiments reported herein, glomerular filtration rate was lower for sheep on a low nitrogen diet. Ergene and Pickering (1976) found that GFR of sheep fed a low

protein diet was apparently influenced by the concentration of plasma urea, however changes in GFR and plasma urea do not follow the same time course. Gans and Mercer (1962) observed a progressive increase in GFR in five of eight experiments with sheep, in which urea solutions were given after a period of protein deprivation. Contrary to the above findings, other researchers (Schmidt-Nielsen et al., 1958; McIntyre and Williams, 1970; Pang, 1971) found no significant differences in GFR when dietary protein level was varied. Rabinowitz et al.(1973) fed ewes 4.9% and 14% protein diets and found GFR was lower for the low protein diet, a result confirmed in the present studies. Lower GFR and plasma urea-N resulted in a lower quantity of urea filtered in LN compared to HN diets. Ergene and Pickering (1978a) regarded this as a renal response to low protein feeding which in conjunction with an increase in the fraction of filtered urea reabsorbed restricts urinary losses of urea and thus contributes to the nitrogen economy.

Acidosis in the present study appeared to have no effect on glomerular filtration rate. This finding agrees with the results reported by McIntosh et al. (1979) using human subjects. A decrease in GFR would have been advantageous to the increased ammonia excretion in acidosis, since more time would be allowed for filtered glutamine to be reabsorbed by the tubular cells. However since some glutamine is also extracted across the peritubular membrane of the blood vessels, this extraction may be influenced by the renal blood flow (Pilkington et al., 1970). Metabolic acidosis induced in sheep by oral or intra-ruminal acid infusion has been reported by other workers to produce variable responses in renal plasma flow. In the present study, no significant changes were found in the renal plasma flow either by infusion of 125 - 150 mEq or

three days later after infusion of 575 - 600 mEq. McIntosh et al. (1973) found a mean reduction in blood pH of sheep from 7.45 to 7.20 accompanied by a decrease in renal blood flow. It seems however that factors other than systemic acidosis could have been involved in the effect on RBF. In their study, one animal had a small decrease in blood pH (7.49 to 7.45) and a marked reduction in renal blood flow, whereas another animal showed the opposite effect; one sheep showed no change in RBF, and two others were in a state of inanition. Heitman and Bergman (1978) found that RBF was not altered when the blood pH of sheep was reduced from 7.52 to 7.35. Intravenous infusion of hydrochloric acid to sheep, which reduced blood pH from 7.45 to 7.40, had no effect on renal plasma flow (Williams and Pickering, 1980). Huber (1969) caused acute lactic acidosis in sheep severely reducing blood pH by 0.55 units and decreasing renal blood flow by 40.6%. The lack of effect on RPF found in the present study and that of Heitman and Bergman (1978) could have been due to lesser increases in the plasma H^+ concentration. Plasma pH was reduced by 0.06 and 0.07 units for LN and HN sheep after the loading dose was given, and by 0.09 and 0.03 units for LN and HN respectively three days later. Heitman and Bergman (1978) decreased blood pH by 0.17 units. Huber (1969) caused a decrease from 7.43 to 6.88, within 40 minutes of the start of lactic acid infusion. Telle and Preston (1971) also studying acute lactic acidosis in sheep found that hematocrit increased markedly and heart rate increased 10 to 50%. A possible reduction in circulating blood volume due to dehydration could cause decreased RBF.

No effect of dietary nitrogen level on renal plasma flow was found. Although glomerular filtration rate was lower for sheep on the low nitrogen diet as was the fraction of plasma that was filtered through the glomeruli,

a change in renal plasma flow was not the causative factor. These findings are similar to those of Ergene and Pickering (1978b), who found that a reduction in dietary nitrogen of sheep caused a significant reduction in GFR with no change in RPF. It can only be concluded that the dietary nitrogen level has some effect on GFR by a yet undetermined mechanism. The most possible factor affected by dietary nitrogen intake is plasma colloid osmotic pressure, which is one of the factors which can affect glomerular filtration rate. An increase in this pressure somehow caused by the low nitrogen diet could cause decreased filtration. An examination of the effects of low nitrogen diets on plasma colloid osmotic concentration, plasma and urine osmolalities, and osmolar clearances would be necessary.

To maintain an increased ammonia production in the kidney, the supply of precursor, glutamine must be increased. Since renal plasma flow, GFR, and arterial plasma glutamine concentration were not changed during acidosis, extraction rate of glutamine from renal blood must have been increased to supply the kidney with adequate amounts of glutamine. In the present experiment, no significant difference was found between plasma glutamine levels of the HN and LN treatments. In fact, for the low nitrogen treatment mean glutamine level tended to decrease during acidosis. The HN group showed no such trend as there seemed to be a slightly elevated glutamine concentration during acidosis. Heitman and Bergman (1978) found that mean arterial glutamine level was 210 $\mu mol.l^{-1}$ for normal sheep, while acidotic sheep had a lower concentration of 178 $\mu mol.l^{-1}$. They suggested that this decrease in glutamine concentration caused the body to increase its glutamine release and thus compensate for the changes in renal metabolism. Plasma glutamine concentration

remained unaltered in acidotic human subjects and in acidotic dogs (Fine et al., 1977; Pitts, 1972). Pitts suggested that although the amount of glutamine presented to the kidney remained unaltered, the amount of glutamine extracted and utilized each minute increased markedly. Heitman and Bergman (1975) demonstrated that there was a dramatic shift from net renal glutamine release in normal sheep to net uptake in acidosis. Bergman and Heitman (1978) found that release of glutamine from muscle was also increased in acidosis. In the present experiment, it appeared for LN sheep that non-renal release of glutamine was not sufficient to keep pace with its increased renal uptake and arterial glutamine concentration decreased as acidosis progressed. Presumably the HN sheep would have had more readily available protein stores and therefore increased glutamine release and renal glutamine uptake could have kept pace with the increased extraction from arterial blood. As discussed previously, fractional reabsorption of urea-N was increased during acidosis for HN sheep, and this recirculated nitrogen would perhaps have been converted into glutamine by the liver. It was also noted that plasma ammonia-N levels followed similar trends to plasma glutamine. For the LN sheep, as glutamine concentration decreased in acidosis, ammonia-N concentration decreased, and vice-versa for the HN sheep. This relationship can be more easily explained for the HN sheep. Addae and Lotspeich (1968) found that glutamine utilization by the gut increased during acidosis, which would increase the release of ammonia into plasma and raise its concentration. This ammonia-N is a substrate for glutamine synthesis in the liver and may act to stimulate liver glutamine production. Sheep on LN had a slight decrease in arterial plasma ammonia-N during acidosis in Experiment 2, however in Experiment 1, venous ammonia-N increased. This

difference may be due to the greater variabilty among sheep in Experiment 2 or may be due to the loading dose used in this experiment which may have caused withdrawl of ammonia from blood to form glutamine and thus counteract quickly the sudden acid stress.

The acidosis and level of dietary nitrogen were confounded by the varying degrees of acidosis found on the two diets, which precluded the assessment of the experimental objectives as originally proposed. The first hypothesis was that urinary ammonia-N excretion is at the expense of urea-N excretion, with no change in total-N excretion. Low nitrogen sheep had an increase in ammonia-N excretion at the expense of other-N excretion, with no change in total-N excretion. High nitrogen sheep had an increase in ammonia-N excretion, an increase in other-N excretion, and a decrease in urea-N excretion with no change in total-N excretion. Whether these differences between the dietary treatments were due to nitrogen level alone or to the varying degree of acidosis could not be determined. The second hypothesis was that in acidosis, an increased supply of glutamine to the kidney occurs because of an increased plasma concentration of glutamine and/or an increased renal plasma flow. Plasma glutamine concentration and renal plasma flow were not increased during acidosis for either dietary treatment. It can be tentatively concluded that an increased extraction of glutamine by the kidneys occurred in acidosis and was maintained by an increased release of glutamine by the liver. More detailed examination is required to assess the turnover rate of glutamine in the liver during acidosis.

SUMMARY AND CONCLUSIONS

1. Sheep on the low nitrogen diet had a greater decrease in blood acid-base values than sheep consuming the high nitrogen diet and receiving the same intra-ruminal dose of hydrochloric acid. This suggests decreased buffering ability is caused by a low nitrogen diet, possibly in the intracellular protein buffering.
2. Urine pH decreased and excretion of net acid increased to a greater degree for the low nitrogen sheep. This increase in net acid excretion on a low nitrogen diet could be a response to the greater decrease shown in blood bicarbonate in these sheep. Presentation of increased H^+ to the tubular cells would have stimulated increased H^+ secretion and a concommittant increase in the reabsorption of bicarbonate from tubular fluid. After infusion of 600 mEq of acid, about 37% had appeared in urine of LN sheep and 20% in the urine of HN sheep as net acid. Some of the infused acid may have been excreted in feces. Assessment of the contribution of fecal H^+ excretion to acid-base balance would involve measurement and identification of fecal H^+.
3. Excretion of acid as titratable acid appeared to be a limited response to acidosis in sheep on either high or low nitrogen diets. Titratable acid excretion did not increase above 0.020 $mEq.hr^{-1}.kg\ BW^{-1}$ suggesting that excretion of phosphate buffer was limited and saturation of the buffer present had occurred.
4. Sheep on the low nitrogen had both an immediate rise in ammonia production and a steady increase throughout acid infusion. Excretion increased thirty-fold. However this rate of ammonia excretion only represented 26% of the rate of acid infusion. For high nitrogen sheep ammonia

excretion rate increased seven-fold which was 10% of the rate of acid infusion. There appeared to be greater stimulation and therefore adaptation to increased ammonia excretion occurred sooner for the low nitrogen sheep.

5. For sheep on the low nitrogen diet, total-N and urea-N were unchanged during acidosis, while other-N excreted decreased and ammonia-N excreted increased. For sheep on the high nitrogen diet, some nitrogen normally excreted as urea was apparently rerouted to glutamine synthesis, and thence to ammonia.
6. Sheep on the low nitrogen diet had lower plasma urea nitrogen levels and creatinine and urea clearances. In spite of this, fractional reabsorption of urea-N was higher. Superimposing acidosis on the limitation of dietary nitrogen caused no change in the fractional reabsorption of urea. Sheep on the high nitrogen diet during acid infusion increased the fraction of filtered urea reabsorbed and decreased the amount of urea excreted.
7. No change in renal plasma flow was apparent between high and low nitrogen treatments before and during acid infusion. Renal plasma flow was therefore not a causative factor in the significantly lower glomerular filtration rate and filtration fraction found for the low nitrogen sheep.
8. Plasma glutamine and ammonia-N decreased as acid infusion progressed in low nitrogen sheep but both parameters increased in high nitrogen sheep. It is suggested that for low nitrogen sheep, increased peripheral glutamine production and/or release did not keep pace with the increase in glutamine utilization in the kidney for ammonia production. The adaptive response of increased ammonia production occurred as rapidly in low nitrogen sheep as in high nitrogen sheep

although the mechanism which triggers increased production is unknown. Also unknown is the mechanism whereby possible release of glutamine from other tissues occurred in the low nitrogen sheep. For high nitrogen sheep, increased blood ammonia-N may have triggered increased glutamine release.

9. The effects of acidosis and nitrogen level were confounded by the varying degree of acidosis found, although both treatments received the same acid load. Thus, the assessment of the original objectives was affected. The relationship between urea-N and ammonia-N may be affected by the degree of acidosis as well as dietary nitrogen. Glutamine concentration and renal blood flow were not increased in acidosis. Assessment of liver production and release of glutamine during acidosis is necessary.

REFERENCES

Adam, W. P., and D. P. Simpson. 1973. Accelerated transport of glutamine by renal mitochondria in metabolic acidosis. Clin. Res. 21:226A.

Addae, S. K., and W. D. Lotspeich. 1968. Relation between glutamine utilization and production in metabolic acidosis. Am. J. Physiol. 215:269-277.

Al-Awqati, W. 1978. H^+ transport in urinary epithelia. Am. J. Physiol. 235:F77-F88.

Al-Awqati, W., B. R. Mueller, and A. Steinmetz. 1975. Site of stimulation of H^+ secretion by aldosterone in turtle bladder. Clin. Res. 23:345A.

Alleyne, G. A. O., and G. H. Schullard. 1969. Renal metabolic response to acid-base changes. I. Enzymatic control of ammoniagenesis in the rat. J. Clin. Invest. 48:364-370.

Balagura, S., and R. F. Pitts. 1962. Excretion of ammonia injected into renal artery. Am. J. Physiol. 203:11-14.

Balagura-Baruch, S., L. Shurland, and T. C. Welbourne. 1970. Effects of α-ketoglutarate on renal ammonia release in the intact dog. Am. J. Physiol. 218:1070-1075.

Bennett, F. I., N. McFarlane-Anderson, and G. A. Alleyne. 1979. The relationship of plasma glutamine to renal metabolism in acute acidosis. Biochem. Med. 22:375-382.

Berliner, R. W. 1952. Renal excretion of potassium and hydrogen ions. Fed. Proc. 11:695-700.

Bone, J. F., J. Metcalfe, and J. T. Parer. 1962. Surgical preparation of a carotid loop in sheep. Am. J. Vet. Res.23:1113-1116.

Burnell, J. M., E. J. Teuber, and D. P. Simpson. 1974. Metabolic acidosis accompanying potassium deprivation. Am. J. Physiol. 227:329-333.

Canessa-Fischer, M., R. Shalhoub, S. Glabman, J. deHaas, and R. F. Pitts. 1963. Effects of infusions of ammonia, amides, and amino acids on excretion of ammonia. Am. J. Physiol. 204:192-196.

Christensen, H. W. 1965. Body fluids and the acid-base balance. W. B. Saunders Co., Philadelphia.

Clark, R. C., A. M. Beal, O. E. Budtz-Olsen, R. B. Cross, J. J. French, J. M. Nave, and M. J. O'Callaghan. 1968. The effects of intravenous infusion of acid, alkali, and phosphate in sheep. Aust. J. Exp. Biol. Med. Sci. 46:P-15.

Davies, B. M. A., and J. Yudkin. 1952. Studies on biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. Biochem. J. (Lond) 52:407-412.

Elam, C. J. 1976. Acidosis in feedlot cattle: Practical observations. J. Anim. Sci. 43:898-909.

Elkington, J. R. 1962. Hydrogen ion turnover in health and in renal disease. Ann. Intern. Med. 57:660-684.

Ergene, N., and E. C. Pickering. 1976. The effect of reducing dietary nitrogen and of infusing urea on glomerular filtration rate and renal plasma flow in sheep. J. Physiol. (Lond) 254:73-74P.

Ergene, N., and E. C. Pickering. 1978a. The effects of reducing dietary nitrogen and increasing sodium chloride intake on urea excretion and reabsorption and on urine osmolality in sheep. Q. J. Exp. Physiol. Cong. Med. Sci. 63:67-76.

Ergene, N., and E. C. Pickering. 1978b. The effects of urea infusion on glomerular filtration rate and renal plasma flow on sheep fed low and high protein diets. Q. J. Exp. Physiol. Cong. Med. Sci. 63:77-81.

Fine, A., F. I. Bennett, and G. A. O. Alleyne. 1978. Effect of acute acid-base alterations on glutamine metabolism and renal ammoniagenesis in the dog. Clin. Sci. Mol. Med. 54:503-508.

Fine, A., J. E. Carlyle, and E. Bourke. 1977. The effects of administrations of HCl, NH_4Cl, and NH_4HCO_3 on the excretion of urea and ammonium in man. Eur. J. Clin. Invest. 7:587-589.

Ganong, W. F. 1977. Review of medical physiology, 8th edition. Lange Medical Publications, Los Altos.

Gans, J. H., and P. F. Mercer. 1962. Effect of osmotic diuretics and vasopressin on renal function in the sheep. Am. J. Vet. Res. 23:230-235.

Glabman, S., R. M. Klose, and G. Giebisch. 1963. Micropuncture study of ammonia excretion in the rat. Am. J. Physiol. 205:127-132.

Goldstein, L. 1965. Actinomycin D inhibition of the adaptation of renal glutamine-deaminating enzymes in the rat. Nature (Lond) 205:1330-1331.

Goldstein, L. 1975. Regulation of renal glutamine deamination. Med. Clin. N. Am. 59:667-680.

Goldstein, L., J. M. Boylan, and T. A. Boyd. 1977. α-Ketoglutarate regulation of glutamine transport and deamidation in renal mitochondria. In Current problems in clinical biochemistry. Vol. 8. Biochemical nephrology. Page 273-279. Hans Huber Pub., Bern.

Goldstein, L., and J. M. Schooler. 1967. Regulation of ammonia production in the rat kidney. Adv. Enzyme. Regul. 5:71-86.

Goodman, A. D., R. E. Fuisz, and G. F. Cahill. 1966. Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency. Its possible role in regulation of renal ammonia production. J. Clin. Invest. 45:612-619.

Guyton, A. C. 1976. Textbook of medical physiology, 5th edition. W. B. Saunders Co., Philadelphia.

Harvey, R. J. 1974. The kidneys and the internal environment. Chapman and Hall, London.

Heitman, R. N., and E. N. Bergman. 1975. Effect of acidosis or fasting on liver, kidney, and peripheral glutamine metabolism and gluconeogenesis in sheep. Fed. Proc. 34:481A.

Heitman, R. N., and E. N. Bergman. 1978. Glutamine metabolism, interorgan transport, and glucogenicity in the sheep. Am. J. Physiol. 234:E197-E203.

Hems, D. A. 1974. Biochemical aspects of renal ammonia formation in metabolic acidosis. Enzyme 20:359-380.

Hems, D. A., and J. T. Brosnan. 1971. Effects of metabolic acidosis on the content of intermediary metabolites in rat kidney. Biochem. J. (Lond) 123:391-397.

Houpt, T. R. 1977. Water, electrolytes, and acid-vase balance. In Duke's physiology of domestic animals, 9th edition. Page 443-492. M. J. Swenson, ed. Comstock Pub. Assoc., Ithaca.

Huber, T. L. 1969. Lactic acidosis and renal function in sheep. J. Anim. Sci. 29:612-615.

Hughey, R. P., B. B. Rankin, and N. P. Curthoys. 1980. Acute acidosis and renal arteriovenous differences of glutamine in normal and adrenalectomized rats. Am. J. Physiol. 238:F199-F204.

Kaufman, C. F., and E. N. Bergman. 1971. Renal glucose, free fatty acid, and ketone body metabolism in the unanesthetized sheep. Am. J. Physiol. 221:967-972.

Kovačević, Z. 1977. Mechanism of carrier-mediated glutamine transport across the inner mitochondrial membrane. In Current problems in clinical biochemistry. Vol. 8. Biochemical nephrology. Page 254-264. Hans Huber Pub., Bern.

Kronfeld, D. S. 1976. Metabolic and respiratory adjustments of acid-base balance and the burden of exogenous acid in ruminants. In Buffers in ruminant physiology and metabolism. Page 32-48. M. S. Weinberg and A. L. Sheffer, eds. Church and Dwight Co. Inc., N. Y.

Lee, E. A., and R. B. Cross. 1969. The role of salivary urea in nitrogen conservation in protein-deficient sheep. Aust. J. Exp. Biol. Med. Sci. 47:P- 6.

Lemann, J., and E. J. Lennon. 1972. Role of diet, gastrointestinal tract and bone in acid-base homeostasis. Kidney Int. 1:275-279.

Linzell, J. L. 1963. Carotid loops. Am. J. Vet. Res. 24:223-224.

MacKenzie, D. D. 1967. Production and utilization of lactic acid by the ruminant. A. review. J. Dairy Sci. 50:1772-1786.

Mahnensmith, R., S. O. Thier, C. R. Cooke, A. Broadus and R. A. DeFronzo. 1979. Effect of acute metabolic acidemia on renal electrolyte transport in man. Metab. Clin. Exp. 28:831-842.

Malnic, G. 1974. Tubular handling of H^+. In Kidney and urinary tract physiology. Vol. 6. Page 107-127. K. Thuraw, ed. Butterworths and Co., London.

Malnic, G. and G. Giebisch. 1972. Mechanism of hydrogen ion secretion. Kidney Int. 1:280-296.

Malnic, G., and F. L. Vieira. 1972. The antimony microelectrode in kidney micropuncture. Yale J. Biol. Med. 45:356-367.

McCance, R. A., and E. M. Widdowson. 1942. The chemical composition of foods, 3rd edition. H. M. Stationary Office, London.

McIntosh, G. H., O. H. Filsell, and I. G. Jarrett. 1973. Kidney function and net glucose production in normal and acidotic sheep. Aust. J. Exp. Biol. Med. Sci. 26:1389-1394.

McIntyre, K. H., and V. J. Williams. 1970. The role of the kidney in nitrogen conservation in sheep. Aust. J. Exp. Biol. Med. Sci. 48:81-91.

Montgomery, H. and J. A. Pierce. 1937. The site of the acidification of the urine within the renal tubule in amphibia. Am. J. Physiol. 118:114-152.

Morris, C. 1979. Renal tubular acidosis: Pathophysiology and therapy. In Regulation of acid-base balance. Page 23-40. W. M. Hale and P. Meinhardt, eds. Church and Dwight Co. Inc., Piscataway.

Morris, S., and S. C. Ray, 1939. The fasting metabolism of ruminants. Biochem. J. (Lond) 33:1217-1230.

Nash, T. P., and S. R. Benedict. 1921. The ammonia content of blood and its bearing on the mechanism of acid neutralization in the animal organism. J. Biol. Chem. 48:463-488.

National Research Council. 1975. Nutrient requirements of sheep, 5th edition. National Academy of Sciences, Washington, D. C.

Oliver, J., and E. Bourke. 1975. Adaptations in urea ammonium excretion in metabolic acidosis in the rat: a reinterpretation. Clin. Sci. Mol. Med. 48:515-520.

Orloff, J., and R. W. Berliner, 1956. The mechanism of the excretion of ammonia in the dog. J. Clin. Invest. 35:223-235.

Pang, C. Y. 1971. Mechanisms of urea excretion in sheep. MSc. Thesis. Univ. of Manitoba. Winnipeg, Manitoba.

Peters, J. P., and D. D. Van Slyke. 1932. Quantitative clinical chemistry: Interpretations. Williams and Wilkins Co., Baltimore.

Phenix, P., and T. C. Welbourne. 1975. Renal glutaminases: Diamox inhibition of glutamyltransferase. Am. J. Physiol. 228:1269-1275.

Phillips, G. D. 1968. Studies on the regulation of the reaction of body fluids in ruminants. PhD. Thesis. Univ. of Liverpool, Liverpool, England.

Phillips, G. D., E. C. Pickering, and K. Wilkinson. 1975. Renal responses of the cow to alteration of the dietary intake of nitrogen and sodium chloride. J. Physiol. (Lond) 245:95-96P.

Phillipson, A. T. 1977. Ruminant digestion. In Duke's physiology of domestic animals, 9th edition. Page 250-286. Comstock Pub. Assoc., Ithaca.

Pilkington, L. A., J. Welch, and R. F. Pitts. 1965. Relationship of pHN_3 of tubular cells to renal production of ammonia. Am. J. Physiol. 208:1100-1106.

Pilkington, L. A., T. K. Young, and R. F. Pitts. 1970. Properties of renal and antiluminal transport of plasma glutamine. Nephron 7:51-60.

Pitts, R. F. 1945. The renal regulation of acid-base balance with special reference to the mechanism for acidifying the urine, I and II. Science 102:49-54 and 82-85.

Pitts, R. F. 1948. Renal excretion of acid. Fed. Proc. 7:418-426.

Pitts, R. F. 1950. Acid-base regulation by the kidneys. Am. J. Med. 9:356-372.

Pitts, R. F. 1964. Renal production and excretion of ammonia. Am. J. Med. 36:720-742.

Pitts, R. F. 1971. The role of ammonia production and excretion in the regulation of acid-base balance. N. Engl. J. Med. 284:32-38.

Pitts, R. F. 1972. Control of renal production of ammonia. Kidney Int. 1:297-305.

Pitts, R. F. 1973. Production and excretion of ammonia in relation to acid-base regulation. In Renal physiology. Section 8. Handbook of physiology. Page 455-496. J. Orloff and R. W. Berliner, eds. Am. Physiol. Soc., Washington, D.C.

Pitts, R. F. 1976. Physiology of the kidneys and body fluids, 3rd edition. Yearbook Medical Pubs., Chicago.

Pitts, R. F. and R. S. Alexander. 1945. The nature of the renal tubular mechanism for acidifying the urine. Am. J. Physiol. 144:239-254.

Pitts, R. F., J. L. Ayer, and W. A. Schiess. 1949. The renal regulation of acid-base balance in man. III. The reabsorption and excretion of bicarbonate. J. Clin. Invest. 28:35-44.

Pitts, R. F., W. D. Lotspeich, W. D. Schiess, and J. L. Ayer. 1948. The renal regulation of acid-base balance in man. I. The nature of the mechanism for acidifying the urine. J. Clin. Invest. 27:48-56.

Pitts, R. F., L. A. Pilkington, M. B. MacLeod, and E. Leal-Pinto. 1972. Metabolism of glutamine by the intact functioning kidney of the dog. J. Clin. Invest. 51:557-565.

Pollak, V. E., H. Mattenheimer, H. DeBruin, and K. J. Weinman. 1965. Experimental acidosis: The enzymatic basis of ammonia production by the dog kidney. J. Clin. Invest. 44:169-181.

Rabinowitz, L., R. A. Gunther, E. S. Shoji, R. A. Freedland, and E. H. Avery. 1973. Effects of high and low protein diets on sheep renal function and metabolism. Kidney Int. 4:188-207.

Rector, F. C. 1973. Acidification of the urine. In Renal physiology. Section 8. Handbook of physiology. Page 431-454. J. Orloff and R. W. Berliner, eds. Am. Physiol. Soc., Washington, D. C.

Rector, F. C., N. W. Carter, and D. W. Seldin. 1965. The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. J. Clin. Invest. 44:278-290.

Rector, F. C. and J. Orloff. 1959. The effect of the administration of sodium bicarbonate and ammonium chloride on the excretion and production of ammonia. The absence of alterations in the activity of renal ammonia producing enzymes in the dog. J. Clin. Invest. 38:366-372.

Rector, F. C., D. W. Seldin, and J. H. Copenhaver. 1955. The mechanisms of ammonia excretion during ammonium chloride acidosis. J. Clin. Invest. 34:20-25.

Rector, F. C., D. W. Seldin, A. D. Roberts, and J. S. Smith. 1960. The role of plasma CO_2 tension and carbonic anhydrase activity in the renal reabsorption of bicarbonate. J. Clin. Invest. 39:1706-1721.

Relman, A. S. and R. G. Nairns. 1975. The control of ammonia production in the rat. Med. Clin. N. Am. 59:583-593.

Roxe, D. M. 1972. Renal gluconeogenesis after NH_4Cl, $NaHCO_3$, hypoglycemia, or pregnancy. Am. J. Physiol. 222:55-60.

Sartorius, O. W., J. C. Roemmelt, and R. F. Pitts. 1948. The renal regulation of acid-base balance. IV. The nature of the renal compensations in ammonium chloride acidosis. J. Clin. Invest. 28:423-439.

Sayre, F. W., and E. Roberts. 1958. Preparation and some properties of a phosphate-activated glutaminase from kidneys. J. Biol. Chem. 233:1128-1134.

Schiess, W. A., J. L. Ayer, W. D. Lotspeich, and R. F. Pitts. 1948. The renal regulation of acid-base balance in man. II. Factors affecting excretion of titratable acid by the normal human subject. J. Clin. Invest. 27:57-64.

Schloeder, F. X., and B. J. Stinebaugh. 1977. Urinary ammonia content as a determinant of urinary pH during chronic metabolic acidosis. Metab. Clin. Exp. 26:1321-1331.

Schmidt-Nielsen, B., and H. Osaki. 1958. Renal response to changes in nitrogen metabolism in sheep. Am. J. Physiol. 193:657-661.

Schmidt-Nielsen, B., H. Osaki, H. V. Murdaugh, and R. O'Dell. 1958. Renal regulation of urea excretion in sheep. Am. J. Physiol. 194:221-228.

Schoolwerth, A. C., B. L. Nazar, and K. F. LaNoue. 1977. The role of glutamate metabolism in ammonia formation by rat kidney mitochondria. In Current problems in clinical biochemistry. Vol. 8. Biochemical nephrology. Page 265-272. Hans Huber Pub., Bern.

Schoolwerth, A. C., B. L. Nazar, and K. F. LaNoue. 1978. Glutamate dehydrogenase activation and ammonia formation by rat kidney mitochondria. J. Biol. Chem. 253:6177-6183.

Schwartz, W. B., R. L. Jenson, and A. S. Relman. 1954. The disposition of acid administered to sodium depleted subjects: The renal response and the role of the whole body buffers. J. Clin. Invest. 33:587-598.

Scott, D. 1969. Renal excretion of potassium and acid by sheep. Q. J. Exp. Physiol. Cogn. Med. Sci. 54:412-422.

Scott, D. 1972. Excretion of phosphorous and acid in the urine of sheep and calves fed either roughage or concentrate diets. Q. J. Exp. Physiol. Cogn. Med. Sci. 57:379-392.

Scott, D., and G. D. Mason. 1970. Renal tubular reabsorption of urea in sheep. Q. J. Exp. Physiol. Cogn. Med. Sci. 55:275-283.

Scott, D., F. G. Whitelaw, and M. Kay. 1971. Renal excretion of acid in calves fed either roughage or concentrate diets. Q. J. Exp. Physiol. Cogn. Med. Sci. 56:18-32.

Sebastian, A., M. Schambelan, S. L. Lindenfield, and R. C. Morris. 1977. Amelioration of metabolic acidosis with fludrocortisone therapy in hydroreninemic hypoaldosteronism. N. Engl. J. Med. 297:576-583.

Sendroy, J., S. Seelig, and D.D. Van Slyke. 1934. Studies on acidosis. XXIII. The carbon dioxide tension and acid-base balance of human urine. J. Biol. Chem. 106:479-500.

Seyama, S., T. Saheki, and N. Katunuma. 1971. Adaptation of nitrogen metabolism in kidney for acidosis. Symp. Chem. Physiol. Path. Proc. 11:165-169.

Shalhoub, R., W. Webber, S. Glabman, M. Canessa-Fischer, J. Klein, and R. F. Pitts. 1963. Extraction of amino acids from and their addition to renal blood plasma. Am. J. Physiol. 204:181-186.

Smith, H. 1937. The physiology of the kidney. Oxford Univ. Press. N. Y.

Somers, M. 1961. Factors influencing the secretion of nitrogen in sheep saliva. 4. The influence of injected urea on the recovery of urea in the parotid saliva and the urinary excretions of sheep. Aust. J. Exp. Biol. Med. Sci. 39:145-156.

Steenbock, H., V. E. Nelson, and E. B. Hart. 1914. Acidosis in omnivera and herbivora and its relation to protein storage. J. Biol. Chem. 19:339-419.

St. Omer, V. V., and W. K. Roberts. 1967. Some effects of dietary potassium upon digestibility, serum electrolytes, and utilization of potassium, sodium, nitrogen, and water in heifers. Can. J. Amin. Sci. 47:39-46.

Swan, R. C., and R. F. Pitts. 1955. Neutralization of infused acid by nephrectomized dogs. J. Clin. Invest. 34:205-212.

Tannen, R. L. 1969. The relationship between urine pH and acid excretion, the influence of urine flow rate. J. Lab. Clin. Med. 74:757-769.

Tannen, R. L. 1977. Relationship of renal ammonia production and potassium homeostasis. Kidney Int. 11:453-465.

Tannen, R. L. 1978. Ammonia metabolism. Am. J. Physiol. 235:F265-F277.

Tannen, R. L., and A. S. Kunin. 1976. Effect of pH on ammonia production by renal mitochondria. Am. J. Physiol. 231:1631-1637.

Tannen, R. L., and A. S. Kunin. 1981. Effect of pH on metabolism of α-ketoglutarate by renal cortical mitochondria. Am. J. Physiol. 240:F120-F126.

Tannen, R. L., and B. D. Ross. 1979. Ammoniagenesis by the isolated perfused rat kidney: The critical role of urinary acidification. Clin. Sci. Mol. Med. 56:353-364.

Telle, P. P., and R. L. Preston. 1971. Ovine lactic acidosis: intraruminal and systemic. J. Anim. Sci. 33:698-705.

Thornton, R. F. 1970. Factors affecting the urinary excretion of urea nitrogen in cattle. I. Sodium chloride and water loads. Aust. J. Agric. Res. 21:131-144.

Tobin, R. E. 1956. Plasma, extracellular and muscle electrolyte responses to acute metabolic acidosis. Am. J. Physiol. 186:131-138.

Uhart, B. A., and F. D. Carroll. 1967. Acidosis in beef steers. J. Anim. Sci.27:1195-1198.

Van Slyke, D. D., and G. E. Cullen. 1917. Studies of acidosis. I. The bicarbonate concentration of the blood plasma; its significance, and its determination as a measure of acidosis. J. Biol. Chem. 30:289-346.

Van Slyke, D. D., R. A. Phillips, P. B. Hamilton, R. M. Archibald, P. H. Futcher, and A. Hiller. 1943. Glutamine as source material of urinary ammonia. J. Biol. Chem. 150:48-482.

Varley, H. 1969. Practical clinical biochemistry. Whitefriars Press.

Vieira, F. L., and G. Malnic. 1968. Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony microelectrode. Am. J. Physiol. 214:710-718.

Warnock, D. G., and F. C. Rector. 1979. Proton secretion by the kidney. Annu. Rev. Physiol. 41:197-210.

Welbourne, T. C. 1975. Mechanism of renal ammonia production adaptation to chronic acidosis. Med. Clin. N. Am. 59:629-648.

Welbourne, T. C. 1977. Cytoplasmic γ-glutamyl transferase: isolation, product formation, and physiological role. In Current problems in clinical biochemistry. Vol 8. Biochemical nephrology. Page 201-215. Hans Huber Pub., Bern.

Williams, J. H., and E. C. Pickering. 1980. Effects of an intravenous infusion of hydrochloric acid on renal function in sheep. Res. Vet. Sci. 28:347-350.

Wolfe, J. E., E. N. Bergman, and H. H. Williams. 1972. Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. Am. J. Physiol. 223:438-446.

Yablon, S. B., and A. S. Relman. 1977. Glutamine as a regulator of renal ammonia production in the rat. Kidney Int. 12:546A.

Yoshimura, H., H. Fujimoto, O. Okumura, J. Sugimoto, and T. Kuwada. 1961. Three step regulation of acid-base balance in body fluid after acid load. Jpn. J. Physiol. 11:109-125.

Young, V. R., G. P. Lofgreen, and J. R. Luick. 1966. The effects of phosphorous depletion and of calcium and phosphorous intake on the endogenous excretion of these elements by sheep. Br. J. Nutri. 20:795-805.

APPENDIX

Tables 1 - 6. Data on urinary flow rate, pH, titratable acid, ammonia, and nitrogenous constituents excreted, for Experiment 1.

Table 1

Sheep no. 275 - Low N diet

Day	Time	Acid infused mEq	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	3.29	7.49	-0.103	2.50	1.71	0.0099
	2100-0100	"	2.87	7.48	-0.060	2.42	1.43	0.0083
	0100-0500	"	2.52	7.43	-0.056	2.65	1.37	0.0073
	0500-0900	"	2.76	7.40	-0.068	2.44	1.41	0.0124
	0900-1300	"	3.30	7.33	-0.108	2.66	1.36	0.0086
	1300-1700	"	3.04	7.47	-0.091	3.20	1.42	0.0103
2	1700-0900	0-100	3.27	7.32	0.057	2.02	0.99	0.0092
	0900-1300	100-125	2.88	7.01	0.045	2.23	0.99	0.0242
	1300-1700	125-150	3.19	5.66	0.055	2.32	1.15	0.0720
3	1700-2100	150-175	3.26	5.81	0.024	2.87	0.96	0.3017
	2100-0100	175-200	4.40	6.13	0.026	3.44	1.52	0.4575
	0100-0500	200-225	4.59	6.19	0.030	4.12	1.18	0.3988
	0500-0900	225-250	4.22	5.89	0.017	2.80	0.67	0.2434
	0900-1300	250-275	3.27	6.01	0.029	2.24	0.75	0.3131
	1300-1700	275-300	3.23	5.98	0.019	2.05	0.68	0.3611
4	1700-0900	300-400	2.03	6.05	0.005	1.57	0.53	0.2652
	0900-1300	400-425	- [1]	-	-	-	-	-
	1300-1700	425-450	0.98	5.42	0.009	1.63	0.62	0.2610
5	1700-0900	450-550	1.88	5.40	0.006	1.92	1.13	0.4184
	0900-1300	550-575	0.64	5.64	0.003	1.09	0.46	0.2564
	1300-1700	575-600	0.85	5.78	0.002	1.04	0.50	0.2292

1 Sample lost.

Table 2

Sheep no. 254 - Low N diet

Day	Time	Acid infused (mEq)	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	5.75	7.38	-0.099	3.75	2.35	0.0290
	2100-0100	"	4.82	7.43	-0.061	3.91	2.05	0.0304
	0100-0500	"	3.62	7.47	-0.082	2.90	1.49	0.0308
	0500-0900	"	2.67	7.43	-0.082	3.50	1.44	0.0305
	0900-1300	"	3.62	7.42	-0.098	3.68	1.85	0.0196
	1300-1700	"	3.60	7.51	-0.097	3.76	1.77	0.0155
2	1700-0900	0-100	2.25	5.85	0.023	3.44	1.21	0.0352
	0900-1300	100-125	1.12	5.23	0.028	2.28	0.74	0.1110
	1300-1700	125-150	1.81	4.96	0.019	3.29	0.81	0.1653
3	1700-2100	150-175	4.39	5.76	0.012	3.80	1.43	0.2147
	2100-0100	175-200	3.60	5.51	0.009	2.92	1.41	0.2003
	0100-0500	200-225	3.39	5.45	0.009	3.78	1.33	0.1925
	0500-0900	225-250	2.89	5.56	0.008	3.39	1.27	0.2451
	0900-1300	250-275	3.20	5.92	0.010	5.04	2.36	0.4140
	1300-1700	275-300	5.26	5.66	0.012	5.53	2.94	0.2297
4	1700-0900	300-400	2.96	5.71	0.017	4.47	2.05	0.2229
	0900-1300	400-425	3.02	5.79	0.009	4.50	2.20	0.1934
	1300-1700	425-450	5.43	5.53	0.009	5.57	2.97	0.2111
5	1700-0900	450-550	4.41	5.60	0.023	2.86	1.50	0.1041
	0900-1300	550-575	3.86	5.59	0.033	2.94	1.48	0.2775
	1300-1700	575-600	2.63	5.20	0.011	2.29	1.00	0.2008

Table 3

Sheep no. 299 - Low N diet

Day	Time	Acid infused (mEq)	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	1.91	7.75	-0.097	1.89	0.78	0.0073
	2100-0100	"	1.91	7.44	-0.141	1.88	0.80	0.0092
	0100-0500	"	0.98	7.46	-0.126	1.61	0.61	0.0100
	0500-0900	"	1.72	8.14	-0.125	1.63	0.95	0.0066
	0900-1300	"	1.44	8.02	-0.109	2.11	0.81	0.0059
	1300-1700	"	1.14	7.58	-0.093	2.96	0.81	0.0188
2	1700-0900	0-100	2.48	6.80	0.041	6.87	3.54	0.2590
	0900-1300	100-125	3.22	5.95	0.016	7.20	3.77	0.3185
	1300-1700	125-150	4.17	5.98	0.021	7.63	4.71	0.4121
3	1700-2100	150-175	3.89	5.76	0.021	6.86	4.04	0.3701
	2100-0100	175-200	3.82	5.65	0.029	7.01	4.22	0.4606
	0100-0500	200-225	3.10	5.41	0.047	7.33	3.88	0.6371
	0500-0900	225-250	2.98	5.31	0.035	6.84	3.65	0.6275
	0900-1300	250-275	2.61	5.67	0.043	5.84	2.93	0.5599
	1300-1700	275-300	2.52	5.49	0.051	6.29	3.16	0.7127
4	1700-0900	300-400	4.28	5.85	0.039	6.66	4.02	0.5632
	0900-1300	400-425	4.52	6.01	0.030	5.80	3.46	0.7880
	1300-1700	425-450	4.90	5.89	0.036	5.36	3.55	0.8700
5	1700-0900	450-550	2.22	5.62	0.037	4.49	2.43	0.9700
	0900-1300	550-575	3.77	5.66	0.043	5.77	3.24	0.8634
	1300-1700	575-600	3.40	5.64	0.024	4.13	2.14	0.9264

Table 4

Sheep no. 261 - High N diet

Day	Time	Acid infused (mEq)	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	13.66	7.16	-0.090	8.23	6.59	0.0342
	2100-0100	"	13.04	7.11	-0.091	8.34	6.50	0.0443
	0100-0500	"	11.44	7.01	-0.091	7.09	6.02	0.0349
	0500-0900	"	11.64	7.24	-0.087	6.86	5.70	0.0419
	0900-1300	"	11.61	6.89	-0.047	6.47	5.29	0.0557
	1300-1700	"	11.54	7.38	-0.086	8.36	6.12	0.0577
2	1700-0900	0-100	5.45	6.23	0.012	6.83	4.87	0.0665
	0900-1300	100-125	2.77	5.81	0.016	6.13	3.62	0.1438
	1300-1700	125-150	2.56	5.14	0.023	7.31	3.77	0.3026
3	1700-2100	150-175	4.45	5.44	0.027	6.02	4.37	0.2775
	2100-0100	175-200	7.02	6.15	0.022	6.38	5.21	0.2921
	0100-0500	200-225	5.90	5.95	0.012	6.54	4.37	0.2578
	0500-0900	225-250	3.84	6.79	0.013	5.72	3.72	0.2398
	0900-1300	250-275	3.73	6.65	0.007	7.37	4.34	0.4222
	1300-1700	275-300	1.79	5.35	0.007	7.85	3.43	0.2881
4	1700-0900	300-400	0.91	5.25	0.003	5.74	2.99	0.2269
	0900-1300	400-425	2.21	5.12	0.008	4.93	2.90	0.2601
	1300-1700	425-450	1.77	5.56	0.009	6.19	2.98	0.3041
5	1700-0900	450-550	2.13	5.32	0.005	7.38	3.65	0.3217
	0900-1300	550-575	1.03	5.15	0.005	6.87	3.65	0.2119
	1300-1700	575-600	0.98	5.53	0.010	7.22	3.19	0.2782

Table 5

Sheep no. 330 - High N diet

Day	Time	Acid infused (mEq)	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	16.06	7.31	-0.152	11.08	9.19	0.0193
	2100-0100	"	13.26	7.21	-0.129	9.74	7.77	0.0186
	0100-0500	"	14.79	7.21	-0.054	9.09	5.80	0.0133
	0500-0900	"	12.21	7.18	-0.145	7.72	5.49	0.0134
	0900-1300	"	8.72	7.33	-0.084	7.10	5.88	0.0192
	1300-1700	"	11.70	7.36	-0.128	8.58	5.78	0.0199
2	1700-0900	0-100	7.35	7.25	-0.022	6.47	5.15	0.0169
	0900-1300	100-125	10.66	7.12	-0.003	7.35	6.05	0.0288
	1300-1700	125-150	10.28	6.98	0.001	7.65	5.20	0.0360
3	1700-2100	150-175	10.33	6.93	0.001	7.16	4.81	0.0403
	2100-0100	175-200	10.21	6.79	0.002	8.93	5.31	0.0490
	0100-0500	200-225	6.40	7.08	-0.013	9.15	6.24	0.0762
	0500-0900	225-250	6.24	5.93	0.029	9.75	5.87	0.3278
	0900-1300	250-275	12.74	6.25	0.028	12.29	10.32	0.4040
	1300-1700	275-300	13.85	6.33	0.041	11.23	8.04	0.2175
4	1700-0900	300-400	9.67	6.02	0.063	9.45	5.94	0.1557
	0900-1300	400-425	8.51	5.03	0.032	7.70	5.43	0.1915
	1300-1700	425-450	12.85	5.78	0.065	8.32	6.25	0.2455
5	1700-0900	450-550	10.26	5.60	0.038	6.84	4.82	0.1590
	0900-1300	550-575	10.11	5.78	0.044	7.38	5.06	0.1947
	1300-1700	575-600	8.29	5.50	0.021	7.46	4.98	0.1916

Table 6

Sheep no. 312 - High N diet

Day	Time	Acid infused (mEq)	Flow rate ($ml.h^{-1}.kgBW^{-1}$)	pH	Titratable acid ($mEq.h^{-1}.kgBW^{-1}$)	Total-N	Urea-N	Ammonia-N
						($mg.h^{-1}.kgBW^{-1}$)		
1	1700-2100	none	8.87	7.49	-0.151	9.54	8.34	0.0204
	2100-0100	"	10.87	7.22	-0.091	9.70	7.50	0.0315
	0100-0500	"	9.05	7.34	-0.136	8.78	7.10	0.0217
	0500-0900	"	8.77	7.53	-0.097	8.59	7.11	0.0184
	0900-1300	"	8.82	7.46	-0.106	8.29	6.97	0.0229
	1300-1700	"	9.52	7.43	-0.130	8.61	6.38	0.0181
2	1700-0900	0-100	6.44	7.18	-0.122	8.19	6.52	0.0367
	0900-1300	100-125	7.43	7.05	-0.066	9.41	6.79	0.0520
	1300-1700	125-150	8.30	7.23	0.002	8.27	6.72	0.0772
3	1700-2100	150-175	5.49	6.11	0.002	8.56	5.09	0.1114
	2100-0100	175-200	7.27	5.89	0.028	8.66	5.89	0.1395
	0100-0500	200-225	6.10	5.27	0.029	9.03	4.88	0.1459
	0500-0900	225-250	6.00	5.13	0.040	9.64	5.94	0.2244
	0900-1300	250-275	7.23	5.50	0.028	9.74	6.80	0.2560
	1300-1700	275-300	8.51	5.80	0.037	11.82	7.04	0.1327
4	1700-0900	300-400	7.49	6.30	0.027	11.52	7.86	0.0973
	0900-1300	400-425	11.87	6.51	0.023	11.15	7.41	0.0962
	1300-1700	425-450	11.08	6.98	0.042	9.86	5.32	0.1097
5	1700-0900	450-550	7.81	6.75	0.061	6.69	4.78	0.0922
	0900-1300	550-575	6.99	6.99	0.043	5.67	4.26	0.0915
	1300-1700	575-600	6.33	6.08	0.002	6.60	4.31	0.0873

Tables 7 - 12. Data on blood acid-base parameters, plasma ammonia-N and urea-N concentrations and creatinine clearances for Experiment I.

Table 7

Sheep no. 275 - Low N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Urea-N (mg.dl^{-1})	Creatinine clearance (ml.min^{-1}.$kgBW^{-1}$)
1	1900	7.47	39.7	27.9	5.3	5.77	3.4	1.58
	2300	7.45	38.1	25.6	3.0	6.20	4.3	1.53
	0300	7.43	42.5	27.0	3.5	5.73	3.3	1.34
	0700	7.43	37.6	23.8	0.9	5.73	3.4	1.59
	1100	7.40	42.3	25.3	1.4	5.77	3.2	1.65
	1500	7.43	40.7	25.9	2.7	5.57	3.2	1.69
2	1100	7.41	35.5	21.6	-1.2	5.67	3.5	1.47
	1500	7.39	36.5	21.6	-1.6	5.67	3.0	2.15
3	1900	7.36	36.1	19.6	-4.1	5.60	3.0	2.09
	2300	7.35	38.9	21.0	-3.1	6.00	2.7	1.74
	0300	7.34	40.0	20.6	-4.0	5.33	2.5	1.64
	0700	7.31	38.4	18.7	-6.1	5.57	2.2	1.24
	1100	7.33	35.5	18.2	-5.9	6.33	2.1	1.37
	1500	7.34	35.3	18.4	-5.7	5.90	1.7	1.30
4	1100	7.31	36.7	17.7	-7.0	7.13	5.4	[1]
	1500	7.30	35.0	16.6	-8.1	7.40	5.6	0.66
5	1100	7.27	34.6	15.4	-9.8	8.27	5.2	0.44
	1500	7.26	34.5	14.8	-9.9	7.97	6.0	0.40

1. Sample lost.

Table 8

Sheep no. 254 - Low N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- ($mEq.l^{-1}$)	BE ($mEq.l^{-1}$)	Ammonia-N ($mg.l^{-1}$)	Urea-N ($mg.dl^{-1}$)	Creatinine clearance ($ml.min^{-1}.kgBW^{-1}$)
1	1900	7.38	40.1	22.8	-1.0	5.87	4.5	2.00
	2300	7.39	38.4	22.3	-1.3	5.67	4.7	2.02
	0300	7.39	37.5	22.0	-1.3	5.80	4.1	1.15
	0700	7.37	38.4	21.6	-2.2	5.83	4.3	2.18
	1100	7.34	41.3	21.7	-2.9	6.30	4.5	2.28
	1500	7.37	32.3	21.0	-2.7	5.60	4.4	1.38
2	1100	7.39	34.7	20.1	-3.0	5.77	3.8	1.45
	1500	7.36	36.0	19.5	-4.3	5.53	2.5	2.16
3	1900	7.35	38.0	20.5	-3.5	4.20	2.0	2.12
	2300	7.33	37.3	19.1	-5.2	5.67	2.2	1.72
	0300	7.33	38.9	19.9	-4.6	5.63	2.8	2.03
	0700	7.32	39.9	19.7	-5.3	5.70	3.1	1.95
	1100	7.35	36.1	19.0	-5.0	6.50	6.2	1.79
	1500	7.37	36.1	20.3	-3.3	5.27	4.1	2.54
4	1100	7.34	38.5	20.3	-4.0	5.53	6.2	2.28
	1500	7.35	36.9	19.6	-4.4	5.50	4.9	2.43
5	1100	7.31	37.4	18.2	-6.6	5.83	2.6	2.46
	1500	7.31	34.9	16.8	-7.8	5.30	2.2	2.05

Table 9

Sheep no. 299 - Low N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Urea-N (mg.dl^{-1})	Creatinine clearance (ml.min^{-1}.$kgBW^{-1}$)
1	1900	-[1]	-	-	-	-	-	-
	2300	7.46	32.5	22.2	0.3	4.07	2.8	1.58
	0300	-[1]	-	-	-	-	-	-
	0700	7.49	34.4	25.4	3.5	4.27	3.4	1.16
	1100	7.50	30.3	22.8	1.9	4.13	3.5	1.49
	1500	7.41	38.0	23.5	0.2	3.67	3.5	1.64
2	1100	7.42	25.9	16.2	-5.4	6.00	11.0	1.63
	1500	7.44	35.5	23.0	0.4	4.07	12.5	2.15
3	1900	7.40	31.9	18.9	-3.9	3.90	9.4	2.70
	2300	7.37	37.0	20.7	-3.0	4.20	10.9	1.67
	0300	7.36	37.0	20.0	-4.0	3.97	10.8	1.43
	0700	7.29	38.7	17.9	-7.3	5.13	11.3	1.36
	1100	7.31	34.5	16.9	-7.4	3.77	11.0	1.48
	1500	7.29	32.6	15.2	-9.5	5.60	9.5	1.73
4	1100	7.27	33.1	14.8	-9.8	6.83	8.6	2.16
	1500	7.27	36.9	16.3	-9.1	6.43	7.9	1.73
5	1100	7.26	32.5	14.0	-9.9	7.13	7.9	1.79
	1500	7.30	31.0	14.6	-9.8	6.33	8.5	1.41

1. Sample not available.

Table 10

Sheep no. 261 - High N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Urea-N (mg.dl^{-1})	Creatinine clearance (ml.min^{-1}.$kgBW^{-1}$)
1	1900	7.44	38.2	25.4	2.7	5.80	10.4	2.09
	2300	7.39	43.3	25.8	1.8	5.40	10.2	1.74
	0300	7.42	38.1	23.7	0.4	4.60	10.4	2.02
	0700	7.38	44.5	25.3	0.8	4.57	9.8	1.96
	1100	7.37	41.4	22.9	-1.2	5.07	10.5	1.72
	1500	7.43	38.6	24.7	1.6	4.40	10.5	1.76
2	1100	7.32	44.2	22.5	-2.6	5.57	9.3	1.46
	1500	7.35	37.3	19.8	-4.4	5.50	8.6	2.06
3	1900	7.34	44.4	23.1	-2.0	3.67	8.3	1.76
	2300	7.33	43.1	22.2	-2.7	5.17	7.5	2.11
	0300	7.41	35.5	21.8	-1.1	5.30	7.5	2.07
	0700	7.35	44.2	23.8	-1.0	5.33	7.2	1.99
	1100	7.35	42.7	22.7	-1.9	5.57	8.6	1.78
	1500	7.28	48.8	22.3	-4.0	5.57	9.3	1.96
4	1100	7.37	40.7	25.5	-1.6	5.33	7.4	2.02
	1500	7.38	38.0	22.0	-1.7	4.57	7.5	2.21
5	1100	7.38	40.7	23.1	-0.9	4.97	12.8	1.80
	1500	7.38	38.9	22.3	-1.5	4.83	12.5	2.03

Table 11

Sheep no. 330 - High N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Urea-N (mg.dl^{-1})	Creatinine clearance (ml.min^{-1}.$kgBW^{-1}$)
1	1900	7.41	42.4	26.2	2.6	4.33	14.2	2.18
	2300	7.44	35.5	23.6	1.2	4.33	13.1	1.71
	0300	7.42	36.0	22.5	-0.2	4.73	11.6	2.09
	0700	7.42	40.2	25.3	2.0	4.13	11.8	1.88
	1100	7.39	41.4	24.3	0.3	4.43	10.3	1.79
	1500	7.41	36.2	22.2	-0.9	4.57	9.0	2.22
2	1100	7.41	31.6	19.6	-2.6	4.57	10.5	1.87
	1500	7.43	34.2	21.8	-0.8	4.67	10.1	2.46
3	1900	7.43	35.7	22.8	0.1	5.17	11.5	2.15
	2300	7.40	33.1	19.9	-2.8	5.00	12.0	1.74
	0300	7.42	32.1	20.2	-2.0	4.80	14.8	2.04
	0700	7.33	35.6	18.0	-6.2	5.30	14.0	1.94
	1100	7.39	34.7	20.2	-3.0	5.80	14.2	2.15
	1500	7.39	34.4	20.0	-3.1	4.56	14.5	2.37
4	1100	7.35	33.5	19.1	-4.7	5.17	9.0	2.69
	1500	7.38	36.1	20.4	-3.2	5.50	9.2	2.44
5	1100	7.37	35.7	20.1	-3.4	5.50	7.6	2.25
	1500	7.41	35.1	21.4	-1.5	5.27	8.7	2.20

Table 12

Sheep no. 312 - High N diet

Day	Time	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Urea-N (mg.dl^{-1})	Creatinine clearance (ml.min^{-1}.$kgBW^{-1}$)
1	1900	7.51	33.4	25.7	4.7	3.60	13.0	2.44
	2300	7.44	31.3	20.7	-1.1	3.47	12.3	2.39
	0300	7.47	34.0	24.1	2.2	3.73	12.0	2.19
	0700	7.47	36.0	25.1	3.0	3.60	11.4	1.90
	1100	7.44	36.4	24.1	1.6	3.73	10.4	2.26
	1500	7.44	37.0	24.5	1.9	3.03	11.3	1.88
2	1100	7.44	35.5	23.4	0.9	3.90	12.1	2.00
	1500	7.44	34.0	22.5	0.3	3.63	10.2	2.55
3	1900	7.41	35.1	21.5	-1.4	3.23	10.5	1.57
	2300	7.38	35.4	20.6	-2.6	3.97	9.8	2.04
	0300	7.40	40.5	24.3	-0.6	3.93	10.4	1.94
	0700	7.38	38.8	22.2	-1.6	4.13	11.9	2.10
	1100	7.35	39.2	21.1	-3.2	4.60	13.4	2.14
	1500	7.38	39.5	22.7	-1.1	3.77	13.0	2.44
4	1100	7.41	37.1	22.6	-0.5	4.50	11.0	2.55
	1500	7.42	37.0	23.1	0.0	4.17	10.1	2.14
5	1100	7.42	34.7	21.9	-0.7	4.33	8.4	1.81
	1500	7.39	34.0	20.0	-3.1	3.93	9.7	1.97

Table 13. Data on acid-base parameters of venous blood samples taken at 1030 and on plasma ammonia-N and glutamine of arterial blood samples taken at 1330 for Experiment 2.

Day	pH	pCO_2 (mmHg)	HCO_3^- (mEq.l^{-1})	BE (mEq.l^{-1})	Ammonia-N (mg.l^{-1})	Glutamine (µmol.l^{-1})
Sheep no. 268 - Low N diet						
1	7.47	36.2	25.5	3.4	6.68	240
2	7.37	31.7	18.0	-5.0	4.98	138
5	7.32	40.0	20.3	-4.4	5.63	126
Sheep no. 254 - Low N diet						
1	7.44	40.4	27.0	3.9	8.05	231
2	7.40	37.3	22.7	-0.5	6.85	245
5	7.36	38.3	21.1	-2.9	7.90	173
Sheep no. 275 - Low N diet						
1	7.43	34.7	22.5	0.1	5.43	90
2	7.37	32.4	18.3	-4.9	6.80	122
5	7.37	35.6	20.3	-3.1	5.68	91
Sheep no. 268 - High N diet						
1	7.36	33.4	18.4	-4.9	6.15	162
2	7.30	30.3	14.8	-9.2	5.93	159
5	7.41	40.8	25.5	2.0	5.90	154
Sheep no. 254 - High N diet						
1	7.40	31.0	22.6	-0.3	5.45	158
2	7.39	30.4	19.4	-3.3	8.05	248
5	7.34	36.5	19.7	-4.2	7.70	285
Sheep no. 275 - High N diet						
1	7.45	29.4	20.0	-1.3	5.40	194
2	7.29	28.6	13.5	-9.7	4.28	166
5	7.37	33.3	19.0	-4.1	5.63	152

Table 14. Data on urinary pH, titratable acid and ammonia excreted, urine flow rate, creatinine and PAH clearances and filtration fraction for the interval 1130 - 1530 for Experiment 2.

Day	pH	Titratable acid	Ammonia	Flow rate	Clearance Creatinine	Clearance PAH	FF
		($mEq.h^{-1}.kgBW^{-1}$)		($ml.min^{-1}.kgBW^{-1}$)			(%)
Sheep no. 268 - Low N diet							
1	6.34	0.003	0.025	0.051	1.97	11.4	17
2	5.30	0.027	0.028	0.087	1.82	10.0	18
5	5.23	0.029	0.030	0.089	1.63	12.9	13
Sheep no. 254 - Low N diet							
1	7.80	-0.044	0.003	0.059	2.29	8.6	25
2	5.86	0.009	0.005	0.020	2.17	8.7	25
5	5.12	0.015	0.023	0.051	2.43	10.3	23
Sheep no. 275 - Low N diet							
1	7.91	-0.035	0.002	0.062	2.74	13.2	21
2	5.36	0.008	0.024	0.016	2.02	10.8	19
5	5.35	0.020	0.025	0.044	2.76	11.9	23
Sheep no. 268 - High N diet							
1	5.60	0.014	0.007	0.070	2.24	7.9	28
2	4.80	0.049	0.039	0.136	3.15	10.5	30
5	7.11	0.002	0.012	0.093	2.67	8.0	34
Sheep no. 254 - High N diet							
1	7.89	-0.037	0.006	0.041	3.56	14.2	25
2	6.79	0.004	0.006	0.038	2.55	13.1	20
5	6.71	0.006	0.011	0.100	4.08	13.7	30
Sheep no. 275 -High N diet							
1	8.03	-0.018	0.004	0.036	1.85	10.7	17
2	5.38	0.006	0.007	0.034	2.02	8.3	24
5	4.92	0.030	0.022	0.051	3.39	12.6	26