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Effect of ketone bodies on the renal excretion of ammonia

Gary Desir
Yale University

EFFECTS OF KETONE BODIES ON THE RENAL EXCRETION OF AMMONIA

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by

Gary Desir

BA New York University 1976

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To my parents

Gerard and Claude Desir

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I INTRODUCTION.

The urinary excretion of ammonium increases markedly during both diabetic (1,2) and starvation (3) ketoacidosis. The rise in renal tubular hydrogen ion concentration that results from the increased production of ketoacids is believed to stimulate ammoniagenesis (4). Surprisingly however, a significant decrease in urinary ammonium excretion has been reported during acute ketone infusion in chronically acidemic dogs (5). The fall in ammonium excretion was not related to changes in urinary pH or in systemic acid base status. It was, however, accompanied by a striking drop in glutamine extraction by the kidney. Whether hyperketonemia also inhibits ammonium excretion in man is not known. Consequently, the present study was carried out to determine the effects of sodium β-hydroxybutyrate on the urinary excretion of ammonium in humans made chronically acidemic by ammonium chloride loading.

II. LITERATURE REVIEW.

A. Renal ammoniagenesis.

In man ingesting a normal protein intake, 1-1.5 meq/kg of strong acids are produced daily. This results primarily from the generation of phosphoric and sulfuric acid during the metabolism of phospholipids and sulfur containing amino acids. The hydrogen ion is initially buffered by extracellular bicarbonate and the accompanying anion is transported to the kidney and is filtered. Those filtered anions can then serve as proton acceptors for distally secreted hydrogen ions. These titratable acids (TA) account for about half the amount of hydrogen ion which is excreted daily. The remaining half of daily hydrogen ion production is excreted with ammonia which because of its high pKa is an excellent urinary buffer. The excretion of titratable acids and ammonium permits the reabsorption of fixed cations and the restoration of the body's buffer stores.

During metabolic acidemia the major increase in hydrogen ion excretion is accounted for by an increase in ammonia production by the kidney. As a buffer, ammonia is unique for several reasons (6): 1) Unlike phosphate which is drawn from cells and bones only at the expense of interference with function and structure, ammonia can be supplied from waste nitrogen and its production and release are metabolically inexpensive. In experimental acidemia, ammonia pro-

duction increases markedly and the ratio of titratable acids to ammonium ion falls from 1:2.5 to 1:5 - 1:10. The ability of titratable acid excretion to increase during acidemia is limited by the glomerular filtration rate (GFR) and the depletion of total body phosphate stores. 2) In solution, an equilibrium exists between ammonia (NH_3) which is readily diffusable and ammonium ion (NH_4+)which is poorly diffusable. The ratio of NH_3/NH_4+ is determined by the pH of the solution. Acidification increases and alkalinization decreases the ratio. It follows that an acid urine acts as a sink for trapping NH_3 in its cationic form thereby preventing its back diffusion into the renal tubular cell and thereby enhancing its secretion into the urine. 3) NH_3 production is capable of increasing 10-20 fold in chronic metabolic acidemia unlike titratable acids (TA) excretion which is limited by GFR.

Biochemistry of renal ammoniagenesis.

Approximately 90% of ammonia produced in the kidney is derived from glutamine. Other amino acids such as alanine, glycine and histidine account for 10% of renal ammoniagenesis (7). The metabolism of glutamine has been extensively studied. In the rat, the pathway of glutamine metabolism has two parts: The first consists of the reactions leading to the formation of ammonia and the second is concerned with the disposal of α-ketoglutarate, the carbon skeleton

of glutamine, (figure 1). α-ketoglutarate enters the tricarboxylic acid (TCA) cycle where it is converted to carbon dioxide and water. Alternatively it can be transported to the cytosol where it is converted to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK) and then toglucose via several reactions. As will be discussed later, gluconeogenesis may play an important role in the control of ammonia production.

Ammonia can be produced from glutamine through several reactions (Figure 1):

a) Deamidation of glutamine:

$$\text{glutamine} \xrightarrow{\text{glutaminase I}} \text{glutamate} + NH_3$$

b) Deamination of glutamate:

$$\text{glutamate} \xrightarrow{\text{glutamic dehydrogenase}} \alpha\text{-ketoglutarate} + NH_3$$

c) Transamination followed by hydrolysis:

$$\text{glutamine} + \text{pyruvic acid} \xrightarrow[\text{transaminase}]{\text{glutamine keto acid}} \text{alanine} + \alpha\text{-ketogl.}$$

$$\alpha\text{-ketoglutaramate} \xrightarrow{\omega \text{ amidase}} \alpha\text{-ketoglutarate} + NH_3$$

The deamidation of glutamine is catalyzed by glutaminase I. In 1935 Krebs (8) described enzymes in liver and kidney that hydrolized glutamine to ammonium and glutamate. Since the pH optimum of the liver enzyme (8.8) differed from that of the kidney, he inferred that they were different enzymes and anticipated the discovery of glutaminase I (kidney) and glutaminase II (liver). Glutaminase I exists as two isoenzymes; one requires phosphate for activity and is termed

phsophate dependent glutaminase (PDG); the other does not and is called phosphate independent glutaminase (PIG) (9). For the most part deamidation is catalized by (PDG). This isoenzyme has been well characterized: it is located in the inner mitochondrial membrane (10), has a pH optimum between 7.8 and 8.5, is heat labile, is inhibited by ammonia and glutamate and is activated by phosphate, sulfate and several TCA cycle intermediates (11). PIG also catalizes the deamidation of glutamine. Its pH optimum is between 7.4 and 7.6; it is heat labile and is inhibited by its end products (12, 13).

Unlike deamidation, much less is known about the deamination reaction which is believed not to play a major role in renal ammoniagenesis (10). This reaction involves an oxidative process and is catalized by glutamate dehydrogenase (GD) a mitochondrial enzyme. GD is inhibited by α-ketoglutarate (14,15) and is activated by an elevated NAD+/NADH ratio (16).

Significant species difference are known to exist among the reactions. Although both deamidation and deamination are known to occur in both rats and men, transamination takes place only in rats. Furthermore, transamination has been shown to be important in renal ammoniagenesis in the rat. Welbourne has demonstrated that in the normal rat kidney this reaction contributes 70% of the total ammonia production (17). Glutamine glutamyl transferase (GT) catalyzes

the transamination reaction. This enzyme complex is found in the cytosol and is bound to the endoplasmic reticulum (18) is non specific, is competitively inhibited by acetazolamide, exhibits both hydrolytic and transferring activities and catalyzes the following reactions:

$$2 \text{ glutamine} \xrightarrow{\text{glutamine glutamyl trans.}} 2 \ NH_3 + \gamma\text{-glutamyl glutam.}$$

$$\text{glutamine} + \text{hydroxylamine} \xrightarrow{GT} NH_3 + \text{glutamyl hydroxamate}$$

As noted above, in the normal rat kidney, those reactions account for most of the ammonia produced. However, in the acidotic rat, the deamination of glutamate and the deamidation of glutamine are responsible for 80% of total ammonia production (17).

Sites of ammonia production and excretion.

Since the glutaminase enzymes are of major importance in ammonia production, their localization in the nephron has been used to identify the sites of ammoniagenesis. Goldstein et al (19,20) showed that although glutaminase I activity was highest in the renal cortex and inner medulla in rats, enzyme activity in the collecting duct was more than sufficient to account for all the ammonia excreted in the urine. They concluded that ammoniagenesis was primarily a function of the collecting duct. However, several experiments suggest that the proximal and perhaps the distal tubule as well, contribute significantly to ammoniagenesis

in the acidemic rat. The major increase in glutaminase I activity following metabolic acidemia occurs in the renal cortex, not in the renal medulla (21). Furthermore, Seyama (22) demonstrated that the increase in glutamic dehydrogenase (GD) activity in acidosis is largely associated with epithelial cells of the proximal convoluted tubule. Micropuncture studies done by Glabman, Klose and Giebisch (23) conclusively demonstrated that ammonia secretion actually occurs throughout the entire nephron. Hayes (24) confirmed those findings and documented that 70% of urinary ammonia was secreted by the proximal tubule and that most of the remaining 30% was added by the distal tubule. It should be noted, however, that the sites where ammonia is secreted need not be identical to those at which it gains final access to the urine. Indeed, Pitts (25) has provided evidence that considerable recycling of NH_3 occurs in the medullary interstitium. Ammonia produced and secreted in the proximal tubule diffuses out of the loop of Henle into the papillary interstitium and eventually gains entry into the collecting duct cell from where it can diffuse into the tubular lumen. In summary, the available evidence suggests that in acidosis most of the production and secretion of ammonia is carried out by the proximal tubule.

Mechanism of ammonia secretion.

It is now well established that ammonia secretion is

passive and effected by a mechanism of non ionic diffusion (26, figure 2). The renal tubular membrane is fully permeable to ammonia and this enables the free base to diffuse down very small concentration gradients into acid urine where it is trapped as ammonium ion. Since diffusion occurs over small distances, it is never a limiting factor in ammonia secretion.

Source of renal ammonia.

The various sources of renal ammonia are shown in figure 3. Van Slyke et al (28) were the first to show that in acidemic dogs glutamine is extracted by the kidney and that its amide nitrogen alone could account for approximately 2/3 of the total amount of ammonium excreted. During metabolic acidemia in men (29) and in dogs (30), glutamine extraction by the kidney increases and greatly exceeds that of any amino acids. Using labeled glutamine, several groups (7,31-35) have demonstrated that the amide nitrogen of glutamine accounts for approximately 43% and the amino nitrogen for another 18% of the excreted ammonium. About 35% of the ammonium excreted comes from arterial blood filtered by the glomeruli. Only a small percentage, approximately 4% of urinary ammonium is derived from the amino nitrogen of alanine and glycine. Thus about 90% of renal ammonia production is derived from the metabolism of glutamine.

Regulation of ammonia production and secretion.

During chronic metabolic acidemia, ammoniagenesis increases by 10-15 fold and large amounts of ammonium are excreted in the urine. The rate at which ammonium is excreted is determined by three factors: 1) the acidity of the urine 2) the rate of urine flow and 3) the amount of ammonia produced by the renal tubular cell. As discussed previously, the permeability of cellular membranes to ammonia is so great that diffusion is never a limiting factor in ammonium excretion. Pitts (36) showed that in normal dogs and in dogs made acutely acidemic, ammonium excretion increased significantly although ammonia production did not change. The increase in excretion was found to be negatively correlated with urine pH. Since hydrogen excretion increases markedly during metabolic acidemia, in the tubular fluid ammonia is immediately converted to ammonium ion. A favorable gradient for ammonia diffusion is thus maintained. The relationship between urine flow rate and ammonium excretion is more complex. If the urine is strongly acidic, ammonia secretion is independent of urine flow (37). However, if the urine is weakly acidic or alkaline, it becomes directly proportional to urine flow. Although it is clear that urine pH and flow rate are important variables, during chronic metabolic acidemia the rate of ammonia production is the primary determinant of ammonium excretion (38).

Several factors appear to affect renal ammoniagenesis: a) plasma concentration of glutamine, b) renal blood flow, c) transport of glutamine, d) glutaminase activity, e) cellular concentration of hydrogen ion, f) intracellular concentration of potassium, g) intracellular concentration of glutamate and NAD+ NADH ratio, h) concentration of ammonia, i) concentration of α-retoglutarate, j) renal gluconeogenesis, k) availability of oxidizable substrates.

A) Effect of plasma concentration of glutamine on NH_3 production

During chronic acidemia the extraction of glutamine, the major precursor of ammonia, increases significantly. However, the plasma concentration of glutamine remains constant since the liver,which is the major source of extrarenal glutamine,increases its production (39,40). In the normal dog, plasma glutamine is increased and ammoniagenesis is stimulated by intravenous infusions of glutamine (41). This suggests that the amount of precursor delivered to the kidney determines how much ammonia is producdd.

B) Effect of renal blood on NH_3 production

Olert and Nagel (42) have postulated that although renal glutamine delivery varied with GFR, substrate supply was independent of renal blood flow since the amount of glutamine filtered and reabsorbed in the normal dog was

sufficient to account fully for the amount of ammonia produced. In metabolic acidemia, however, glutamine uptake increases markedly and filtered glutamine is not sufficient to meet the demand for ammonia producing substrate. Consequently, in metabolic acidemia large amounts of glutamine are actively taken up at the peritubular surface of the renal tubular cell (43,30). Lemieux et al (44) documented that in the acidemic dog, 60% of ammonia produced is derived from glutamine extracted at the antiluminal surface. Thus, under conditions of metabolic acidemia, renal ammonia production becomes very much dependent upon renal blood flow.

C) Effect of glutamine transport on NH_3 production

Although the delivery of glutamine to the kidney and its transport into the peritubular cell cytoplasm are essential for ammoniagenesis, other factors are also important. The availability of glutaminase I plays a primary role in the regulation of ammonia production. Since this enzyme is located within the mitochondrion, (45) glutamine must reach the mitochondrial inner membrane and changes in membrane permeability can affect ammonia production. The presence of a variety of transport systems for ions, substrates and products across the inner mitochondrial membrane has been demonstrated. There are group specific transport systems for dicarboxylic acids (malate,

malonate, succinate), tricarboxylic acids (citrate, isocitrate), α-ketoglutarate and for glutamate (46). Simpson demonstrated an increase in glutamate formation and ammonia production in mitochondrial and submitochondrial preparations from acidemic dogs in the absence of changes in glutaminase I activity (47). He has also provided evidence suggesting that passive movement of glutamine across the inner mitochondrial membrane did not occur and has postulated that a carrier for glutamine exists in the inner membrane (48). Crompton et al have shown that glutamine transport is rate limiting in the deamidation reaction by intact mitochondria from hog kidney (49). It has, thus, been suggested that the rise in ammoniagenesis observed in metabolic acidemia was due to an increase in glutamine transport by the carrier transport located within the inner mitochondrial membrane (48).

Glucocorticoids may modulate the activity of the carrier system. The evidence supporting the role of glucocorticoids in glutamine transport is indirect and rests on the fact that the two glutamine utilizing systems are located in separate cellular compartments. In the rat, glutamyl glutamine transferase is located in the cytoplasm and glutaminase I is found in the mitochondrion. In the normal rat, approximately 70% of ammonia production takes place in the cytoplasm and 30% in the mitochondria (17). One mole of ammonia

is produced by the reaction catalyzed by GT, while two moles of ammonia are derived from each mole of glutamine metabolized in the mitochondria (since glutamine undergoes both deamidation and deamination). The ammonia/glutamine ratio varies with the activity of the cytoplasmic and mitochondrial enzymes, and is equal to 1.3 under normal circumstances (17). If glutaminase I is inhibited the ratio falls and equals 1.0. On the other hand, in metabolic acidemia, since 80% of ammonia produced is derived from glutamine metabolized in the mitochondrion the NH_3/glutamine ratio rises to 1.5. In normal rats, bilateral adrenalectomy resulted in a 30% decrease in ammonia excretion despite the development of metabolic acidemia. The NH_3/glutamine ratio fell from 1.3 to 1.1 (50). This suggests that adrenalectomy inhibited the mitochondrial metabolism of glutamine. Conversely, the ingestion of triamcinolone increased ammonia production by 3 fold in the face of a metabolic alkalemia (50). The NH_3/glutamine ratio increased from 1.3 to 1.5. This is consistent with an increase in the intramitochondrial metabolism of glutamine. Since in vitro studies with kidneys from adrenalectomized rats show the initial effect of triamcinolone to be apparent within 15 minutes, it is unlikely that the synthesis of additional molecules of glutaminase I was responsible for the increase in ammoniagenesis. These results are more consistent with an increase in mitochondrial permeability to glutamine (50).

D) Effect of glutaminase activity on NH_3 production

Davies and Yudkin (21) were the first to demonstrate an adaptive increase in glutaminase I activity during chronic metabolic acidosis. However, the significance of this increase is unclear since during the first 24 hours of ammonium chloride administration, ammonium excretion and production increased without changes in glutaminase I activity (51). Furthermore,the inhibition of protein synthesis by actinomyosin D blocked the adaptive increase in renal glutaminase I activity without altering the increase in ammonia production (52). Interestingly, the increase in ammonia production that occurs during acid loading and actinomyosin D administration has been correlated with an increase in the activity of the purine nucleotide cycle enzymes namely adenylosuccinate synthetase adenylate deaminase and adenylo succinase (53). It has thus been suggested that the production of ammonia from fumarate via the purine nucleotide cycle may contribute significantly to the renal pool of ammonia in acidosis. The net reaction for one turn of the cycle is:

Aspartate + GTP + H20 $\longrightarrow$ Fumarate + NH_3 + GDP + Pi

Experimental evidence demonstrating glutaminase II activity (the liver enzyne) has not been forthcoming (54).

E) Effect of acidemia on NH_3 production

Robinson (55) and Holmes (56) demonstrated that kidney

slices incubated in acid medium displayed increased ammonia production and concluded that the intracellular acidemia per se was responsible for the increase in ammoniagenesis. However, other studies employing renal slices and mitochondrial systems have shown the initial effect of acidemia to be an inhibition of glutaminase I activity and a fall in ammonia production (57,58). As the duration of acidemia increased, the initial inhibition of glutaminase I was overcome and ammonia production increased. Consequently, acidemia per se does not appear to stimulate ammoniagenesis.

F) Effect of potassium hemeostasis on NH_3 production

Potassium depletion has been shown to alter ammonia production in several ways: a) it stimulates glutamine I and II, (59-62), b) it facilitates the entry of glutamine into the mitochondrion, c) it increases the production of ammonia from both glutamine and glutamate (63-66), and d) it inhibits the conversion of glutamate to glutamine thereby increasing the availability of ammonia for excretion (63-66). Potassium loading on the other hand inhibits ammonia production (67).

G) Effect of renal concentration of glutamate and NAD^+/ NADH ratio

Glutamate inhibits the reaction catalyzed by glutaminase During acidosis, the intracellular concentration of gluta-

mate falls and glutaminase I activity rises. Preuss has proposed that the initial reduction in glutamate concentration is caused by an increase in the redox state of pyridine nucleotides (68-69). Direct and indirect measurements of NAD^+/NADH and $NADP^+$/NADPH ratios showed them to be increased in both acute and chronic acidemia. An increase in the NAD^+/NADH ratio will facilitate the conversion of glutamate to α-ketoglutarate and thereby cause a fall in glutamate concentration:

$$\text{glutamate} \xrightarrow[\text{NAD}^+ \quad \text{NADH+}]{\text{glutamate dehydrogenase}} \alpha\text{-ketoglutarate} + NH_3$$

H) Effect of plasma ammonia concentration on NH_3 production

Like glutamate, ammonia inhibits the deamidation of glutamine (13). Pitts has shown that infusions of ammonium chloride into the renal artery of dogs although increasing urinary ammonia excretion, significantly inhibited renal ammoniagenesis (70). Similar results were obtained with infusions of N15 ammonium lactate (71). One cannot conclude that the rise in arterial ammonia concentrations caused the decline in ammonia production observed with ammonium lactate infusion since it is known that lactate alone inhibits renal ammoniagenesis in vivo and in vitro (71). More importantly, the concentration of ammonia necessary to achieve 50% inhibition of glutaminase I is two orders of magnitude greater than those observed in vivo. Consequently, it is likely that this effect is not important

physiologically (13).

I) Effect of α-ketoglutarate concentration on NH_3 production.

α-ketoglutarate which provides the carbon skeleton of glutamine also inhibits glutaminase I and glutamic dehydrogenase. Its cellular concentration decreases during metabolic acidemia and increases in metabolic alkalemia (14,15). Furthermore, infusions of α-ketoglutarate cause a significant decrease in ammonia production (72). Although glutamate concentration rises with α-ketoglutarate, the rise is not sufficient to account for the entire decrease in ammonia production. Consequently, a direct inhibition of glutaminase I by α-ketoglutarate has been postulated (72).

J) Effect of renal gluconeogenesis on NH_3 production

In metabolic acidemia, gluconeogenesis is stimulated (73-75). This observation suggests that glucose formation increases the utilization of glutamine and reduces tissue glutamate concentration, thereby activating glutaminase I. However, more recent experiments indicate that gluconeogenesis and ammoniagenesis are dissociable. Churchill et al showed that the inhibition of gluconeogenesis has no effect on ammonia production in acidemia (76) and Pitts documented that only 25% of the glutamine used by the

kidney is converted to glucose (77). It is now believed that the increase in gluconeogenesis observed with metabolic acidemia is secondary to the rise in renal ammonia production and is dependent upon the availability of glutamate (78).

K) Effect of oxidizable substrate concentration on NH_3 production

Ketone bodies, free fatty acids and lactate are all oxidized by the kidneys and changes in their availability have been shown to alter renal ammonia production. Lemieux demonstrated a 50 to 60% fall in ammonia production and excretion during ketone body infusions in ammonium chloride loaded acidemic dogs (5). A simultaneous decrease in the renal extraction of glutamine was also noted in these studies. The inhibitory effect of ketones was independent of urinary pH or acid base changes. Similar results have been obtained with infusions of sodium citrate (79), sodium octanoate and levarterenol (80). The inhibitory effect of ketone bodies appears to be linked with their oxidation and can be eliminated by blocking the TCA cycle with fluoracetate (81). Lactate inhibits ammoniagenesis by kidney slices of acidemic rats independent of changes in pH. (71). It is thus clear that in animal experiments, both in vivo and in vitro, an increase in substrate availability for oxidation by the

kidney can cause a decrease in the rate of ammoniagenesis.

In summary, in chronic metabolic acidemia, the following alterations are thought to increase ammonia production: a) decreased intracellular pH, b) decreased cellular potassium concentration, c) a decrease in the renal concentration of glutamate, d) an increase in the NAD^+/NADH ratio, e) a fall in the concentration of ammonia and α-ketoglutarate, f) the stimulation of renal gluconeogenesis, g) an increase in the availability of oxidizable substrate.

B. Ketone body metabolism

Regulation of peripheral uptake.

Ketone bodies are an important source of energy during starvation (82) and long term exercise (83). The rate of uptake and oxidation of beta-hydroxybutyric acid varies directly with its plasma concentration (84,85). During prolonged starvation and diabetic ketoacidosis, however, the peripheral uptake of ketones is depressed in spite of their very high plasma levels (86,87). These observations have stimulated interest in the role of insulin as a regulator of ketone metabolism. Acetoacetate uptake by the liver and skeletal muscles is reduced in streptozotocin induced diabetes in rats and is restored to normal with insulin administration (88). Balasse has shown that ketone body uptake by dog skeletal muscle is stimulated by insulin

(89). In man, Felig and Sherwin have demonstrated that hyperinsulinemia stimulates beta-hydroxybutyrate utilization (90). Thus, both the plasma ketone and insulin concentrations appear to play important regulatory roles in modulating ketone uptake by peripheral tissues.

Effects of ketone bodies on glucose metabolism.

Ketone bodies appear to inhibit glycolysis. In both normal and diabetic subjects, infusions of Na β-OHB while not affecting the plasma concentration of insulin are associated with a fall in plasma glucose (90). Randal has shown that in the perfused rat heart and isolated rat diaphragm, ketone bodies inhibit glucose metabolism at the level of phosphofructokinase (91). On the other hand, inhibition of glucose metabolism in the rat hindquarter could not be demonstrated (91). Interestingly, lactate and pyruvate oxidation and the activity of glutamate dehydrogenase are markedly inhibited by ketone bodies (92). Gluconeogenesis is also inhibited by ketone bodies (93). Using renal cortical slices from acidemic and normal dogs, Lemieux showed that β-OHB and acetoacetate in concentrations ranging from 0.5 to 5 mM induced a 20 to 30% decrease in glucose production when either glutamine or glutamate was used as substrate (93).

Effects of ketones on amino acid release.

Felig et al have demonstrated that in the postabsorptive state and in prolonged starvation, intravenous fusion of ketone bodies may inhibit the peripheral release of alanine and results in a significant decrease in the plasma alanine concentration (90,94). The urinary excretion of ammonia was also reduced. In the diabetic rat, no effect of acetoacetate (1.5 mM) or Na β-OHB (1.9 mM) on plasma alanine concentration could be demonstrated in the absence of insulin (95). As the plasma ketone concentration was increased further, however, a significant decrease in plasma alanine concentration occurred in the absence of insulin. Since alanine is the principal amino acid extracted by the liver for gluconeogenesis, (96) inhibition of its peripheral release may explain the reduction in glucose production during ketone infusion.

III. MATERIALS AND METHODS.

A. Infusion studies.

Seven healthy, (4 males, 3 females) normal weight volunteers (body mass index = 20 ± 2), ranging in age from 19 to 36 years were studied. Over the three day period prior to study, each subject consumed a weight maintaining diet containing at least 200 grams of carbohydrate per day and ingested 0.1 g/kg body weight of ammonium chloride daily. The ammonium chloride was administered in 4 divided doses at six hour intervals, the last dose being administered at twelve on the night prior to study. All the subjects tolerated the ammonium chloride well without nausea or vomiting. Venous pH and bicarbonate concentration were measured prior to starting ammonium chloride. Subjects remained fasting after midnight on the evening prior to study. On awakening at 7 am on the morning of study, they drank 600 ml of tap water and reported to the Clinical Research Center at 8 am. A water load of 20 ml/kg was administered from 8 to 8:30 am and the volume of each subsequently voided urine was quantitatively replaced to achieve a steady state water diuresis of 9-12 ml/min.. Small polyethylene catheters were inserted under local xylocaine anesthesis into each antecubital vein, one for the infusion of all test substances and the other for withdrawal of blood. Priming doses of insulin (40 mg/kg) and PAH (12 mg/kg) were administered and were immediately followed

by a continuous infusion of insulin and PAH at a rate of 1.11 ml/min. calculated to maintain plasma levels of 20 mg% and 2 mg% respectively. The subjects remained supine throughout the study except to void. After a 60 minute equilibration period, 3 consecutive 30 minute urine volumes were collected. Subjects then received a 3 hour intravenous infusion of sodium β-hydroxubutyrate (n=7), sodium bicarbonate (n=3) or sodium lactate (n=4) as described below. Urine was collected at 30 minute intervals throughout the study period. Venous blood samples were drawn without the use of a tourniquet at the end of each urine collection. All blood losses were replaced quantitatively with intravenous normal saline.

B. Infusion of test substances.
Sodium β-hydroxybutyrate (Na β-OHB) studies.

Sodium β-hydroxybutyrate (Na β-OHB was administered intravenously as a priming dose (1mmole/kg) over 20 minutes) followed by a continuous infusion at a rate of 2.2 ml/min. which delivered 0.01 mmole/kg min. of Na β-OHB. The Na β-OHB (Nutritional Biochemical Co., Cleveland, Ohio) was prepared as a 40% solution in sterile water, passed through a 22 μm filter apparatus (Millipore Corp., Bedford, Mass.) and stored at $4^{o}C$ until used. Each lot was tested for pyrogenicity (97) and sterility prior to use. Sterile pyrogen free water was added to the 40% solution to achieve the desired ketone concentration. By varying the concentration

of the infusate, a continuous delivery rate of 2.2 ml/min. was used in all patients.

Sodium bicarbonate (Na HC03) studies.

3 patients were restudied as previously described except that they received an infusion of Na HC03. These subjects served as control for volume expansion and for the degree of alkalemia which was associated with Na β-OHB infusion. The rate of bicarbonate infusion varied from 0.52 to 0.85 meq/min. and was calculated to simulate the rise in plasma bicarbonate observed during the infusion of Na β-OHB. Insulin and PAH were not infused in this or the subsequent study with sodium lactate. Endogenous creatinine clearance was used as an index of glomerular filtration rate (GFR).

Sodium lactate studies.

Four subjects were restudied with sodium lactate to simulate the change in intracellular redox potential (NAD^+/NADH) that occurs during the conversion of β-hydroxybutyrate (β-OHB) to Acetoacetate (AcAc). The oxidation of lactate to pyruvate causes a fall in the NAD^+/NADH ratio. These subjects were restudied in a similar manner to that previously described except that they received an infusion of sodium lactate, given as a prime (1 mmole/kg over 20 minutes) - continuous unfusion (0.01 mmole/kg min.).

The purpose and potential risks of the study were explained to each subject and written, voluntary consent was obtained prior to their participation.

C. Analytical methods.

Plasma and urine sodium and potassium concentrations were determined by flame photometry (Flame Photometer 143 Instrumentation Lab. Inc., Watertown, Mass.). Urine and plasma chloride concentrations were determined with a silver electrode titrating chloridometer (Buchler-Cotlove, Chloridometer Buchler Instruments, Ft. Lee, N.J.). Plasma bicarbonate concentration was determined by using a modification of the Van Slyke method. Whole blood pH and PCO_2 were measured on a blood micro pH meter (Radiometer, Copenhagen). Insulin concentrations in plasma and urine were quantified by the method of Walser et al (98) adapted to the autoanalyzer by Fuhr et al (99). Plasma and urine PAH were measured by the method of Smith (100) using a Gilford spectrophotometer at 540 nm. Creatinine was determined by the method of Folin and Wu (101) adapted to the Technicon Autoanalizer. Plasma and urine β-OHB and acetoacetate concentrations were measured according to the method of Williamson and Krebs (102). Plasma lactate was determined using an enzymatic assay (103). Plasma glucose was measured by the glucose oxidase method (104) on a Beckman Glucose analyser (Beckman Instrument Inc. Science Essentials Co., Mountainside, N.J.).

Plasma free fatty acids were measured using the modified Doles' Titrimetric method (105). Plasma glutamate was determined by enzymatic reaction with glutamate dehydrogenase (GDmp4) (L glutamate, NADP oxydoreductase deaminating, EC 1.4.13)(106). Plasma glutamine concentration was quantified by enzymatic hydrolysis with purified glutaminase (L glutamine aminohydrolase E.C. 3512), followed by spectrophotometric detection of glutamate at 340 nm(106). The methods used for the determination of plasma immunoreactive insulin and glucagon andplasma acidic and neutral amino acids have been previously described (107). Urine was collected under oil and analyzed for ammonium, titratable acidity and pH on the day of study. Urinary pH was measured wtih a glass electrode pH meter (PHm 71 Radiometer, Copenhagen, Denmark). Urinary ammonium and titratable acidity were determined using the microdiffusion method of Conway (108) and the method of Peters and Van Slyke (109) respectively.

D. Calculations.

For the statistical analysis of the data, observations from the 3 control periods before the administration of test substances were averaged and compared to the values obtained following the infusions (paired t-test), (110). Each subject thereby served as his own control. Differences between the hydroxybutyrate, lactate and bicarbonate groups

were compared using the unpaired t-test. Absolute urinary excretion of electrolytes were determined using the formula:

Excretion = Urinary concentration urine flow (μmol/min.).

The clearances of creatinine, PAH and Insulin were calculated using the formula: Cx = Ux.V/Px. All values represent mean ± SEM.

IV. RESULTS

1. NA β-OHB infusion studies

A. Chronic ammonium chloride loading. (Table 1): After 3 days of ammonium chloride ingestion, the venous pH fell from 7.36 ± 0.02 to 7.31 ± 0.12 ($p < 0.01$) and the plasma bicarbonate concentration decreased from 25 ± 1 to 20 ± 1 meq/L ($p < 0.01$). There were no significant changes in body weight and plasma concentration of sodium, chloride and potassium.

B. Infusion of Na β-OHB: During the infusion of Na β-OHB, the rise in urinary β-OHB excretion paralleled the increase in plasma ketone concentration. Within 30 minutes, plasma ketone concentration increased from 81 ± 12 to 530 ± 20 μM ($p < 0.001$) and then gradually declined to 193 ± 29 μM ($p < 0.001$) by the third hour. Urinary β-OHB excretion rose from 0.7 ± 0.008 to 136 ± 48.4 μmol./min ($p < 0.002$) at 30 minutes and then gradually fell to a nadir of 23.7 ± 6 μmol./min ($p < 0.01$) at 120 minutes, (Table 2 and 3, Figure 4B). The pattern of change in plasma acetoacetate concentration was similar to that of β-OHB. Throughout the study, urine flow rate and creatinine, Inulin and PAH clearances remained unchanged from baseline (Table 2). Baseline urine pH (5.63 ± 0.06) fell to 5.34 ± 0.056 ($p < 0.02$) at 30 minutes and then gradually rose reaching a value of 5.97 ± 0.008 ($p < 0.02$) at the end of 3 hours. Urinary ammonium excretion fell by 26% ± 2

($p < 0.01$) within 30 minutes after starting the infusion of Na β-OHB, despite a significant fall in urine pH (Table 2). Thereafter, ammonia excretion continued to decrease reaching a nadir of 54 ± 5.5 μeq/min. ($p < 0.002$). This represented a 43% decrease in ammonia excretion at 120 minutes. During the last hour of the study ammonium excretion remained suppressed (Table 2, Figure 5). Over the entire study period, the mean decrease in ammonium excretion was 35% less than the basal excretion rate ($p < 0.001$). The excretion of titratable acid followed a slightly different pattern. No significant change from baseline (41 ± 3 μeq/min.) was observed at 30 minutes but thereafter it gradually fell reaching a level of 28.3 ± 1.7 μeq/min. ($p < 0.02$) at 3 hours (Table 2). Urinary sodium excretion ($U_{Na}V$) fell from a baseline value of 221 ± 18 to 124 ± 11 μeq/min. ($p<0.01$) (Table 4, Figure 6) without a change in plasma sodium concentration (P_{Na}) or Inulin clearance (C_{IN}). Consequently, the fractional excretion of sodium (C_{Na}/C_{IN}), fell proportionately to the decrease in $U_{Na}V$. The pattern and the magnitude of change in $U_{Cl}V$, C_{Cl} and C_{Cl}/C_{IN} paralleled that of sodium. The plasma chloride concentration decreased from 103.44 ± 0.9 to 98.8 ± 0.65 meq/L ($p < 0.01$), (Table 4). The plasma potassium concentration fell from 4.18 ± 0.09 to 3.55 ± 0.05 meq/L ($p < 0.001$) at 3 hours (Table 5). Urinary potassium excretion ($U_K V$) remained unchanged during the first hour but thereafter fell progressively, reaching a value 31% below baseline values

at 3 hours. Since urinary potassium excretion fell in concert with the decrease in P_K, there was no significant change in C_K and C_K/C_{IN}.

The infusion of Na β-OHB was accompanied by a gradual rise in venous pH from 7.31 ± .012 to a peak of 7.38 ± 0.01 ($p < 0.01$) and bicarbonate concentration also increased from 19.4 ± 1 to 24.8 ± 1.1 meq/L ($p < 0.02$), (Table 6). The plasma concentration of glucose, insulin, glucagon and free fatty acids remained unchanged (Table 7). Plasma amino acid concentration remained the same for that of alanine which decreased by 20% at 150 minutes. Glutamine and glutamate concentrations were unchanged. (Table 8).

2. $NaHCO_3$ infusion studies.

A. Chronic ammonium chloride loading: Following the 3 day period of ammonium chloride administration, venous pH and plasma bicarbonate concentration decreased from 7.36 ± 0.03 to 7.30 ± 0.004 ($p < 0.05$) and from 25.4 ± 0.8 to 19.4 ± 1.1 meq/L ($p < 0.01$) respectively. There were no significant changes in body weight and plasma sodium, chloride and potassium concentration (Table 1).

B. Infusion of $NaHCO_3$: Following $NaHCO_3$ infusion venous pH rose from 7130 ± 0.01 to 7.38 ± 0.01 ($p < 0.01$) and plasma bicarbonate concentration from 19.5 ± 0.3 to 26 ± 0.2 μeq/L $p < 0.002$). These increases were similar to those observed

in the Na β-OHB infusion studies, (Table 6 and 11). Urine flow and creatinine clearance were unchanged from baseline. Urine pH gradually increased from 5.64 ± 0.06 to 6.1 ± 0.06 ($p < 0.01$) at 180 minutes (Table 9). The rise in urine pH was similar to that observed with Na β-OHB. Ammonium excretion was unchanged from baseline, 92 ± 8 μeq/min, at 30 minutes, compared to a 26% decrease ($p < 0.01$) observed following Na β-OHB infusion. At 120 minutes following $NaHCO_3$ infusion, ammonium excretion decreased slightly, 84 ± 5 μeq/min.. This decrease was not statistically significant. At 180 minutes, however, ammonium excretion fell further to 74 ± 5 μeq/min. ($p < 0.05$) (Table 9). The decline in ammonium excretion was only half as great as that observed with Na β-OHB infusion ($p < 0.01$). Over the entire study period, the mean fall in ammonium excretion was 8% of the baseline value, compared to a 35% decline observed following Na β-OHB ($p < 0.002$). No significant change in titratable acid excretion occurred. $U_{Na}V$ decreased from a control value of 210 ± 16 to 112 ± 11 μeq/min at 180 minutes ($p < 0.01$) without any change in plasma sodium concentration (Table 10). Sodium clearance declined from a baseline value of 1.6 ± 0.12 to 0.9 ± 0.1 ml/min ($p < 0.01$). (Table 19, Figure 7). Plasma chloride concentration fell from 104.0 ± 0.2 to 99.0 ± 0.4 meq/L ($p < 0.02$) and this was paralleled by a decrease in chloride excretion from 222 ± 36 to 163 ± 19 μeq/min ($p < 0.05$). The

clearance of chloride decreased similarly from 2.1 ± 0.2 to 1.64 ± 0.1 ml/min ($p < 0.05$), (Table 10). $U_K V$ and C_K did not change significantly even though the plasma potassium concentration decreased from 4.54 ± 0.03 to 3.95 ± 0.1 meq/L ($p < 0.05$). There was no change in plasma ketone glucose, insulin, glucagon, free fatty acids and amino acid concentration (Table 12,13).

3. Na Lactate infusion studies.

A. Chronic ammonium chloride loading: During the three day ammonium chloride loading, venous pH fell from 7.36 ± 0.01 to 7.30 ± 0.01 ($p < 0.05$) and the plasma bicarbonate concentration decreased from 25.4 ± 0.8 to 18.3 ± 1.5 meq/L ($p < 0.01$). There was a small and statistically insignificant rise in plasma chloride and potassium concentration. No significant changes in plasma sodium concentration and body weight were observed (Table 1).

B. Na Lactate infusion: Plasma lactate concentration rose from 6.9 ± 0.5 to 13.7 ± 1 mM ($p < 0.002$) within 30 minutes and reached a plateau of approximately 10 mM by 60-90 minutes. No change in plasma ketone concentration was observed (Table 14, Figure 6A). Venous pH increased from 7.30 ± 0.01 to 7.38 ± 0.01 ($p < 0.01$) and plasma bicarbonate concentration from 18.3 ± 1.5 to 25.8 ± 0.8 meq/L ($p < 0.01$) (Table 15). Creatinine clearance and urine flow were unchanged from base-

line. Urinary pH fell from 5.12 ± 0.018 to 5.02 ± 0.025 at 30 minutes and then gradually increased reaching a value of 6.03 ± 0.16 ($p < 0.01$) at 180 minutes (Table 16). Urinary ammonium excretion decreased by 10% at 30 minutes and progressively fell thereafter, reaching a value which was 40% below baseline at 150-180 minutes ($p < 0.01$) (Table 16, Figure 8). The mean decrease in ammonium excretion during the 3 hour study was 35% ($p < 0.01$). The change in ammonium excretion was similar to that observed following Na β-OHB infusion. Urinary sodium excretion and sodium clearance fell significantly by 30 minutes and reached a plateau value, 60% below baseline, at 120 minutes ($p < 0.001$), Figure 7. No change in plasma sodium concentration occurred (Table 16). Urinary chloride excretion and chloride clearance paralleled those of sodium. Plasma chloride concentration fell significantly from 102 ± 1 to 98 ± 1 meq/L ($p < 0.05$). Urinary potassium excretion and potassium clearance fell by 55% ($p < 0.05$) and 46% ($p < 0.05$) at 150 minutes, and this was associated with a decrease in plasma potassium concentration from 4.23 ± 0.2 to 3.5 ± 0.15 meq/L ($p < 0.05$), (Table 17). There was no change in plasma glucose, insulin, glucagon free fatty acids and amino acid concentration, (Table 18,19).

V. DISCUSSION

The present study demonstrates that the intravenous infusion of Na β-OHB or lactate to acidemic subjects acutely inhibits urinary ammonium excretion. The maximum increase in plasma ketone concentration, 530 μM, was modest and comparable to levels seen in mild fasting and diabetic ketosis (111). A similar effect of ketone infusion on urinary ammonium excretion in acidemic dogs has been reported by Lemieux et al (5). They showed that an increase in total plasma ketone concentration to 4.50 mM led to an inhibition of ammonia production and excretion which was associated with a decrease in glutamine uptake.

The factors known to affect renal ammonium excretion and production are listed in table 20, and are shown schematically in figure 10. The sources of excreted ammonium are shown in figure 3.

In the present study, blood and urine pH rose following Na β-OHB infusion. Although those changes could potentially explain the decrease in ammonium excretion, several observations suggest that other mechanisms must also be involved. The initial (0-30 min) effect of Na β-OHB infusion was to decrease urine pH ($p<0.002$), which should have increased ammonium excretion since the lower urine pH would trap more ammonia in the ionized form (26). Instead ammonium excretion fell by 27% ($p<0.01$) during the first 30 minutes. Addition-

ally, neither blood pH nor plasma bicarbonate concentration changed significantly during this time period. To further examine the potential contribution of changes in blood and urine pH to the decrease in ammonium excretion, 3 subjects were restudied with an infusion of sodium bicarbonate designed to increase the plasma bicarbonate concentration and blood and urine pH to levels similar to those observed during Na β-OHB infusion. Despite a similar or greater plasma bicarbonate concentration (23 meq/L with Na β-OHB vs 26 with $NaHCO_3$), blood pH (7.36 with Na β-OHB vs 7.38 with $NaHCO_3$) and urine pH (5.97 with Na β-OHB vs 6.10 with $NaHCO_3$) the mean decline in urinary ammonium excretion was 3.5 times greater with Na β-OHB than with $NaHCO_3$. Thus, it appears likely that factors other than the rise in plasma bicarbonate concentration and blood and urine pH must have contributed to the decline in ammonia excretion following Na β-OHB infusion.

Since urine flow did not change during our experiments, an increase in back diffusion of ammonia (37) or a change in the concentration gradient from the tubular lumen to the renal tubular cell cannot explain the observed decrease in ammonia excretion. Since there is no evidence to suggest that ketones inhibit hepatic ammonia production, it is unlikely that decreased arterial ammonium concentration can explain the decline in ammonium excretion observed during

Na β-OHB infusion. Since RBF and GFR were unchanged and since plasma levels of glutamine were unchanged, it is unlikely that decreased substrate delivery can account for the fall in ammonia excretion.

Lemieux et al have shown previously that ketones inhibit renal ammoniagenesis both in vivo and in vitro (5,87). Although the renal extraction of glutamine was significantly depressed in the in vivo experiments, the uptake of glutamine by the renal tubular cell from the renal tubular lumen (5) was not affected. These authors postulated that the metabolism of ketone bodies by the renal tubular cell caused intracellular changes that inhibited ammonia production. In vitro studies suggest that ketone bodies inhibit glutamine metabolism through a direct metabolic effect linked with ketone oxidation in the tricarboxylic acid cycle (81). In renal cortex slices from acidotic dogs, ketones inhibit glutamine uptake, ammoniagenesis and gluconeogenesis and these effects can be completely reversed by the addition of fluoracetate which blocks the TCA cycle. Fluoracetate is known to act by inhibiting the enzyme aconitase which converts citrate to isocitrate (81). However, although it seems that oxidation of ketones via the TCA cycle results in the inhibition of renal ammoniagenesis, the exact mechanism by which this occurs is not clear at present.

Ketones could interfere with ammonia production in two ways: first, by increasing the intracellular α-ketoglutarate

level or secondly by decreasing the $NAD^+/NADH$ ratio in the renal tubular cell. Ketone bodies are first oxidized to Acetyl CoA which subsequently enters the TCA cycle. Citrate synthetase then catalizes the formation of citrate from Acetyl CoA and oxaloacetate (Figure 1). Citrate is in turn converted to α-ketoglutarate which plays an important role in ammonia production. Increased levels of α-ketoglutarate inhibit both the transport and the deamidation of glutamine by renal mitochondria (14,15). The intravenous infusion of α-ketoglutarate into chronically acidotic dogs decreases the renal production of ammonia and increases the amount of glutamate and α-ketoglutarate that is released in the renal vein (72). It is thought that an increase in cellular α-ketoglutarate concentration inhibits the deamination of glutamate. Since glutamate is an inhibitor of glutaminase I, a rise in its concentration will inhibit the deamidation of glutamine (Figure 1). Although it is clear that an increase in α-ketoglutarate concentration can result in a fall in ammonia production, it has not been proven that the oxidation of ketone bodies causes a significant rise in α-ketoglutarate concentration. Indeed, when Lemieux incubated renal cortical slices from acidemic dogs with ketone bodies he observed a significant decline in ammonia production but could not detect any change in α-ketoglutarate concentration (87).

The second mechanism by which hyperketonemia may inhibit

ammonia production is related to a decrease in the NAD^+/NADH ratio which has been postulated to occur during ketone oxidation:

$$\beta\text{-OHB} \xrightarrow{NAD^+,NADH} \text{Acetoacetate (AcAc)}$$

As β-OHB is converted to AcAc the concentration of NAD^+ decreases while that of NADH increases. This results in a decline in the NAD^+/NADH ratio. Both Pitts (27) and Preuss (68,69) have underscored the theoretical importance of the redox state of nicotinamide dinucleotide in the control of ammonia production. Preuss has demonstrated an increase in the NAD^+/NADH ratio in both acute and chronic acidemia. Glutamate dehydrogenase which catalyzes the deamination of glutamate to α-ketoglutarate is in equilibrium with the β-OHB dehydrogenase system and is therefore influenced by the NAD^+/NADH ratio (16). A rise in the ratio inhibits the deamination of glutamate leading to the accumulation of glutamate which can in turn depress glutaminase I activity. This will decrease the rate at which glutamine is deaminated and will result in decreased ammonia production (68,69).

To determine whether an alteration in the NAD^+/NADH ratio might play some role in the fall of ammonia production observed following hyperketonemia, we infused Na lactate into chronically acidemic subjects to stimulate the conversion of NAD^+/NADH. Lactate is converted to pyruvate in the cytosol and NADH is generated from NAD^+ in the process:

$$\text{Lactate} \xrightarrow{NAD^+\ NADH} \text{Pyruvate}$$

Following lactate infusion, urinary ammonium excretion declined by 35% ($p < 0.01$) and this decline was similar to that observed with Na β-OHB. Although Lemieux et al could not demonstrate a fall in ammonia production in acidemic dogs during ketone body infusion, other investigators have previously reported the inhibition of ammonia production by lactate both in vivo (71) and in vitro (111,112). Thus the results observed with lactate infusion are consistent with the possibility that the decrease in the $NAD^+/NADH$ ratio is responsible for the decline in ammonia excretion.

Potassium homeostasis is thought to be closely related to ammonia production. Both in vivo and in vitro studies have demonstrated that potassium depletion stimulated ammonia production (63-66). Several mechanisms have been shown to play a role in the increase in ammonia production following potassium depletion: 1) Stimulation of glutaminase I activity (59-62), 2) Stimulation of glutaminase II activity (59-62), 3) inhibition of glutamine synthetase activity and of the conversion of glutamate to glutamine (66). In contrast, potassium loading has been shown to depress ammonia production (67). This inhibitory effect of potassium on ammoniagenesis has not been well studied. The only available evidence suggests that potassium loading does not affect glutaminase I activity (113).

Plasma potassium concentration decreased significantly following both Na β0OHB ($p < 0.001$) and Na lactate ($p < 0.002$) while urinary potassium excretion decreased or remained the

same. The decline in plasma potassium concentration is most likely related to the increases in blood pH and plasma bicarbonate concentration with a subsequent shift of hydrogen ion out of cells in exchange for potassium (114-116). Measurements of the potassium transport pool in distal tubular cells of rats using isotopic potassium have shown that alkalosis increases the intracellular pool size of potassium (117). Consequently, in the present study, it is likely that cellular potassium concentration increased during both Na β-OHB and Na lactate infusion. This could represent a situation similar to that observed following potassium loading. Thus, it is possible that the decline in urinary ammonium excretion was in part due to an increase in intracellular potassium concentration. This explanation is unlikely for several reasons:

1) ammonium excretion falls before any change in plasma potassium concentration occurs. 2) Tannen has shown that acute changes in intracellular or extracellular potassium concentration or in the transcellular potassium gradient do not influence ammoniagenesis (118). 3) In the present study although $NaHCO_3$ infusion resulted in a significant rise in blood pH and a decrease in plasma potassium concentration, the decline in urinary ammonium excretion was significantly less than observed with Na β-OHB or Na lactate.

Several studies have suggested that ammonia production is linked to gluconeogenesis (73-75). Since ketones are

known to inhibit renal gluconeogenesis (73,119) it is possible that the decrease in ammonium excretion observed following β-OHB in our studies was related to its inhibitory effect on glucose production. Using renal cortical slices from normal and acidotic dogs, Lemieux has shown the addition of β-OHB or AcAc (0.5-5 mM/L) leads to a 20% decrease in glucose production and a 50% decline in ammonia production (93). These authors postulated that decreased glucose production led to increased tissue glutamate and decreased glutaminase I activity and ammonia production (Figure 1). Conversely, it has been argued that enhanced gluconeogenesis would stimulate ammonia production by reducing tissue glutamate and increasing glutaminase I activity. However, not all of the available evidence supports such a strong link between ammoniagenesis and glucose production. First, Churchill and Malvin were able to clearly dissociate glucose and ammonia production in vivo and in vitro (76). Second, although lactate increases glucose production it depresses ammoniagenesis (111).

In the present study, Na β-OHB infusion caused a 20% decrease in plasma alanine concentration. A similar fall in plasma alanine level following ketone infusion has been reported by Felig in subjects fasted overnight as well as following prolonged starvation (94). These authors suggested that ketone bodies may inhibit muscle efflux of alanine or possibly enhance hepatic alanine uptake. Studies by Pitts

have shown that about 5% of ammonia production is derived from alanine. Since plasma alanine levels dropped by only 20% it is difficult to see how decreased alanine availability could account for a 35% decrease in ammonia production following Na β-OHB infusion. Although Felig has suggested that decreased muscle efflux or increased hepatic uptake of alanine may be responsible for β-OHB induced hypoalaninemia, another possibliity needs to be considered. Experiments in which carboxyl ^{14}C labeled pyruvate was infused into the renal artery of acidemic dogs have shown that alanine is produced by the kidney by the transamination of pyruvate with the amino nitrogen of glutamine, either directly or after the conversion of glutamine to glutamate (32,34). Since substantial amounts of alanine are thought to be produced by the kidney and since alanine production is dependent on renal ammonia production, ketone induced hypoalaninemia might result from the inhibition of renal ammoniagenesis by ketone bodies and not vice versa.

In all 3 studies (β-OHB, HCO_3, lactate) urinary sodium and chloride excretion and clearance declined without any change in plasma sodium or potassium concentration. This inhibition of sodium and chloride excretion was observed wtihin 30 minutes and was progressive throughout the 3 hour study period. The fall in sodium and chloride excretion occurred despite expansion of the extracellulary volume (ECV). Indeed, ECV expansion would be expected to

increase both sodium and chloride excretion (120-124). Presumably such ECV increase would have depressed aldosterone levels which in turn would have led to an increase in sodium and chloride excretion. The infusion of a poorly reabsorbable anion such as ketones would also be expected to enhance sodium excretion (125). What then is responsible for the observed decline in sodium and chloride excretion? It is well known that metabolic acidemia inhibits renal tubular sodium reabsorption (126-128) and results in a natriuresis. Alkalemia does the opposite. Thus, the most likely explanation for the decrease in urinary sodium excretion is a change in acid base status from chronic metabolic acidemia to acute metabolic alkalosis. Such an explanation would account for the fall in urinary sodium and chloride observed with Na lactate, $NaHCO_3$ and Na β-OHB.

Urinary potassium excretion decreased with lactate and β-OHB infusions. This finding was somewhat unexpected since 1) both infusions were accompanied by a rise in plasma bicarbonate and venous pH which should have resulted in an increase in intracellular potassium concentration and a subsequent rise in potassium secretion (117, 2) urinary β-OHB and bicarbonate ion excretion increased significantly with Na β-OHB infusion; this should have caused a decrease in the transepithelial potential difference and favored the passive diffusion of potassium out of the cell into the tubular lumen. A possible explanation for the observed

decrease in potassium excretion is related to the fact that urinary sodium excretion fell with β-OHB and lactate infusion. Indeed, a direct relationship has been observed between potassium secretion and distal sodium delivery and reabsorption (129): when sodium delivery is diminished, potassium secretion is reduced. However, this explanation cannot totally account for the decline in potassium excretion: 1) Recent evidence suggests that potassium secretion is dependent on luminal flow rate and not on luminal Na concentration (130). 2) $NaHCO_3$ infusion results in a similar decrease in sodium excretion while slightly increasing potassium excretion.

Summary

This work was undertaken to determine the effects of sodium beta-hydroxybutyrate on urinary ammonium excretion in chronically acidemic human subjects. The following conclusions can be drawn from the above experiments:

1) Naβ-OHB infusion significantly depresses urinary ammonium excretion in chronically acidemic human subjects.

2) This effect of Na β-OHB is unrelated to changes in urine pH or in systemic acid base balance.

3) A fall in NAD^+/NADH ratio may explain the inhibitory effect of Na β-OHB on urinary ammonium excretion.

4) Na β-OHB infusion caused a 20% decrease in plasma alanine concentration.

5) β-OHB, lactate and bicarbonate is accompanied by a fall in urinary sodium excretion.

6) A decrease in potassium excretion is observed with the infusion of β-OHB and lactate.

Other experiments which would help determine the precise nature of the inhibitory effect of β-OHB on ammonia production include: 1) infusing NAD^+ and Naβ-OHB or β-hydroxy butryic acid in order to maintain the subjects in metabolic acidemia. 2) Infusing acetoacetate, thereby bypassing the following reaction:

$$\beta\text{-OHB} \xrightarrow{NAD^+\ NADH} \text{Acetoacetate}$$

3) Directly measuring the NAD^+/NADH ratio, in in vitro studies.

TABLE 1. Effect of a 3 day period of ammonium chloride loading on plasma electrolytes, venous blood gases and body weight.

A. Na β-OHB infusion studies.

	PLASMA				BLOOD			
	Na meq/L	K meq/L	Cl meq/L	HcO_3 meq/L	pH	PCO_2 mmHg	HCO_3 meq/L	Weight kg
Before NH4CL	137.5±1	4.14±.25	101±0.8	24.8±0.7	7.36±.02	42± 3.3	25±0.7	71.4±16
After NH4CL	134.3±1	4.18±.09	103±1.0	19.5±1.0*	7.31±.01'	41± 1.6	20±1.0*	70.4±15

B. Na HCO_3 studies.

Before NH4Cl	136.0±1	4.40±.18	102±0.9	25.2±1.3	7.36±.01	41±2.6	25±0.8	81.0±20
After NH4CL	133.0±1	4.54±0.03	104±0.2	19.5±.3'	7.30±.01'	37±2.0	19±1.1'	80.0±20

C. Na lactate infusion studies.

Before NH4CL	136.0±1.	4.20±.30	100±0.8	25.3±1.0	7.37±.01	42±3.0	25±0.7	66.8±6.8
After NH4CL	134.0±1.	4.23±0.20	102±1.3	18.3±1.5'	7.30±.01'	43±0.9	21±1.2	66.0±6.7

Values are mean ± SEM. ' $p<0.05$; * $p<0.01$.

TABLE 2. Effect of Na β-OHB infusion on urine flow, creatinine clearance, inulin and PAH clearance, urine pH., amonia, titratable acid and β-OHB excretion

Time	Urine Flow	Ccr.	C_{In}	C_{PAH}	U pH	$U_{\beta\text{-OHB}}V$	$U_{NH_3}V$	$U_{TA}V$
Minutes	ml/min.	ml/min.	ml/min.	ml/min.		µmole/min.	µEq/min.	µEq./min.
Control	12.8±0.9	124±5	107±6	548±37	5.63±.06	0.70±.08	95.6±4.4	41.2±3.2
0-30	13.0±0.7	128±4	111±4	580±51	5.34±.056"	136.2±48"	70.4±3.9*	44.5±1.8
30-60	12.9±1.0	135±6	106±6	560±44	5.45±.079	68.5±28'	64.1±4.5**	40.2±1.6
60-90	12.3±1.0	133±6	106±4	543±40	5.68±.1	36.6±10*	61.9±4.5**	34.2±1.5
90-120	11.5±0.6	126±6	106±3	549±34	5.78±.08	24.7±6*	54.3±5.5**	31.9±.5'
120-150	12.2±0.8	142±8	111±6	579±60	5.93±.117	28.0±9*	59.4±4.5**	30±1.8"
150-180	11.0±0.8	140±4	109±5	560±35	5.97±.083"	32.7±8*	59.0±6.1*	28.3±1.7"

Values are means ± S.E.M. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Na β-OHB infusion was started at time 0. C_{IN}. = Inulin clearance; C_{PAH}=para amino hippurate clearance; Ccr = creatinine clearance; U_{pH} = urine pH; $U_{\beta\text{-OHB}}V$= β-OHB urinary excretion; $U_{NH_3}V$ = ammonia excretion; $U_{TA}V$ = titratable acids excretion.

' $p < 0.05$; " $p < 0.02$; * $p < 0.01$; ** $p < 0.002$

TABLE 3. Effect of Na β-OHB infusion on blood ketone concentration.

Time Minutes	(β-OHB) μM	(AcAc) μM	(T) μM
Control	53+9	28+6	81+12
0-30	394±30***	136±9***	530±20***
30-60	234±30***	93±10***	327±24***
60-90	178±16***	83±8***	262±10***
90-120	192±13***	74±11***	266±18***
120-150	258±22***	69±9***	327±29***
150-180	193±29***	74±13***	359±40***

Values are mean ± S.E.M.. The control period represents the average of 3 successive measurements obtained during steady water diuresis. (β-OHB) = plasma β-hydroxybutyrate concentration; (AcAc)= plasma acetoacetate concentration; (T) = total plasma ketone concentration

*** $p < 0.001$

TABLE 4. Effect of Na β-OHB infusion on plasma concentration, urinary excretion, clearance and fractional excretion of sodium and chloride.

Time	P_{Na}	$U_{Na}V$	C_{Na}	C_{Na}/C_{IN}	P_{Cl}	$U_{Cl}V$	C_{Cl}	C_{Cl}/C_{IN}
Minutes	meq/L	μeq/min.	ml./min.	%	meq/L	μeq/min.	ml/min.	%
Control	134.3±1.0	221±18	1.65±.13	1.54±.12	103.5±1.0	250±27	2.42±.17	2.26±.13
0-30	134.6±0.9	238±18	1.76±.12	1.59±.10	101.6±1.2	218±27	2.14±.17	1.93±.13
30-60	134.3±1.0	204±22	1.52±.15	1.43±.13	101.1±1.0	188±26	1.85±.16	1.74±.12
60-90	134.6±1.2	176±18	1.31±.12	1.23±.10	101±1.0	163±17'	1.60±.12*	1.50±.10*
90-120	134.4±1.2	151±11"	1.13±.08*	1.06±.07*	100.4±0.9	144±11"	1.43±.08**	1.34±.06**
120-150	134.6±1.4	160±22'	1.19±.16'	1.07±.06*	99.9±1.2	129±17*	1.30±.12**	1.17±.04**
150-180	134.3±1.1	124±11*	0.93±.09*	0.90±.04*	99.8±.07	124±13*	1.35±.10***	1.14±.03**

Values are mean ± S.E.M.. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Na β-OHB was started at time 0. P_{Na} = plasma sodium concentration; $U_{Na}V$ = urinary excretion of sodium; C_{Na}/C_{IN} = fractional excretion of sodium; C_{Na} = sodium clearance; P_{Cl} = plasma chloride concentration; $U_{Cl}V$ = urinary chloride excretion; C_{Cl} = chloride clearance; C_{Cl}/C_{IN} = fractional excretion of chloride.

' $p<0.05$; " $p<0.02$; * $p<0.002$; *** $p<0.001$

TABLE 5. Effect of Na β-OHB infusion on plasma concentration, urinary excretion, clearance and fractional excretion of potassium.

Time	P_K	$U_K V$	C_K	C_K/C_{IN}
Minutes	meq/L	μeq/min	ml/min.	%
Control	4.18±.09	106±11.8	25.4±2.5	23.8±1.7
0-30	4.03±.11	98±11.4	24.3±2.5	21.9±1.6
30-60	3.99±.11	107±12.9	26.8±2.5	25.3±1.8
60-90	3.93±.08	91±9.9	23.1±1.8	21.7±1.4*
90-120	3.71±.08	79±8.0	21.3±1.8	20.0±1.4*
120-150	3.66±.08	79±8.8	21.6±1.9	19.4±1.4*
150-180	3.58±.06'	73±8.1	20.5±1.8	19.0±1.0***

Values are mean ± S.E.M.. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Na. β-OHB infusion was started at time 0. P_K= plasma potassium concentration; $U_K V$ = urinary potassium excretion; C_K= potassium clearance; C_K/C_{IN}= fractional excretion of potassium.

' $p < 0.05$; * $p < 0.01$; ** $p < 0.002$; *** $p < 0.001$.

TABLE 6. Effect of Na β-OHB infusion on venous pH, calculated and measured bicarbonate concentration, and venous carbon dioxide partial pressure.

Time	V pH	Calculated bicarbonate	Measured bicarbonate	V pCO_2
Minutes		meq/L	meq/L	mm/Hg
control	7.31±.012	20.2±1.0	19.4±1.0	42±1.6
0-30	7.33±.006	20.9±1.0	21.6±0.9	41±3.1
30-60	7.33±.01	21.4±1.5	22.2±1.2	41±2.7
60-90	7.36±.012'	21.5±1.2	22.7±1.0	39±2.2
90-120	7.37±.006*	22.1±1.3	23.0±1.1'	39±2.5
120-150	7.38±.01**	22.4±1.1	24.3±1.2"	39±2.4
150-180	7.36±.008"	23.3±1.1'	24.8±1.2"	43±2.0

Values are mean ± S.E.M.. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Na β-OHB infusion was started at time 0.
V pH = venous blood pH; V pCO_2 = venous carbon dioxide partial pressure.
' $p<0.05$; " $p<0.02$; * $p< 0.01$.

TABLE 7. Effect of Na β-OHB infusion on plasma levels of glucose, insulin, glucagon and free fatty acids

Time	Plasma glucose concentration	Plasma insulin concentration	Plasma glucagon concentration	Plasma free fatty acid concentration
Minutes	mg%	U/ml	pg/ml	nM
Control	89±1.6	20±1.4	55±6	310±60
0-30	87±2.3	21±0.6		
30-60	88±2.2	18±1.0	59±3	290±40
60-90	88±1.9	19±1.5		
90-120	87±1.8	16±1.5	53±7	270±25
120-150	85±0.8	18±1.4		
150-180	86±1.1	17±1.2	50±5	300±30

Values are mean ± SEM. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Naβ-OHB infusion was started at time 0.

TABLE 8. Effect of Na β-OHB infusion on plasma amino acid concentration (μM).

Amino acid	Control	0-30 min.	30-90 min.	90-150 min.
Taurine	58±3	64±5	58±4	57±4
Threonine	112±15	121±12	113±15	108±18
Serine	89±9	90±8	88±7	88±8
Glutamic acid	520±30	515±20	519±16	510±13
Glutamate	47±8	51±7	52±6	51±3
Proline	185±18	176±21	165±21	167±23
Citrulline	29±2	30±3	26±3	27±3
Glycine	235±28	220±23	225±20	246±20
Alanine	359±29	319±42	306±41	288±50
Butyric acid	25±2	22±3	21±3	22±3
Valine	233±32	235±32	223±22	231±23
1/2 Cysteine	74±7	70±6	74±6	74±6
Methionine	24±3b	23±4	26±4	28±5
Isoleucine	53±6	52±6	53±5	43±5
Tyrosine	45±6	45±6	45±6	48±6
Phenylalanine	46±6	46±6	47±5	37±6
Leucine	116±11	120±11	123±10	125±10

Values are mean ± S.E.M.. The control period represents the average of 3 baseline measurements obtained during steady water diuresis. Na β-OHB infusion was started at time 0.

TABLE 9. Effect of sodium bicarbonate infusion on urine flow, creatinine clearance urine pH, and ammonia and titratable acid excretiin

Time	Urine flow	C_{Cr}	U_{pH}	$U_{NH_3}V$	$U_{TA}V$
Minutes	ml/min	ml/min		µeq/min	µeq/min.
Control	11.4±1.4	141±7	5.64±.06	92.8±8.7	33.5±5.2
0-30	11.4±2.5	140±2	5.78±.10	92.3±7.0	31.0±4.2
30-60	10.2±1.8	132±6	5.82±.08	89.7±7.9	31.5±5.1
60-90	9.8±1.3	138±7	5.86±.09	85.4±6.7	32.1±5.7
90-120	10.3±1.8	128±7	5.91±.10	84.3±5.6	30.9±5.1
120-150	12.1±1.6	153±5	6.00±.08"	83.4±6.6	32.7±3.4
150-180	11.6±1.6	153±7	6.10±.08*	74.5±5.5	31.7±4.0

Values are mean ± S.E.M.; the control value represents the average of 3 successive baseline measurements obtained during steady water diuresis. C_{Cr} = creatinine clearance; U_{pH}= urine pH; $U_{NH_3}V$=urinary ammonia excretion; U_{TA}= titratable acid excretion.

" $p<0.02$; *$p<0.01$.

TABLE 10. Effect of sodium bicarbonate infusion on plasma concentration, urinary excretion, and clearance of sodium, chloride, and potassium

Time	P_{Na}	$U_{Na}V$	C_{Na}	P_{Cl}	$U_{Cl}V$	C_{Cl}	P_K	U_KV	C_K
minutes	meq/L	µeq/min	ml/min	meq/L	µeq/min	ml/min	meq/L	µeq/min	ml/min
control	133.2±1.2	210±16	1.58±0.12	103.7±0.2	222±37	2.14±.17	4.54±.03	109±10	24.0±2.2
0-30	132.5±0.4	190±17	1.44±0.14	102±0.2	215±48	2.10±.16	4.40±.01	119±21	27.0±2.4
30-60	133.0±0.7	177±15	1.33±0.12	101.5±0.4	191±44	1.88±.12	4.40±.13	95±21	21.7±2.1
60-90	133.5±0.4	143±18	1.06±0.15	100-5±0.4	180±36	1.79±.11	4.30±21	126±21	29.4±2.6
90-120	132-5±0.4	114±12	0.86±0.11	100.5±0.4	148±31	1.67±.10	4.10±.16	114±18	27.9±2.5
120-150	132.5±1.2	120±11	0.91±0.10	98.0±0.2	156±26	1.80±.11	4.15±.04	115±14	27.7±2.7
150-180	134.0±0.7	129±11	0.91±0.10	99.5±0.4	144±20	1.64±.10	3.95±.14	103±15	26.0±2.6

Values are mean ± S.E.M.. The control value represents the average of 3 baseline values obtained during steady water diuresis. Na HCO_3 infusion was started at time 0. P_{Na} = plasma sodium concentration; $U_{Na}V$= sodium excretion; C_{Na} = sodium clearance; P_{Cl} = plasma chloride concentration; $U_{Cl}V$ = chloride excretion; C_{Cl} = chloride clearance; P_K = plasma potassium concentration; U_KV = potassium excretion; C_K = potassium clearance.

' p <0.05; " 0 <0.02; * p <0.01.

TABLE 11. Effect of sodium bicarbonate infusion on venous pH, calculated and measured plasma bicarbonate concentration and venous carbon dioxide partial pressure

Time	V pH	Calculated plasma bicarbonate concentration	Measured plasma bicarbonate concentration	$VPCO_2$
Minutes		meq/L	meq/l	
control	7.30±0.004	19.4±1.1	19.5±0.3	37±2
0-30	7.34±0.009	21.5±1.6	21.7±0.4	42±3
30-60	7.33±0.004	21.5±1.2	23.2±0.3	42±2
60-90	7.36±.012"	23.6±2.6	24.1±0.3"	37±0.4
90-120	7.34±0.116	22.4±1.6	24.8±0.5"	43±0.4
120-150	7.37±0.004*	23.4±0.5	25.6±0.2*	42±0.4
150-180	7.38±0.16**	24.0±0.6	26.0±0.2**	42±0.9

Values are mean ± S.E.M.; the control value represents the average of 3 successive baseline measurements obtained during steady water diuresis.

"p <0.02; *p<0.01: ** p<0.002.

TABLE 12. Effect of $NaHCO_3$ infusion on plasma glucose, insulin, glucagon, free fatty acid and ketone body concentration.

Time	Plasma glucose concentration	Plasma insulin concentration	Plasma glucagon concentration	Plasma FFA Concentration	Plasma β-OHB Concentration	Plasma AcAc Concentration	Plasma AcAc+β-OHB Concentration
Minutes	mg%	U/ml	pg/ml	nM	μM	μM	μM
Control	80±4	22±2	50±3	300±45	63±20	33±10	98±30
0-30	78±3	20±1					
30-60	80±5	19±1	46±3	310±36	69±34	31±9	100±30
60-90	84±6	18±1					
90-120	83±6	19±2	51±4	290±43	77±30	42±9	119±35
120-150	82±7	18±2					
150-180	83±5	18±1	50±6	305±35	62±13	35±20	98±30

Values are mean ± SEM. The control value represents the average of 3 successive baseline measurements obtained during steady water diuresis. $NaHCO_3$ infusion was started at time 0.

TABLE 13. Effect of $NaHCO_3$ infusion on plasma amino acid concentration (μM)

Amino acid	Control	0-30 min	30-90 min	90-150 min
Taurine	50±3	55±4	54±6	49±3
Threonine	117±15	114±16	111±16	120±14
Serine	87±6	90±12	91±11	87±11
Proline	178±23	181±23	188±26	179±19
Citrulline	33±5	30±3	29±2	28±4
Glycine	265±23	259±26	248±34	259±33
Alanine	380±34	379±41	376±33	360±29
Butyric acid	28±2	31±1	32±4	35±3
Valine	229±32	238±33	229±27	240±24
1/2 Cysteine	71±4	75±9	81±2	81±4
Isoleucine	51±7	55±4	53±4	51±1
Tyrosine	34±7	33±3	39±5	40±4
Phenylalanine	47±4	51±6	56±7	52±8
Leucine	122±14	127±10	131±17	126±17
Methionine	19±5	21±5	29±8	25±3

Values are mean ± SEM. The control value represents the average of 3 successive baseline measurements obtained during steady water diuresis. the $NaHCO_3$ infusion was started at time 0.

TABLE 14. Effect of Na. Lactate infusion on plasma lactate and ketone concentration.

Time	Plasma lactate concentration	Plasma β-OHB concentration	Plasma AcAc concentration	Total plasma ketone concentration
Minutes	mM	μM	μM	μM
Control	6.9±0.5	58±8	19±3	77±6
0-30	13.7±1.0**			
30-60	12.5±0.4**	57±21	16±2	72±4
60-90	10.3±1.3'			
90-120	10.0±0.5*	66±7	17±3	83±10
120-150	10.0±0.5*			
150-180	9.3±0.7'	78±15	22±6	94±18

Values are mean ± S.E.M.. The control value represents the average of 3 successive baseline measurements obtained during steady water diuresis. β-OHB=B-hydroxybutyric acid; AcAc=acetoacetic acid.

' $p<0.05$; * $p<0.01$; ** $p<0.002$.

TABLE 15. Effect of Na lactate infusion on venous pH, calculated and measured bicarbonate concentration, and venous carbon dioxide partial pressure.

Time Minutes	V pH	Calculated (HCO_3) meq/L	Measured (HCO_3) meq/L	V pCO_2 mm/Hg
Control	7.30±.013	20.7±1.2	18.3±1.5	42±0.9
0-30	7.33±.012	22.4±1.4	21.1±1.2	43±1.5
30-60	7.35±.017'	24.0±1.9	22.8±1.2	44±1.9
60-90	7.34±.014'	23.6±1.4	23.5±1.2'	44±1.0
90-120	7.37±.008*	24.1±1.2	24.2±1.2	43±1.0
120-150	7.38±.011*	24 ±1.0	25.5±0.8*	43±1.8
150-180	7.38±.010*	25.0±1.0'	25.8±1.0*	43±1.8

Values are mean ± SEM. The control value represents the average of 3 successive baseline measurements obtained during steady water diuresis. (HCO_3) = plasma bicarbonate concentration; ' $p<0.05$; " $p<0.02$; * $p<0.01$.

TABLE 16. Effect of Na lactate infusion on urine flow, creatinine clearance, urine pH, ammonia and titratable acid excretion.

Time	Urine flow	C_{Cr}	U_{pH}	$U_{NH_3}V$	$U_{TA}V$
Minutes	ml/min	ml/min		μeq/min	μeq/min
Control	12.3±0.7	132±5	5.12±.018	92.9±7.8	38.2±1.2
0-30	11.3±0.7	134±5	5.02±.025	82.5±8.8	41.6±1.8
30-60	10.5±0.4	131±7	5.23±.060	67.5±4.7"	42.1±2.2
60-90	10.2±0.5	132±4	5.37±.090	63.7±6.3"	38.4±1.9
90-120	9.3±1.1	138±5	5.49±.120	60.5±7.0"	37.4±2.0
120-150	10.1±0.7	119±9	5.81±.150"	52.7±6.4*	33.2±4.5
150-180	11.8±1.5	149±5	6.03±.160*	56.3±6.2*	35.8±2.1

Values are mean ± SEM. The control value represents the average of 3 successive baseline values obtained during steady water diuresis. C_{Cr}= creatinine clearance; U_{pH}= urine pH; $U_{NH_3}V$ = ammonia excretion; $U_{TA}V$ titratable acid excretion.

' $p < 0.05$; " $p < 0.02$; * $p < 0.01$.

TABLE 17. Effect of sodium lactate infusion on plasma concentration, urinary excretion and clearance of sodium, chloride and potassium

Time	P_{Na}	$U_{Na}V$	C_{Na}	P_{Cl}	$U_{Cl}V$	C_{Cl}	P_K	U_KV	C_K
Minute	meq/L	µeq/min	ml/min.	Meq/L	µeq/min	ml/min.	meq/L	µeq/min	ml/min.
control	133.5±0.3	268±55	2.01±.20	102.4±1.3	383±75	3.74±.30	4.23±0.2	106±24	25.2±3.1
0-30	134.3±0.5	192±35	1.43±.12	99.8±1.3	273±60	2.74±.20"	3.95±0.1	92±22	23.3±2.0
30-60	135.0±0.4	175±39"	1.30±.12"	99.8±0.9	232±64	2.33±19*	3.78±0.1	73±8	19.4±2.4
60-90	134.3±0.3	134±21"	1.00±10*	98.3±0.9	181±34"	1.84±14***	3.65±0.1	68±8	18.7±2.5
90-120	135.0±0.7	112±21"	0.83±.08***	97.3±1.1	152±29*	1.56±.12***	3.48±0.1"	52±5	15.0±2.4
120-150	134.5±0.3	116±24"	0.86±.10**	98.0±1.5	133±31*	1.36±.10***	3.55±.15	48 ±5	13.7±2.4
150-180	135.0±0.8	110±24"	0.86±.10**	97.8±1.7	130±21*	1.36±.10***	3.50±15'	60±11	17.0±2.5

Values are mean ± SEM. The control period represents the average of 3 baseline values obtained during steady water diuresis. Na lactate infusion was started at time 0. P_{Na}= plasma sodium concentration; $U_{Na}V$ = sodium excretion; C_{Na}= sodium clearance; P_{Cl}=plasma chloride concentration; $U_{Cl}V$=chloride excretion, C_{Cl}= chloride clearance; P_K= pleama potassium concentration; U_KV=potassium excretion; C_K = potassium clearance

' $p < 0.05$; " $p < 0.02$; * $p<0.01$; ** $p<0.002$; *** $p<0.001$.

TABLE 18. Effect of Na lactate infusion on plasma glucose, insulin glucagon, and free fatty acid concentration.

Time	Plasma glucose concentration	Plasma insulin concentration	Plasma glucagon concentration	Plasma free fatty acid concentration
Minutes	mg%	U/ml	pg/ml	nM
Control	86±1.6	19±1.2	48±4	295±50
0-30	87±2.1	18±1.1		
30-60	86±1.8	19±1.1	46±5	300±45
60-90	88±1.4	17±1.3		
90-120	86±1.9	18±1.5	50±6	310±53
120-150	85±1.3	16±1.0		
150-180	85±0.9	19±1.2	49±7	290±63

Values are mean ± SEM. The control value represents the average 3 baseline measurements obtained under steady water diuresis. Na lactate infusion was started at time 0.

TABLE 19. Effect of Na lactate infusion on plasma amino acid concentration (μM)

Amino acid	Control	0-30 min.	30-90 mon.	90-150 min.
Taurine	54±4	50±3	57±6	55±2
Threonine	109±12	110±13	120±10	121±12
Serine	91±4	89±3	95±4	89±7
Proline	180±10	192±14	182±12	188±10
Citrulline	32±2	29±5	33±1	33±2
Glycine	240±15	236±17	220±30	229±24
Alanine	355±30	340±23	320±22	300±19
Butyric acid	30±3	30±2	39±5	37±5
Valine	220±24	218±24	209±26	222±23
1/2 Cysteine	65±13	68±12	65±10	69±10
Isoleucine	55±4	51±5	61±7	62±8
Tyrosine	40±5	39±5	35±2	41±2
Phenylalanine	43±3	41±7	49±6	44±3
Leucine	110±23	118±24	110±22	107±2
Methionine	22±1	25±3	22±3	26±5

Values are mean ± SEM. The control value represents the average of 3 baseline measurements obtained during steady water diuresis. Lactate infusion was started at time 0.

TABLE 20. Factors known to influence renal ammonia production and excretion

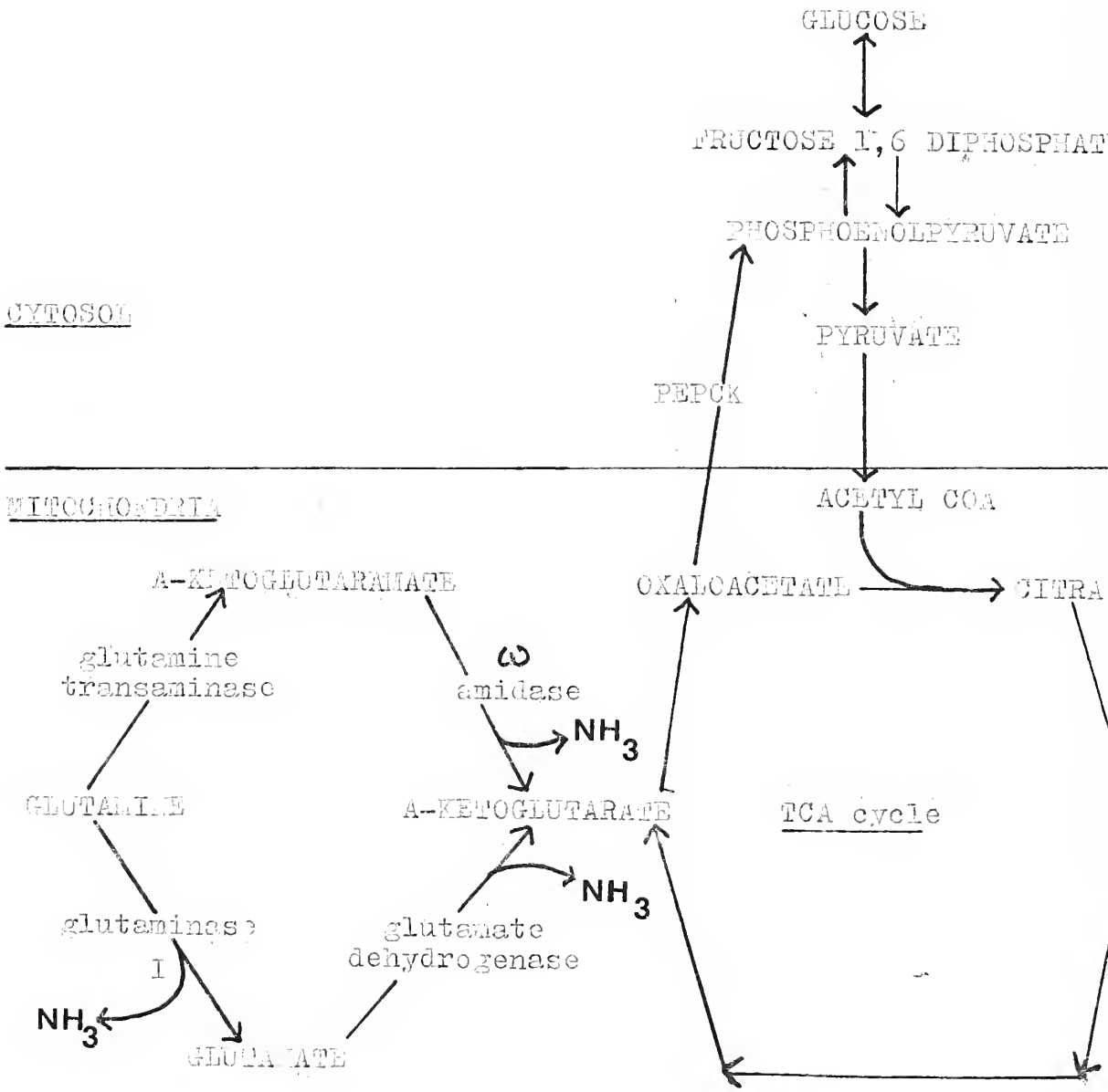
A. Ammonia excretion

1. Urine pH
2. Urine flow
3. Arterial blood ammonia concentration
4. Rate of ammonia production

B. Ammonia production

1. Substrate delivery: glutamine and other amino acids.
2. Enzyme activity: glutaminase I, glutamic dehydrogenase.
3. Cellular concentration of
   a) ammonia
   b) glutamate
   c) α-ketoglutarate
   d) NAD?$NADH^+$
   e) potassium
4. Renal gluconeogenesis

FIGURE 1. Relationship between gluconeogenesis and ammoniagenesis in the kidney. After Goodman et al. (119)



PEPCK= phosphoenolpyruvate carboxykinase

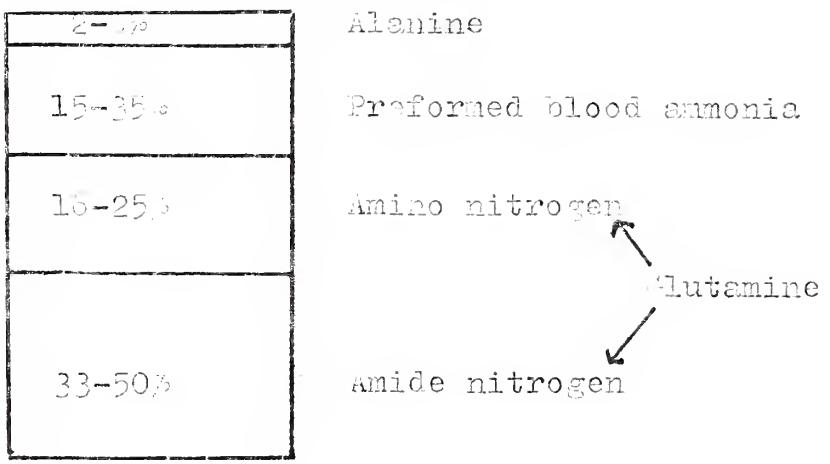


Figure 2. Passive secretion of free base ammonia by non ionic diffusion. The major fraction is trapped in acid urine as relatively non diffusible ammonium ion. (Pitts, 27).

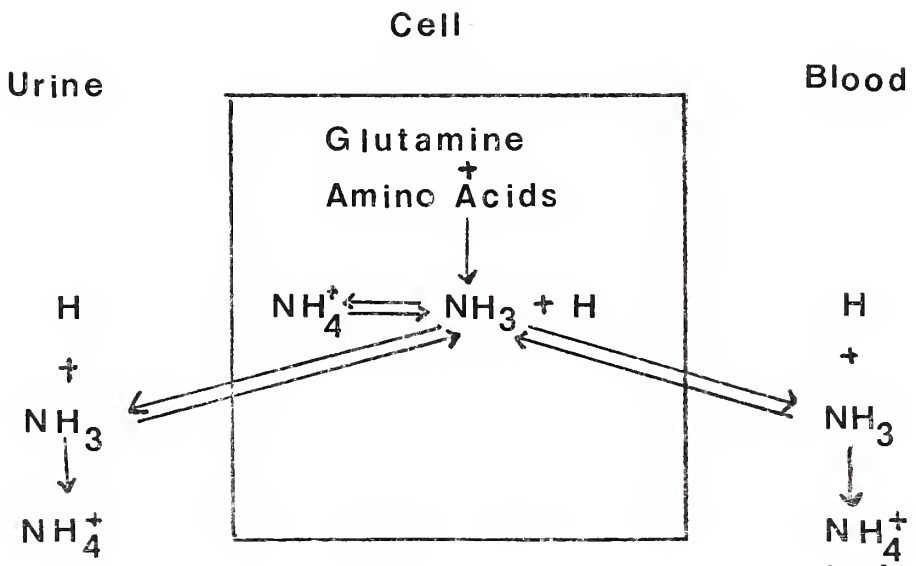


Figure 3 Origin of renal ammonia in dogs in chronic metabolic acidosis.

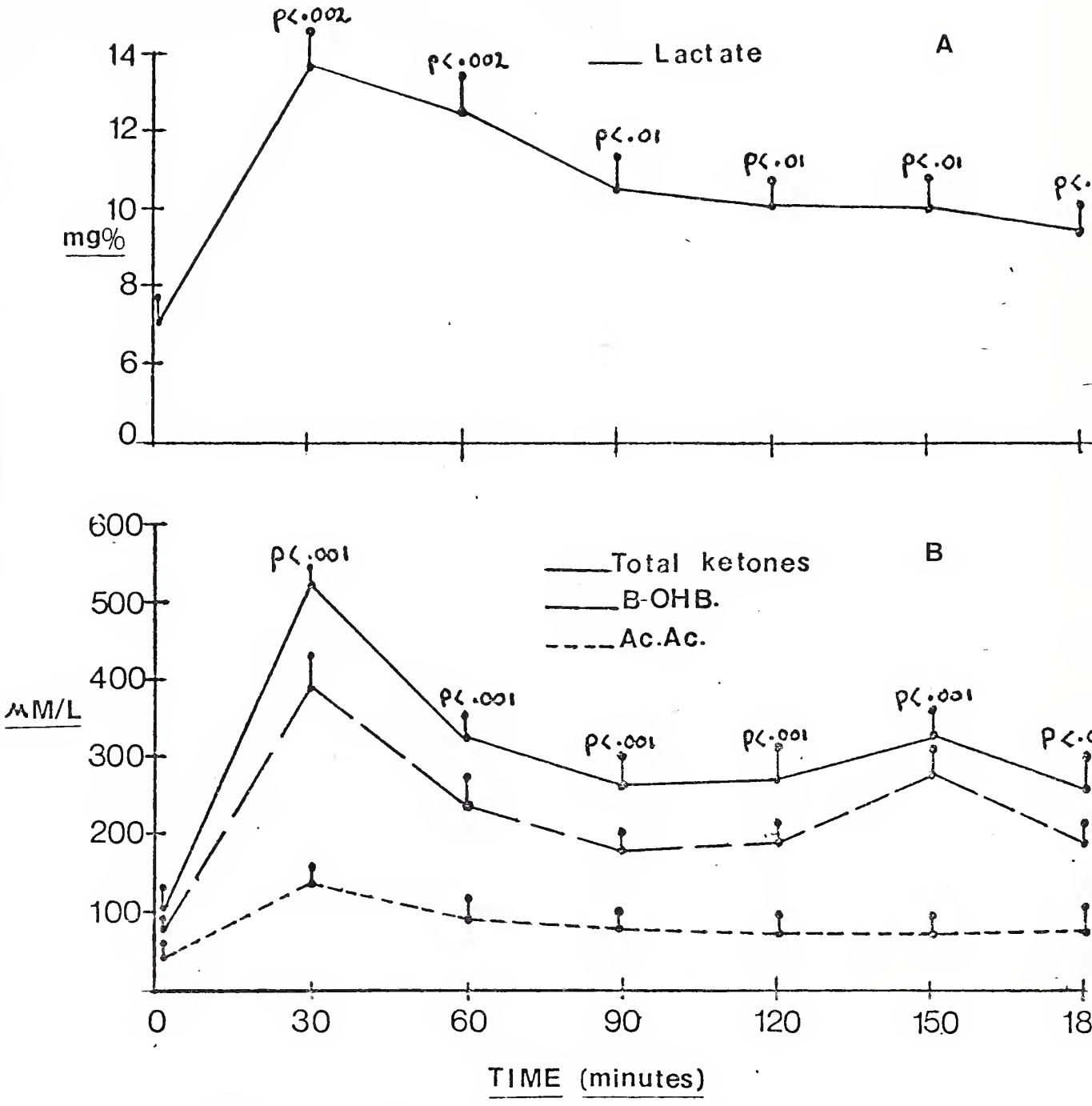


Figure 4A. Effect of lactate infusion on plasma lactate concentration.

Values are mean+/- S.E.M. for 4 acedemic subjects. 3 successive measurements were obtained during steady water diuresis before lactate infusion. These were averaged and represented plasma lactate concentration at time 0 (control value). Lactate infusion was started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.

Figure 4B. Effect of Na B-OHB infusion on plasma ketone concentration.

Values are mean+/- S.E.M. for 9 subjects. Na.B-OHB was started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.

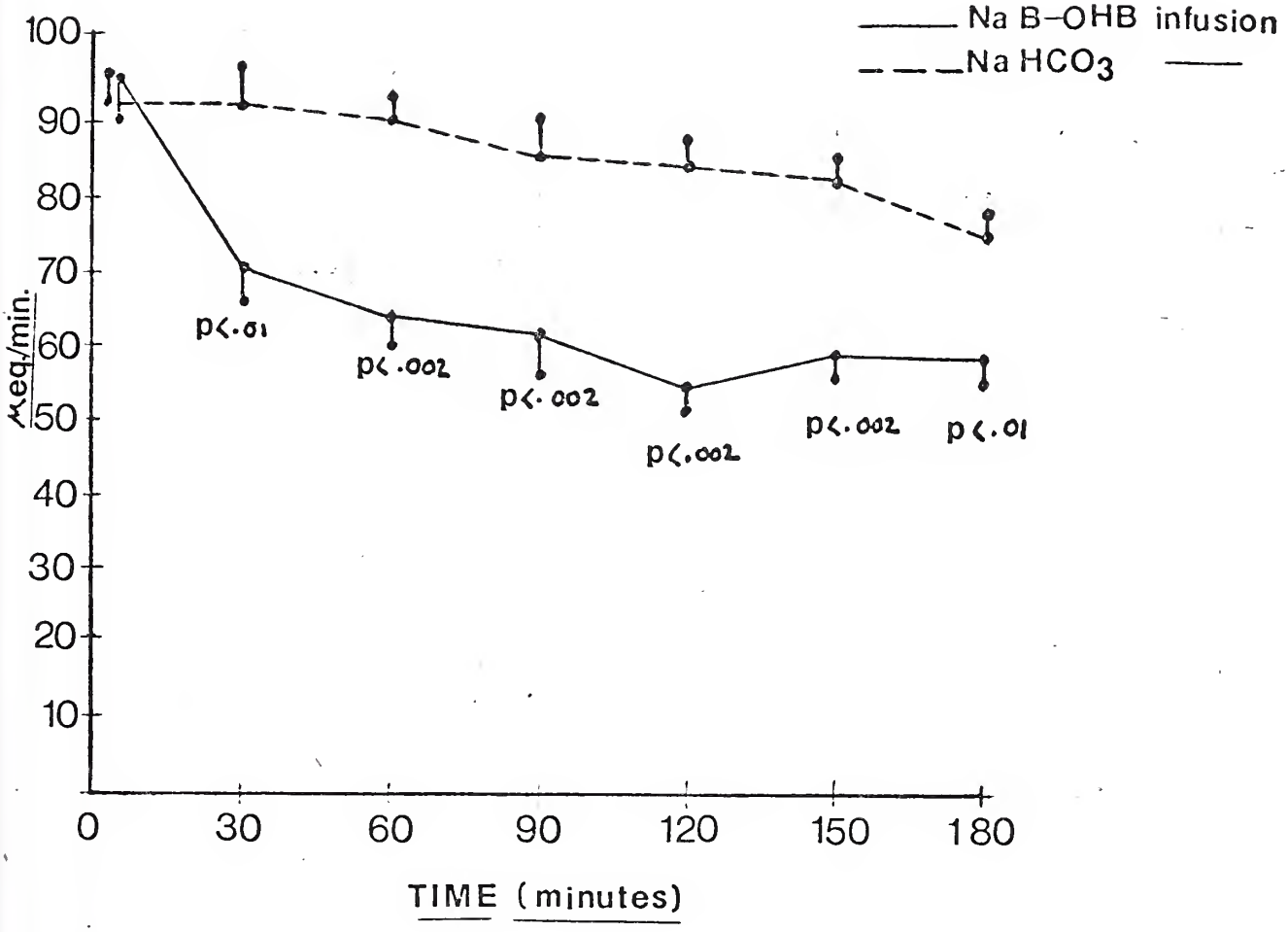


Figure 5. Effect of Na.B-OHB infusion on ammonia excretion.

Values are mean+/- S.E.M. for 9 acidemic subjects. 3 successive measurements were obtained du ing steady water diuresis before Na.B-OHB infusion. These were averaged and represented ammonia excretion at time 0 (control value). Na.B-OHB wa started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.
Ammonia excretion following NaHCO3 infusion in 3 subjects is also shown.
The decline in ammonia excretion was significantly greater with Na.B-OHB than with NaHCO3.

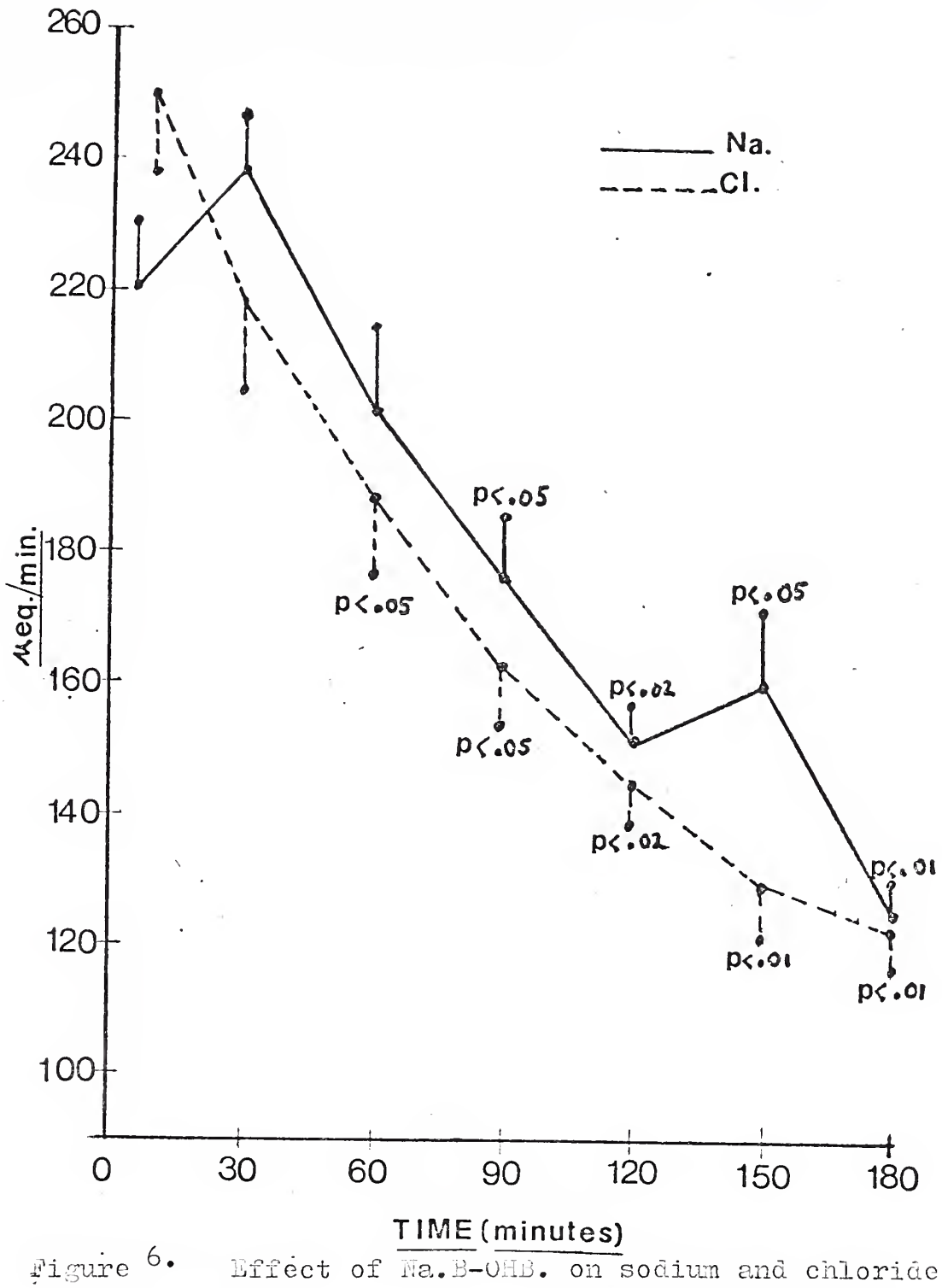


Figure 6. Effect of Na.B-OHB. on sodium and chloride excretio

Values are mean+/- S.E.M. for 9 acidemic subjects. 3 successive measurements were obtained during steady water diuresis before Na.B-OHB infusion. These were averaged and represented excretion values at time 0 (control value). Na.B-OHB was started at time 0. Each value obtined afterwar was compared to the control value using the paired t-test. Sodium and chloride excretion fell significantly during Na.B-OHB infusion.

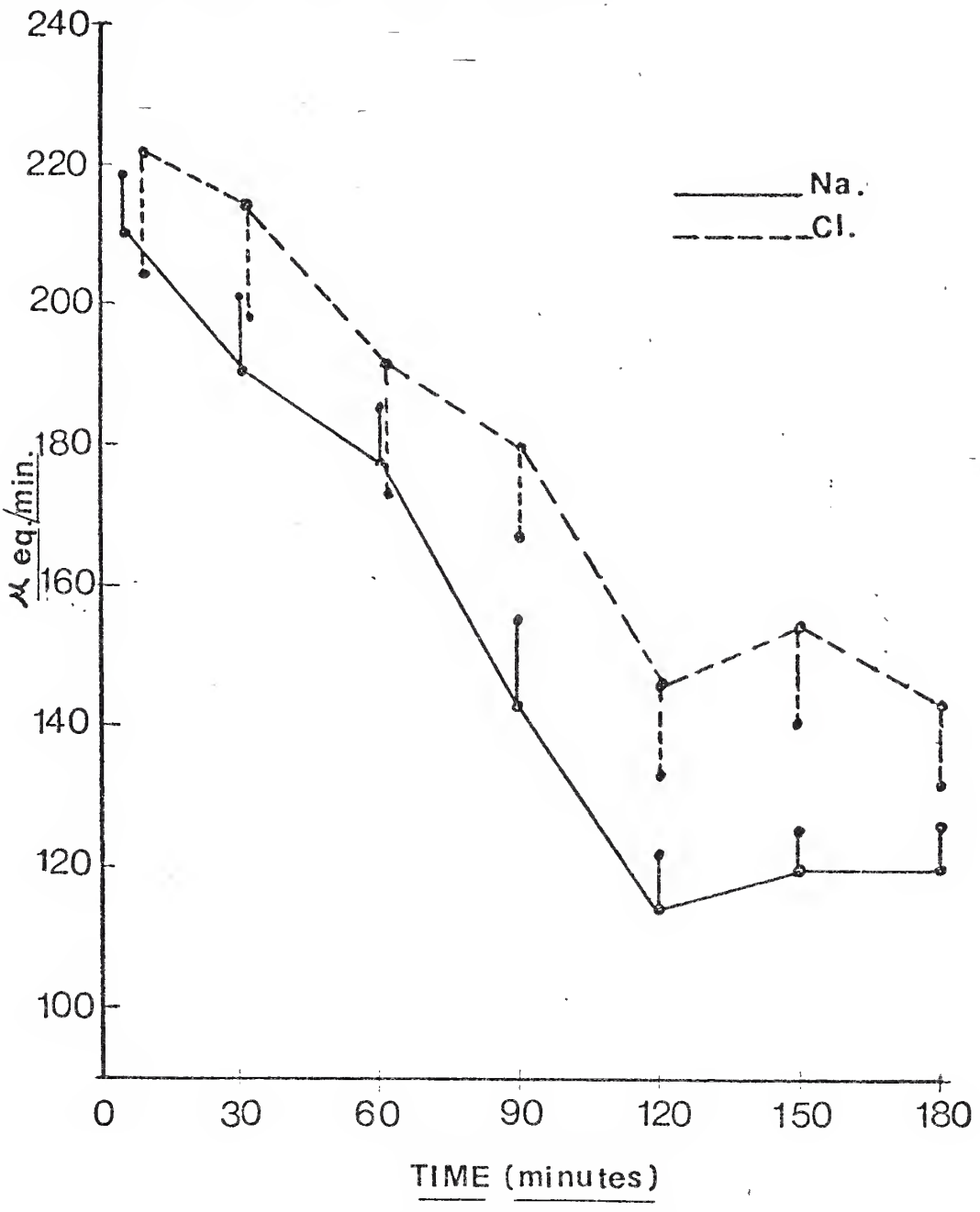


Figure 7. Effect of NaHCO3 infusion on sodium and chloride excretion.

Values are mean+/- S.E.M. for 3 acidemic subjects. 3 successive measurements were obtained during steady diuresis before NaHCO3 infusion. These were averaged and represented excretion value at time 0 (control value). NaHCO3 infusion was started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.
Sodium and chloride excretion fell during NaHCO3 infusion.

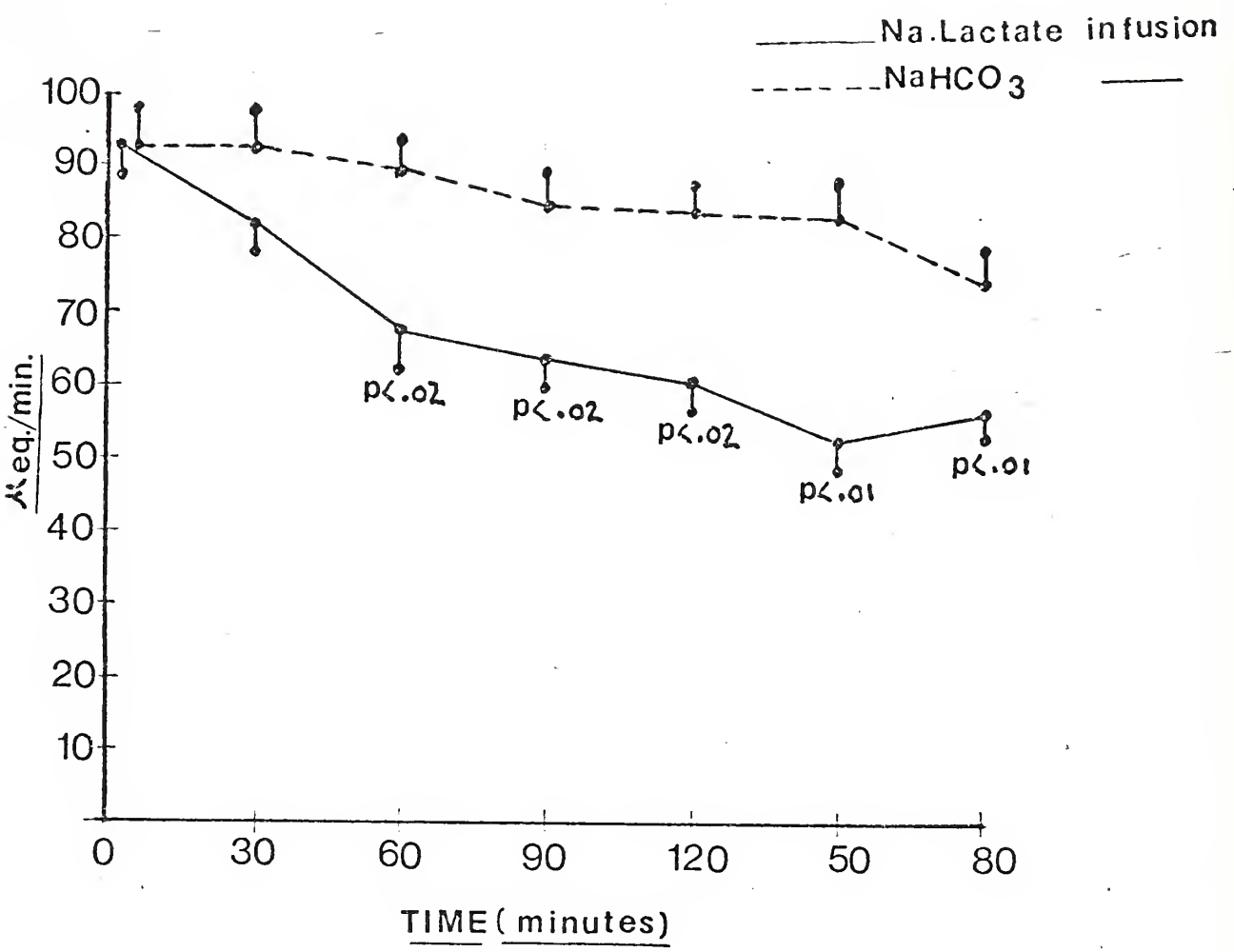


Figure 8. Effect of Na.Lact. on urinary ammonia excretion.

Values are mean+/- S.E.M. for 4 acidemic sujects. 3 successive measurements were obtained during steady water diuresis before lactate infusion. These were averaged and represented ammonia excretion value at time 0 (control value). Lactate infusion was started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.
Urinary ammonia excretion following NaHCO3 infusion in 3 subjects is also shown.
The decline in ammonia excretion was significantly greater with Na.Lact. than with Na.HCO3.

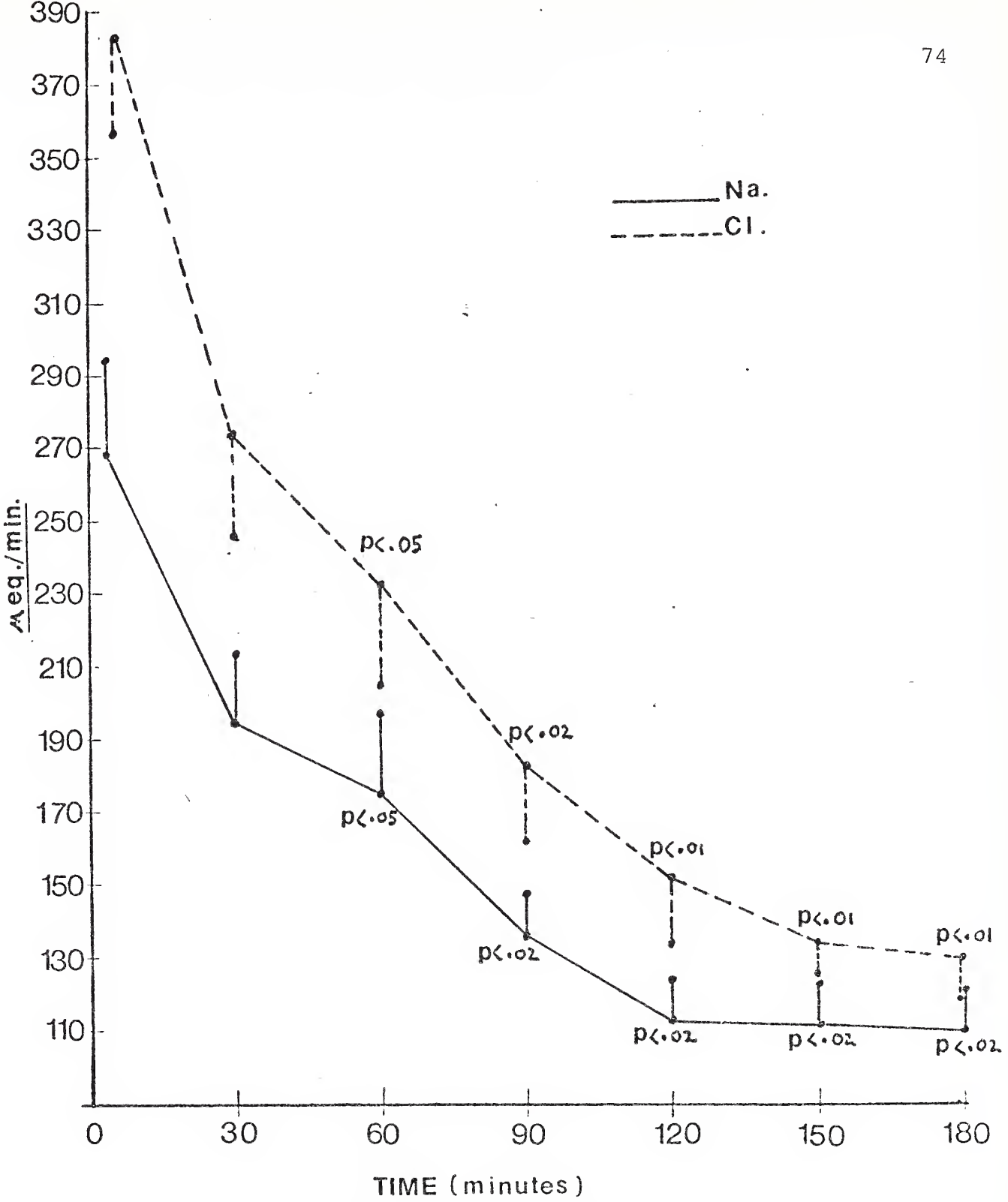
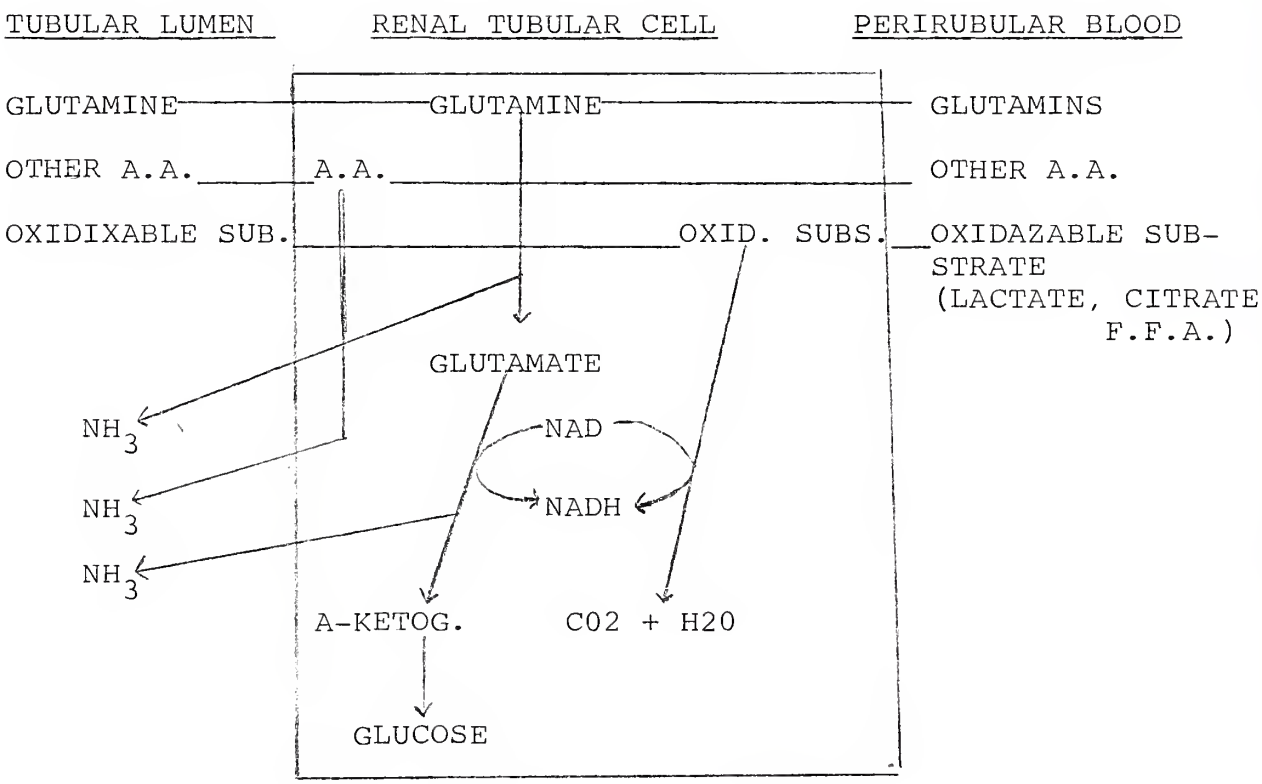


Figure 9. Effect of Na.Lactate on sodium and chloride excretion.

Values are mean+/-S.E.M. for 4 acedemic subjects. 3 successive measurements were obtained during steady water diuresis before Na.Lactate infusion. These were averaged and represented excretion value at time 0 (control value). Na.Lactate infusion was started at time 0. Each value obtained afterwards was compared to the control value using the paired t-test.
Sodium and chloride excretion fell during Na.Lactate infusion



1= glutaminase I; 2= glutamate dehydrogenase
3= phosphoenolpyruvate carboxykinase.

Figure 10. Factors affecting renal ammonia production and excretion.

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