THE MECHANISM OF AMMONIA EXCRETION DURING AMMONIUM CHLORIDE ACIDOSIS [1]

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The response of urinary ammonia to strong acid loads has been repeatedly observed to follow a characteristic pattern (1–3). Initially, ammonia excretion increases to a slight extent, then slowly attains maximum intensity after three to four days, and is maintained at a rate which parallels the magnitude of the acid load. This enhanced rate of ammonia excretion has usually been ascribed to the accelerated transport of ammonia into the tubular urine as a result of a sharp fall in urine pH (4–8). Pitts, however, found that chronic acidosis resulted in the excretion of more ammonia at any given urine pH than did acute acidosis (5) and suggested that prolonged acidosis might stimulate certain adaptive changes in the ammonia-producing system. The subsequent demonstration by Davies and Yudkin (9) of increased activity of renal ammonia-producing enzymes in rats given acid loads strongly supports the thesis that the production of ammonia may also constitute a regulatory mechanism of ammonia excretion. Apparently, therefore, two processes control the excretion of ammonia: 1) The *production* of ammonia in the tubular cell by the action of various ammonia-producing enzymes on precursor amino acids; and 2) the *transport* of the ammonia thus produced from tubular cell into tubular urine.

The purpose of the present investigation was three-fold: 1) To determine the relationship between ammonia excretion and renal glutaminase activity during two circumstances: the early period of adaptation following the administration of an acid load, and the period of maximum ammonia excretion following the administration of different amounts of strong acid; 2) to define, if possible, the factors responsible for the adaptation of renal glutaminase; and 3) to determine the effect of urine pH upon the relationship between ammonia excretion and renal glutaminase activity.

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PROCEDURE

Male Sprague-Dawley rats, weighing 350 to 400 gm., were tube fed 10 ml. of standard electrolyte deficient diet twice daily. Each 10 ml. of diet contained 1.0 gm. of protein, 0.75 gm. of fat, 1.0 gm. of glucose, essential salts and soluble vitamins, but no sodium, potassium, or chloride. Preliminary unpublished observations in this laboratory have shown that variations in dietary intake result in altered excretion of acid by the kidneys. In order to obviate variations in acid excretion arising from differences in dietary intake, rats were tube fed constant amounts of diet. All rats were kept in an air-conditioned room maintained at a constant temperature to avoid any change in the excretion of acid resulting from metabolic response to temperature changes.

The first group of experiments was undertaken to determine the relationship of glutaminase adaptation to the rise in ammonia excretion during the early phase of a constant acid load. In order to avoid variations in NH_3 excretion resulting from different antecedent diets, 32 rats were tube fed the standard electrolyte deficient diet for five days. At the end of this period 4 of these rats were sacrificed and their kidneys analyzed for glutaminase to serve as controls. The remainder then received 2½ mM of NH_4Cl twice daily; the rats were sacrificed in groups of 4 at 24, 36, 48, 72, and 96 hours, 6 days, and 14 days, and their kidneys analyzed for glutaminase activity. Daily twenty-four hour urine samples were collected and analyzed for pH, titratable acidity, and ammonia throughout the experiment.

To determine the relationship between ammonia excretion and renal glutaminase activity in rats receiving different acid loads, a second group of experiments was initiated. Seven groups of 4 rats were tube fed the standard electrolyte deficient diet (SEDD) containing varying amounts of acid. One group received only the SEDD. A second group received SEDD plus 6 mM $NaHCO_3$ daily. Four groups received SEDD plus 2, 3, 5, and 6 mM NH_4Cl daily, respectively. A final group received SEDD plus 6 mM NH_4Cl and 6 mM KCl daily. After 14 days (ammonia excretion having been constant for about eight days), the rats were sacrificed and the activity of renal glutaminase was determined.

METHODS

Daily twenty-four hour urine samples from each rat were collected under mineral oil using phenyl mercuric nitrate and toluene as preservatives. The ammonia in the urine was determined by a modification of the microdiffusion method of Conway (10). The pH of the urine was measured with a Cambridge pH meter using external glass electrodes, and corrected to 37.5°C. by a factor of 0.01 pH unit per degree. The urine was titrated to corrected pH 7.4 with 0.1 N NaOH to determine the titratable acid.

At the end of each experimental period, the rats were anesthetized by an intraperitoneal injection of hexabarbital (Evipal® sodium) and arterial blood was collected anaerobically from the abdominal aorta. Muscle samples were removed from the hind legs. The kidneys were then removed and placed in chilled isotonic saline.

Maximum glutaminase activity was measured by adding 0.2 ml. of cold 5 per cent kidney homogenate (11) to a test tube containing 0.3 ml. of 0.25 M tris (hydroxymethyl) amino methane and 0.4 ml. of 0.6 M sodium phosphate (both solutions were adjusted to pH 8.0), 0.15 ml. of 0.12 M sodium cyanide, 0.25 ml. of 0.2 M glutamine and 0.2 ml. of water. The tubes were then incubated for 30 minutes at 38°C. The reaction was stopped by the addition of 0.15 ml. of 50 per cent perchloric acid. With each determination a blank was run in which the enzyme was replaced with water. The amount of ammonia produced was measured by nesslerization of an aliquot (12). Dry weights were determined by adding an aliquot of the 5 per cent homogenate to a weighed tube and drying for two or more days at 90°C. to a constant weight. The activity of glutaminase was expressed as micro moles of ammonia per 100 mgm. of dry kidney per hour.[3]

The muscle samples were weighed and dried; fat was removed with ether; electrolytes were extracted with 0.75 N nitric acid (13). The content of sodium and potassium in the serum and in the muscle extracts was determined on the flame photometer (14). Chloride was measured in the serum and extracts of muscle by the Hald modification of the open Carius method (15). The CO_2 content was determined on 0.2 ml. serum (15).

RESULTS

Following the start of the constant daily administration of 5 mM NH_4Cl (2½ mM every 12 hours) renal glutaminase activity remained unchanged for 24 hours, then rose in a stepwise manner, reaching maximum activity in approximately six days (Figure 1). The rise in ammonia excretion preceded the rise in glutaminase activity,

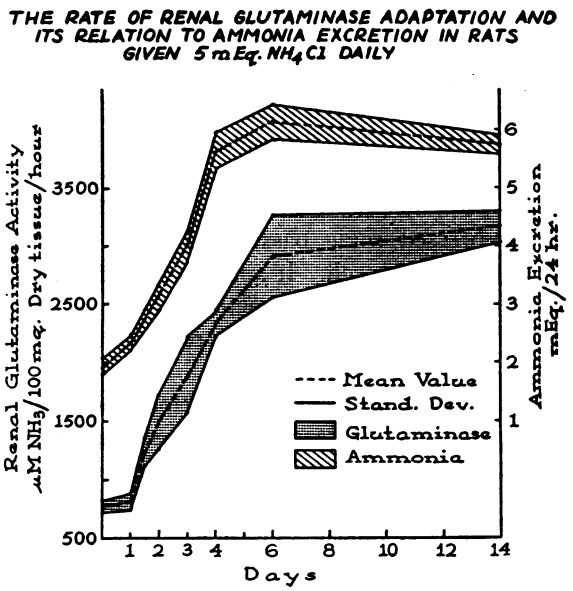

FIG. 1

showing a slight increase during the first 24 hours, but thereafter roughly paralleled the rise in renal glutaminase activity. The progressive rise in ammonia excretion during the early period of acid loading was, therefore, related to a similar rise in the activity of renal glutaminase.

In order to determine whether different loads of acid stimulated ammonia excretion by activating glutaminase, the ammonia excretion and glutaminase activity were compared in seven groups of rats, each group being maintained on a different acid load. Renal glutaminase activity was determined at the end of 14 days, when constant maximum ammonia excretion had been attained. Urine pH did not vary significantly among the rats receiving different loads of NH_4Cl. Under such circumstances, there was an excellent correlation between maximum ammonia excretion and the activity of renal glutaminase (Figure 2). During chronic acid loading the excretion of ammonia, therefore, appears to be more closely related to glutaminase activity than to urine pH.

The effect of increased urine pH upon the relationship between ammonia excretion and renal glutaminase activity is illustrated in Figure 3. The regression line was calculated from the data obtained in studies on rats receiving varying NH_4Cl loads; the urine pH did not vary significantly among these groups. Rats whose urine pH was

[3] It has been demonstrated (Copenhaver, unpublished results) that the conditions outlined here are optimal for the determination of phosphate-activated glutaminase in water homogenates of rat kidney.

elevated, either by the administration of $NaHCO_3$ or the injection and ingestion of 9 mg. of Diamox® every six hours (16), excreted far less ammonia than could be predicted from the level of glutaminase activity. The disproportionately low ammonia excretion in these rats may possibly be linked with the increased urine pH, which may have repressed some passive diffusion of NH_3 out of the tubular cell. It appears, therefore, that in addition to renal enzyme activity, urine pH may play a contributory role in the regulation of ammonia excretion.

In an attempt to identify the factors responsible for the adaptation of renal glutaminase during acid loading, the changes in the electrolyte composition of plasma and muscle were determined; mean values with standard deviations are listed in Table I. The rise of glutaminase activity in rats receiving 6 mM NH_4Cl daily was accompanied by a decrease in muscle potassium from 51.4 mEq. per 100 gm. fat-free dry tissue (FFDT) to 47.3 mEq. per 100 gm. FFDT. The addition of 6 mM of KCl to the diet (Group III) had no significant effect upon the response of glutaminase to the acid load. However, the muscle potassium of rats given KCl + NH_4Cl (Group III) was not significantly different from the muscle potassium of rats given NH_4Cl alone (Group II) owing to the large standard deviation of the former which resulted from a marked divergence from the mean

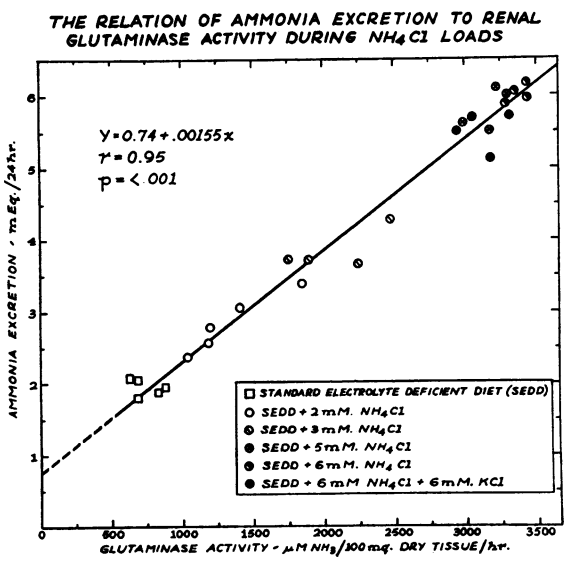

FIG. 2

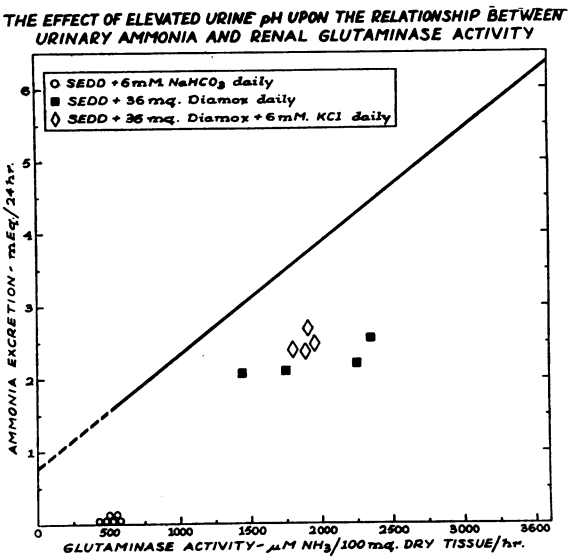

FIG. 3

in one rat. In other studies on rats given Diamox® reported elsewhere (16) correction of the potassium deficiency was accomplished by the administration of KCl without depressing glutaminase activity. It appears likely, therefore, that changes in tissue potassium, as reflected by the potassium content of muscle, are not responsible for the adaptation of renal glutaminase. Although the rise of glutaminase activity in rats receiving 6 mM NH_4Cl was accompanied by a decrease in the serum bicarbonate content from 23.9 mEq. per L. in controls to 17.8 mEq. per L., no significant change in bicarbonate content supervened in rats given 2 mM NH_4Cl daily (Group IV) despite a significant rise in glutaminase activity. Therefore, changes in serum bicarbonate content can not be causally related to the adaptation of renal glutaminase.

DISCUSSION

The excellent correlation between ammonia excretion and the activity of renal glutaminase during both the early period of adaptation in rats given constant acid loads and the period after steady ammonia excretion had been attained in rats given different acid loads strongly suggests that the rate at which ammonia is excreted is, at least partially, regulated by the activity of the ammonia-producing enzymes. The activation of the NH_3-producing enzymes by the administra-

TABLE I

THE RELATION OF SERUM AND MUSCLE ELECTROLYTES TO AMMONIA EXCRETION AND RENAL GLUTAMINASE ACTIVITY

Group	Treatment	Duration Days	SERUM				MUSCLE		MEAN NH_3	GLUTAMINASE
			Na mEq./L	K mEq./L	Cl mEq./L	CO_2 mEq./L	Na mEq./100gm. fat-free dry wt.	K mEq./100gm. fat-free dry wt.	Excretions mEq./24 hr.	μM. NH_3/100 mg. dry tissue/hr.
I (5 rats)	Standard electrolyte deficient diet (SEDD)	14	140.2 ±4.15	5.17 ±.47	105.9 ±1.9	23.9 ±1.2	4.06 ±.41	51.4 ±1.2	1.94 ±.15	788 ±39
II (4 rats)	SEDD + 6mM. NH_4Cl daily	14	140.3 ±4.1	4.99 ±.26	104.1 ±2.5	17.8 ±3.3	3.43 ±.51	47.3 ±.8	5.98 ±.26	3230 ±47
III (4 rats)	SEDD + 6mM. NH_4Cl + 6mM. KCl daily	14	143.7 ±2.2	6.60 ±.50	103.7 ±1.3	17.7 ±3.7	1.43 ±.53	50.0 ±4.9	5.53 ±.26	3131 ±138
IV (5 rats)	SEDD + 2mM. NH_4Cl daily	14		4.64 ±.40		24.0 ±1.2			2.80 ±.37	1327 ±102
V (6 rats)	SEDD + 6mM. $NaHCO_3$ + 1mM. KCl daily	14		4.84 ±.38		25.2 ±1.1			.04 ±.01	508 ±42

tion of strong acids apparently augments ammonia excretion by accelerating the rate at which ammonia is formed within the tubular cell.

Since the administration of NH_4Cl produces a shift of potassium out of the cells and subsequent loss of potassium into the urine (1) and may produce a systemic acidosis, at least three changes in the internal environment might have been causally related to the activation of renal glutaminase: 1) Intracellular potassium deficiency; 2) extracellular acidosis; and 3) changes in intracellular acid-base equilibrium. The cellular content of potassium, as reflected by the potassium content of skeletal muscle, does not appear to be responsible for glutaminase adaptation, since the administration of supplemental potassium corrected the muscle deficit in three out of four rats in the present study and in all rats in a study reported elsewhere (16) (in which Diamox® resulted in glutaminase adaptation), yet prevented neither the rise in ammonia excretion nor the activation of renal glutaminase. However, since it has been demonstrated (17) that during the development of potassium deficiency the cellular content of different tissues may vary widely, the possibility remains that the potassium content of the renal tubular cells may be diminished even when muscle potassium is normal. Such a possibility was not excluded by the present study, since kidneys were not analyzed for their potassium content. Although acidosis of the extracellular fluid resulting from large acid loads (6 mM NH_4Cl) might be implicated as the causative factor for the increased glutaminase activity, rats receiving smaller acid loads (2 mM NH_4Cl) had increased glutaminase activity in the absence of a systemic acidosis. Moreover, other experiments from this laboratory on animals receiving DOCA and high sodium, low potassium diets have demonstrated a similar rise in glutaminase activity in the presence of a severe alkalosis (18). Therefore, no constant relationship between the HCO_3^- content (and probably the pH as well) of the extracellular fluid and glutaminase activity can be established. Changes in intracellular acid-base equilibrium are more difficult to analyze, since there is no satisfactory method for measuring the H^+ content of the tubular cells. A fall in intracellular pH, resulting from an acid load, might be a factor in the activation of renal glutaminase. However, *in vitro* experiments have shown the pH for optimum glutaminase activity to be approximately 8.0 (19). Nevertheless, Robinson (20) has recently demonstrated that the maximum rate of ammonia forma-

tion by kidney slices is attained at pH 5.5. This suggests either that the optimum pH *in vivo* differs radically from the optimum pH *in vitro,* or else that intracellular acidosis stimulates the formation of increased amounts of ammonia producing enzymes. While such *in vitro* data indicate that intracellular pH could modify enzymatic activity, the absence of any information of either the actual pH of the tubular cell or of the impact of pH on the integrated *in vivo* operation of the ammonia producing enzymes renders any inference from *in vitro* experiments to *in vivo* operation highly tenuous at best. The factors, therefore, which are responsible for the activation of renal glutaminase during ammonium chloride acidosis could not be identified. The present experiments indicate only that glutaminase adaptation is proportional to the magnitude of the acid load, but independent of the concentration of serum bicarbonate and the potassium content of cells (as reflected by muscle potassium).

Although the excellent correlation between ammonia excretion and glutaminase activity during NH_4Cl loading strongly suggests that the rate of ammonia excretion is, in part, regulated by the rate of *production* of ammonia within the tubular cell, the manner in which the ammonia thus produced is *transported* into the tubular urine is not precisely clear. Two theories have been formulated: 1) Free molecular ammonia (NH_3) may enter the tubular urine by a process of passive diffusion (4–8); 2) ammonium ions (NH_4^+) within the tubular cell may be transported into the urine by an active process of ion exchange for tubular Na^+ (16, 21). These theories are illustrated in the two model cells of Figure 4.

According to the theory of passive diffusion, the rate of ammonia transport is regulated by the H^+ concentration of the tubular urine, the H^+ promoting the conversion of NH_3 to NH_4^+, thereby establishing a concentration gradient favoring continued passive diffusion of NH_3 out of the tubular

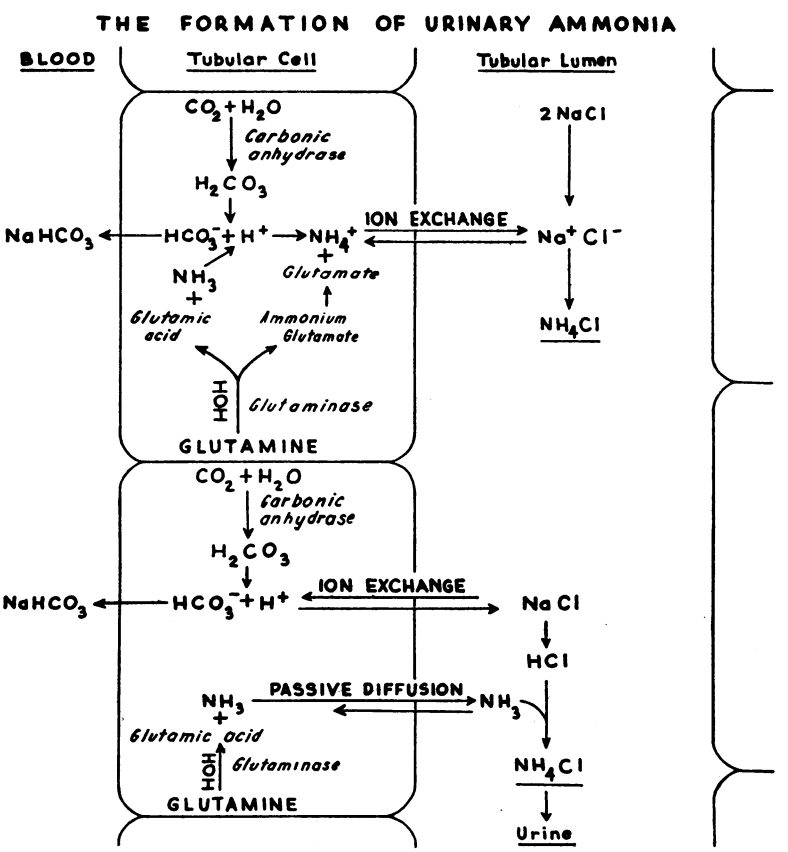

FIG. 4

cell. The data on which this theory is based have been assembled principally during acute experiments of less than 24 hours' duration (4–8). During such short intervals, major changes in the activity of the ammonia producing enzymes—judging from the data in Figure 1—do not occur, and ammonia excretion seems closely correlated with urine pH. However, during chronic acidosis, dogs excrete more ammonia at any given urine pH following an infusion of acid than do previously normal dogs subjected to the same acute acid load (5). Moreover, in the present study, with rats given varying amounts of NH_4Cl for a prolonged period of time, variations in ammonia excretion could not be attributed to any changes in urine pH. Since the rate of ammonia excretion in chronically acidotic rats varies in direct proportion to renal glutaminase activity, it is possible that accelerated formation of NH_3 within the cell may be sufficient to maintain a concentration gradient favorable for increased passive diffusion into the tubular lumen at levels of urine pH which, in acute acidosis (when the ammonia producing enzyme systems are not activated), are associated with much less ammonia excretion. The effect of urine pH on ammonia excretion can be evaluated properly, therefore, only when the rate of ammonia production can be estimated. In order to explore this relationship, ammonia excretion and glutaminase activity were determined in rats whose urine pH was elevated either by the administration of $NaHCO_3$ or Diamox® (Figure 3). These rats excreted less ammonia per unit enzyme than did rats with acid urines. The apparent discrepancy between ammonia excretion and glutaminase activity in these rats may be linked with the elevated urine pH, which could have repressed some passive diffusion of NH_3 out of the tubular cell. If passive diffusion is accepted as the primary means by which ammonia is transported into the urine it is necessary, therefore, to postulate that both the rate of ammonia formation within the cell and the pH of the tubular urine influence the concentration gradient of NH_3 across the cell membrane. This would mean, as Pitts suggested earlier (5), that the rate of ammonia excretion is pH dependent *at any given level of enzymatic activity.*

Since approximately 99 per cent of the ammonia present in the cell at physiologic pH is in the form of ammonium ion,[4] an alternative hypothesis explains the transport of NH_3 into the tubular urine on the basis of an active process of ion exchange, Na^+ of the tubular urine exchanging for NH_4^+ of the tubular cell. Although the accelerated excretion of ammonia under conditions unfavorable to passive diffusion (*i.e.*, increased urine pH) in animals receiving Diamox® (16) is highly suggestive that a mechanism of active ion exchange may be operative, sufficient data comparing urine pH and ammonia excretion with the level of enzymatic activity are not available to validate either the theory of passive diffusion or the theory of active ion exchange.

SUMMARY

1. The administration to rats of a constant NH_4Cl load resulted in a progressive, stepwise increase to a plateau in the activity of renal glutaminase, which was closely paralleled by a similar increase in ammonia excretion.

2. With the administration of different NH_4Cl loads, maximum ammonia excretion rose in direct proportion to increased glutaminase activity.

3. The adaptation of renal glutaminase was proportional to the administered acid load, but independent of the concentration of serum bicarbonate and the content of intracellular potassium.

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[4] The pK for the reaction $NH_4^+ \rightleftharpoons NH_3 + H^+$ is 9.4 (13). Therefore, in simple solution at pH 7.4 the fraction dissociated (2) can be estimated from the equation $7.4 = 9.4 + \log 2/1–2$. Since α is 0.01, 99 per cent of the total ammonia within the cell exists as NH_4^+.

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