MINIREVIEW

Current Evidence for an Ammoniuretic Factor in the Control of Ammonium Excretion (43268)

JOHN C. VANATTA* AND LOY W. FRAZIER[†,1]

Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235 and Department of Physiology,† Baylor College of Dentistry, Dallas, Texas 75246*

There are many reports in the literature that indicate that the adrenal cortex is essential to the normal renal response to a metabolic acidosis. A frequent way of introducing an acute or chronic metabolic acidosis is to administer ammonium chloride. The renal response to such an acidosis is an increase in H^+ excretion and an increase in NH_4^+ production and excretion.

Goth (1), in summarizing the therapeutic effects of NH_4Cl, notes that its natriuretic effect is associated with a "delay of several hours to days" before the kidney develops fully its capacity to form ammonia. The emphasis on this delayed action has served to cause researchers to pay little attention to the lesser, but prompt, response of the body to increases in both NH_4^+ and H^+ excretion within 2 hr or less.

There is good evidence, cited below, that indicates that this early response, as well as the later response, is influenced by the adrenal cortex in some manner. We have reviewed the literature on this subject with these questions in mind.

1. After administration of an acidifying substance such as NH_4Cl, are one or more adrenal cortical hormones released that have a specific effect on the kidney to result in either increased NH_4^+ excretion (ammoniuretic effect) or increased H^+ excretion (protonuretic effect), or both?
2. Is the delay in maximal NH_4^+ and H^+ excretion due to:
   a. different substance(s) excreted by the adrenal cortex that are involved in affecting the kidney? or
   b. a delay in the kidney responding to the ammoniuretic and protonuretic factors that initiate the acute effect?

[1] To whom correspondence and requests for reprints are addressed at Department of Physiology, Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246.

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Adrenal Gland and Control of NH_4^+ Excretion

In the following section, we present some early literature describing the functional renal changes in metabolic acidosis. We then present some of the studies on possible cellular mechanisms which might produce an increase in NH_4^+ excretion, and H^+ excretion. We then present some of a large body of literature that indicates that the adrenal cortex plays a major role in producing these changes in renal function.

In 1949, Sartorius *et al.* (2) reported on studies done on human subjects who had been made acidotic by ingestion of NH_4Cl. They found that NH_4^+ excretion increased in a stepwise fashion after NH_4Cl was given. They noted an increase in NH_4^+ excretion within 15 min after the first dose of NH_4Cl, and excretion increased continuously throughout the next 5 days. After cessation of NH_4Cl ingestion, there was a 3–4 day delay before NH_4^+ excretion returned to pre-acidosis levels.

Ryberg (3) performed two experiments on a human subject, and gives details of one experiment on a dog. He reported ammonia excretion in man on a daily basis, so we cannot evaluate his results during the first 24 hr. There was an increased NH_4^+ excretion during the first 24 hr that increased further, reaching a plateau after 4 days. The dog received HCl by mouth and showed an increase in ammonium excretion within the first 4 hr after the load, at which time excretion had reached a maximum.

Two possible mechanisms of increasing NH_4^+ excretion by the kidney have been suggested. One is the diffusion of NH_3 with subsequent trapping as NH_4^+ in the lumen, and the other transport of NH_4^+ across the membrane. The specific mechanisms involved have been discussed in great detail in another recent review by Knepper *et al.* (4), and we will not discuss such mechanisms further at this time. The second mechanism involves the rate of production of NH_3 by the renal tubular cells and its control. Early work by Pitts (5) reported that in dogs, there is a change in basic cellular secretory capacity for NH_3 during acidosis. These studies showed that at the same urine pH, the dog, when in acidosis, could excrete two to three times more NH_4^+ than when in normal acid-base balance. This indicated that there was an actual change in tubular cellular excretory capacity during metabolic acidosis.

Enzyme adaptation in the kidney has been implicated as part of the adaptation that occurs in response to acidosis. Alleyne and Scullard (6) and Alleyne (7), using rat kidney homogenates prepared from normal and metabolic acidotic rats, have shown that glutaminase II activity is increased during acidosis. Bourke *et al.* (8) have shown also that after daily administration of NH_4Cl to dogs for a period of 10 days, there is an increase in the glutaminase II activity in the kidney.

Contrary to this finding, Pollak *et al.* (9) have reported on a study in which they measured 10 different enzyme systems in kidney tissue homogenates from acidotic dogs. The only enzyme activity that was found to increase during acidosis was aspartate amino transferase. Additionally, Goldstein (10) observed that the administration of actinomycin D, an inhibitor of protein synthesis, to an acidotic rat blocked the adaptive increase in glutaminase I. However, the progressive increase in NH_4^+ excretion continued throughout the acidosis.

In 1981, Welbourne and Dass (11) were able to demonstrate that in rat kidneys, during metabolic acidosis, there was an adaptive change in the renal γ-glutamyltransferase. They showed that there was an increased affinity of the γ-glutamyltransferase for its substrate on both the luminal and antiluminal sides of the kidney tubular cell.

It seems that an enzyme adaptation is not a key factor that would determine the rate of NH_4^+ excretion in the rat or dog. It also seems that the normal amounts of enzymes present in the kidney are there in quantities sufficient to produce the increased NH_3 excreted during acidosis. However, the change in enzyme affinity could account for some of this increased activity. But, even if one accepts the hypothesis that an enzyme adaptation is responsible for the increased NH_4^+ excretion during acidosis, it would not explain the missing agent that must ultimately stimulate the specific enzyme activity.

Sartorius *et al.* (12) were the first to show that renal excretion of NH_4^+, in response to a metabolic acidosis, was reduced in adrenalectomized rats. Similar studies by Harris *et al.* (13) showed that steroid therapy or electrolyte replacement returned the renal excretion of NH_4^+ in rats to normal during a metabolic acidosis. In a later study, Wilson and Seldin (14) measured NH_4^+ excretion, as well as glutaminase activity, in adrenalectomized salt-supported rats. They found an increased NH_4^+ excretion, as well as increased glutaminase activity, in both adrenalectomized rats that were maintained on salt and in normal rats. Their results indicate that adrenal cortical hormones were not necessary for an increase in renal glutaminase in acidosis. Instead, they suggested that adrenal cortical hormones produced an increased Na^+–H^+ exchange in the renal tubules thereby causing increased NH_4^+ excretion in acidosis.

In two different reports in 1974, Welbourne (15, 16) dealt with the role of the adrenal gland in NH_4^+ excretion. In one study using adrenalectomized rats, he perfused isolated kidneys with an artificial plasma containing glutamine as the only nitrogen source (15) and measured NH_4^+ excretion. He reported a 20% reduction from controls in normal rats and a 60% reduction from controls in rats treated with NH_4Cl. When he reperfused with a solution containing elevated levels of glutamine, these differences were abolished between controls and adrenalectomized rats. In the other study (16) he found that acidosis increased glutamine transport into the mitochondria. In light of these findings, he presented the hypothesis that adrenal steroids served to increase mitochondrial glutamine uptake.

In later studies, Welbourne and co-workers (17, 18) reported that triamcinolone administration increased NH_4^+ excretion in normal rats. Additionally, it was shown that this increased NH_4^+ excretion was the result of increased transport of glutamine into the mitochondria and that the adrenal hormone, aldosterone, could mimic this same effect. In similar studies, Tannen and Kumin (19) used isolated renal cortical mitochondria from both normal and metabolic acidotic rats. Their studies supported the findings of Welbourne (17, 18) in that they reported that both mitochondria glutamine entry and increased phosphate-dependent glutaminase activity were important in the adaption to acidosis. By altering pH in their experiments, they were able to exclude a change in pH as the direct stimulator of this adaptive response to acidosis.

Hulter *et al.* (20), using adrenalectomized dogs that were maintained on mineralocorticoid hormones, reported that upon withdrawing mineralocorticoids and maintaining the dogs on glucocorticoids, net acid excretion decreased. Further, they were able to show that this decrease in net acid excretion was due for the most part to a reduction in urinary NH_4^+ excretion. Part of this diminution of NH_4^+ excretion could be explained

by an ensuing K^+ retention and hyperkalemia. However, there still was a portion of the reduced NH_4^+ excretion that appeared to be dependent on the mineralocorticoid. In yet a later study by Hulter *et al.* (21), using adrenalectomized dogs under conditions of constant mineralocorticoid replacement, it was demonstrated that dexamethasone was able to stimulate net NH_4^+ excretion. They were further able to show that with increased dosage of the glucocorticoid, they could significantly modify the severity of metabolite acidosis.

The rat model has been used extensively for studying the adaptive response to metabolic acidosis. Damasco *et al.* (22), using adrenalectomized rats, have shown in 48-hr experiments that corticosterone promotes the excretion of proton and NH_4^+ excretion in these animals. Dubrovsky *et al.* (23), also using adrenalectomized rats, found that they excreted much less net acid than did their paired normal controls. Additionally, the metabolic acidosis produced in these animals after NH_4Cl loading could be blunted by steroid (aldosterone) replacement. Their conclusion was that in the rat, the adrenal gland is essential for normal renal net acid excretion. A recent study by Welbourne *et al.* (24), using adrenalectomized rats, reported results that indicate the signal for acceleration of ammoniagensis during chronic metabolic acidosis is mediated in part by the adrenal glands and specifically by the glucocorticoids. Their results suggest further that the glucocorticoid acts by coupling cellular glutamine transport to its metabolic utilization.

Kinsella *et al.* (25) and Boross *et al.* (26) have reported on two different studies using brush border membrane vesicles from rat proximal tubules. They have reported that Na^+–H^+ exchange activity, phosphate excretion, and NH_4^+ excretion in these vesicles are all increased by metabolic acidosis, and that this increased activity is dependent on intact adrenal function or glucocorticoid treatment. Wilcox *et al.* (27), using intact rats that were normal and acid-loaded, measured pH, titratable acidity, and ammonia excretion. These rats were then selectively treated with dexamethasone or aldosterone. Their findings suggest a differential effect of the two steroids. Mineralocorticoids (aldosterone) were reported to promote urinary acidification, while glucocorticoids (dexamethasone) promoted the excretion of phosphate and ammonia.

Alleyne and Roobol (28) have reported on a nondialyzable factor present in the plasma of acidotic rats that has ammoniuretic activity on renal tissue from normal rats. In a later study (29), this ammoniuretic factor was reported to be present 30 min after induction of acidosis, was dialyzable, was not a protein, and was not of adrenal origin. In addition, the factor was not present 6 hr after induction of acidosis. It is not clear from this study whether the earlier (nondialyzable) factor is the same factor reported on in this later study. The investigators suggest that this "factor" may be important in mediating the acute response to acidosis, while other factors, including the adrenal cortical hormones, may be responsible for mediating the chronic ammoniuretic response to acidosis. It is interesting to note that Frazier and Vanatta (30) and Melton *et al.* (31) have reported ammoniuretic activity from three sources: plasmas of dog and toad, and human urine. In all cases, the animals were in metabolic acidosis. The factors were demonstrated by increasing ammonia excretion on toad bladders *in vitro*. Melton *et al.* (32) have also been able to demonstrate an ammoniuretic factor from dog plasma. This factor was present in both metabolic and respiratory acidosis and was not present when the animal was in metabolic alkalosis.

In the normal human, it has been shown in several studies (33, 34, 35) that administration of mineralocorticoid hormones results in a stimulation of renal H^+ secretion. Acute injection of aldosterone within a time frame of several hours resulted in an increase in net acid excretion (35). Kassirer *et al.* (36) have reported that chronic administration of aldosterone resulted in development of a metabolic alkalosis, indicative of stimulation of net acid secretion. Sebastian *et al.* (37) studied six adrenalectomized patients that were constantly maintained on glucocorticoid therapy. Their studies indicate that mineralocorticoids are necessary in maintaining net acid excretion and plasma bicarbonate concentrations in these patients. Further, their studies were the first to show that net renal acid excretion is controlled by mineralocorticoids at concentrations similar to those prevailing in normal patients on normal acid-producing diets.

The urinary bladder of the amphibian *Bufo marinus* has been recognized as a functional analogue of the mammalian nephron. Frazier and Vanatta (38) have shown that the toad urinary bladder can excrete H^+ and NH_4^+, and that this excretion is stimulated by a metabolic acidosis. Frazier and Zachariah (39) have demonstrated further that the toad bladder *in vitro* increases its production and excretion of NH_4^+ when stimulated by aldosterone or 17β-estradiol. In the same study, it was shown that the glucocorticoid dexamethasone, pregnenolone, and cholesterol had no stimulatory effect on NH_4^+ excretion in this tissue. In a later study, Hill and Frazier (40), using adrenalectomized toads, reported that the adrenal gland was vital in maintaining NH_4^+ excretion in the normal and acidotic toad. In addition, they were able to show in the adrenalectomized toad that the ability to secrete net acid by the bladder could be restored by administration of deoxycorticosterone acetate, a mineralocorticoid, but not by dexamethasone, a glucocorticoid.

Using the toad urinary bladder as the assay system for ammoniuretic activity, Frazier and Vanatta (30) have demonstrated a plasma factor from toads in met-

abolic acidosis that stimulates NH_4^+ excretion. This factor was nondialyzable, lipid soluble, and not present in normal plasma. In the same study, a similar factor was demonstrated in dog plasma during metabolic acidosis. The factor had ammoniuretic activity when tested on the toad urinary bladder. In a later study, Melton *et al.* (31) were able to demonstrate a factor from the urine of human patients who had been put in metabolic acidosis by ingesting NH_4Cl. This factor was lipid soluble, contained a phenolic group, and was not present in these same patients when they were placed in metabolic alkalosis.

Vanatta and Frazier (41) reported ammoniuretic activity of a factor from adrenal cortical extracts. This extract had the same solubility characteristics as the factor from human urine reported by Melton *et al.* (31). They assayed this fraction on the skin of *Rana pipiens.*

Given the fact that the adrenal gland is necessary for acidosis activation of the NH_4^+ excretion by the kidney, one must ask the following questions. Is the adrenal gland stimulated by the fall in pH, or is some other factor involved? Welbourne (42) has reported on the involvement of the pituitary gland in activating the adrenal gland during acidosis. This study, using hypophysectomized and adrenalectomized rats, indicates that acidosis does not stimulate the adrenal gland directly, but instead it requires the intact pituitary gland for complete response to the adaptation to metabolic acidosis.

It seems that the evidence is overwhelming in support of the hypothesis that the adrenal hormones, particularly the mineralocorticoids, are important in the normal response of stimulating NH_4^+ excretion during metabolic acidosis. It is also quite apparent that there is still an as yet unidentified "ammoniuretic factor(s)" that has been demonstrated in plasmas from rat, dog, and toad, as well as in human urine. In each case, the factor was present during metabolic acidosis, but was not detectable in preparations from animals in normal acid-base balance or in metabolic alkalosis.

Specific Steroid Compounds during Acidosis

In this section, evidence suggesting specific known hormones, or unidentified factors, causing ammoniuresis and/or proton excretion are presented. The most comprehensive paper on blood steroids in chronic metabolic acidosis is that of Fazekas and Fazekas (43). They produced chronic acidosis for 5 months in 15 rabbits with NH_4Cl. They reported determinations of concentrations of steroids in blood before treatment and at the end of each month of treatment. In addition, they reported the concentrations of these same steroids in a control group of untreated rabbits at the end of the 5 months, so as to detect any variations due to either season or time. There were no marked changes in concentration detected between the pretreatment controls and the "seasonal" control animals. In addition, they determined concentrations of these same steroids in the adrenal gland in the experimental and the control group at the end of the 5 months. They reported on blood and adrenal concentrations of eight known steroids plus an attempt to determine the concentration of deoxycorticosterone. No deoxycorticosterone was found in any of the determinations on blood or on adrenal tissue. The concentrations of each steroid and important concepts from the literature on each compound are related below; a major discussion of the significance of these findings is reserved for the summary.

Common names of the compounds and abbreviations for them are used throughout the discussion. The actual full chemical names of the compounds are not given, but can be found in the reference of Cost and Vegter (44). Open brackets ([ ]) are used to indicate concentration, and the subscripts b, p, and a are used to indicate blood, plasma, and adrenal tissue, respectively.

Corticosterone. Corticosterone (B) appears to be a good candidate as a mediator of the hormonal effect on the kidney in acidosis. Welbourne *et al.* (24) observed a doubling of the $[B]_b$ 4 hr after intubating rats with NH_4Cl solution. The concentration was maintained and possibly rose a bit by the seventh day after daily administration of the solution.

Fazekas and Fazekas (43) observed $[B]_b$ to be elevated in rabbits for 5 months. The level was not constant, but rose each of the first 2 months from a control of 0.6 μg/dl to 2.3 units and then fell to a level of 0.8 units at the fifth month. This was associated with an increase in $[B]_a$ from 200 μg/100 g to 360 units (control values to treated animals).

Cortisol. Fazekas and Fazekas (43) reported the pretreatment cortisol (F) blood ($[F]_b$) to be 0.53 μg/dl, and it rose to 1.0 units at the first month. The $[F]_b$ then fell to pretreatment levels on the third month, and was below the pretreatment level on the fourth and fifth months. The $[F]_a$ showed a modest increase from 160 to 240 μg/100 G. Schambelan and Sebastian (45) found no significant change in the $[F]_p$ of four men in metabolic acidosis on the second day of administering NH_4Cl.

Fukushima *et al.* (46, 47) injected ^{14}C-labeled cortisol into a man and recovered 75% of the label as steroids in the urine. Of this 75%, 24% was tetrahydrocortisone, 18% tetrahydrocortisol, 1% F, and 0% tetrahydrocorticosterone.

Cortisone. Fazekas and Fazekas (43) reported the pretreatment cortisone (E) blood ($[E]_b$) to be 0.53 μg/dl. However, they were unable to detect any $[E]_b$ at the end of the first month; then, it rose to 1.0 and further to 1.46 units in the second and third months respectively, but returned to the control level by the fifth

month. They did report the $[E]_a$ to increase from 150 μg/100 g in the control to 233 units in the treated rabbits. When labeled E is infused, only about 1% is excreted in the urine as F; however, blood levels of F indicate greater conversion of E to F (47).

Hill and Frazier (40) were unable to reestablish an increase in either the H^+ or NH_4^+ excretion in the urinary bladder of adrenalectomized, acidotic toads by treating them with E. In essence, the evidence is against E being the mediator of the response to acidosis.

Aldosterone. The changes in aldosterone blood ($[Ald]_b$) of this hormone were not striking, as reported by Fazekas and Fazekas (43). The average $[Ald]_b$ did rise from 0.43 to 0.70 μg/dl in the second month, but it could not be considered different from controls in the first, third, and fourth months, and it fell in the fifth month. The $[Ald]_a$ rose from 50 μg/100 g in the control rabbits to 166 units in the experimental animals.

On the other hand, both Perez *et al.* (48) and Schambelan and Sebastian (45) reported increases in aldosterone levels in humans in response to NH_4Cl loading. Perez *et al.* (49) also showed an increase in aldosterone in dogs after injection of HCl. These responses were all within the first 48 hr. Schambelan and Sebastian also reported the aldosterone secretory rate was increased from a control rate of 111 to 513 μg/24 hr on the third day. There is an increase in $[K^+]_p$ in a metabolic acidosis, and there is a decrease in body Na^+ content resulting from the natriuretic effect of NH_4Cl. Both of these changes are known to be stimuli for increased aldosterone production. Aldosterone administration is known to cause a metabolic alkalosis in a normal animal. It does so by causing excretion of an acid urine.

Tetrahydrocorticosterone. Fazekas and Fazekas (43) were unable to detect tetrahydrocorticosterone (THB) in the blood of the rabbits during the control period. The $[THB]_b$ rose to 1.6 μg/dl the first month, stayed at near this level through the third month, then fell. THB is a metabolic product of B, as well as of E and F. It is estimated that about 35% of THB in the urine comes from B. The rise in concentration of THB could then be explained by increased production of B in acidosis. Unexplained, however, is the rise in $[THB]_a$ in acidosis. It rose from 100 to 263 μg/100 g, a greater percentage increase than was noted for $[B]_a$.

Tetrahydrocortisol. Peterson (50) concludes that sizable quantities of tetrahydrocortisol (THF) in the urine are derived from E and F. He also notes that the metabolism of B either does not contribute or contributes very little to the pool of THF. Fazekas and Fazekas (43) were unable to detect THF blood levels in the pretreatment samples, but $[THF]_b$ was present in all of the five monthly samples during treatment. $[THF]_b$ peaked at a concentration of 1.2 μg/dl in the second month, but was in a lower concentration the other four months. The $[THF]_a$ was 40 μg/100 g in the control rabbits, and averaged 183 units in the treated rabbits. Its effect on renal tissue or epithelial tissue is not known.

Tetrahydrocortisone. Tetrahydrocortisone (THE) is a major metabolite of F and probably of E. Peterson estimates that 65% of THE in the urine is from F. Fazekas and Fazekas (43) again found no THE in control blood samples. During the 5 months of treatment, the $[THE]_b$ varied from a high of 0.76 μg/dl to 0.33 units. The $[THE]_a$ was 50 μg/100 g in the control and 216 units in the experimental animals.

11-Dehydroxycorticosterone. Fazekas and Fazekas (43) reported 11-dehydroxycorticosterone (A) blood ($[A]_b$) to be 0.43 μg/dl in the pretreated control. The concentration rose markedly, with average concentrations of 1.03, 1.9, 1.6, 0.7, and 0.7 units in the successive 5 months of treatment. The $[A]_a$ changed from 200 in the control animals to 320 μg/100 g in the treated animals. We are not aware of any other studies relating this specific compound to metabolic acidosis or to its effect on any excretory epithelial tissue. This compound is stated by Cost and Vegter (44) to be metabolic product of B, but they report it is then further metabolized to tetrahydro-11-dehydroxycorticosterone (THA). The urine ratio of THA to A averaged 16 in normal subjects.

Urine Factor. Melton *et al.* (31) reported that a lipid fraction capable of increasing NH_4^+, but not increasing H^+ excretion in the toad bladder, was extractable from the urine of men and women collected during a metabolic acidosis induced by ingesting NH_4Cl. Extracts of urine of these same individuals when they were in a metabolic alkalosis did not have this activity. This factor is extractable from 1,2-dichloroethane into 0.1 N NaOH, but is not extractable from the same solvent into 0.5 N $NaHCO_3$. This solubility characteristic indicates that if it is a steroid, the A ring has three double bonds with a phenolic OH attached to it. Extracts of the adrenal cortex that had these same solubility characteristics were observed to stimulate NH_4^+ excretion in the toad bladder. This strongly suggests that the active fraction is a steroid.

Nothing is known of the blood levels of this substance. None of the substances discussed above, except 17β-estradiol, have either the requisite phenolic group or the unsaturated A ring of a steroid in order to be related to this substance. The fact that the activity is present in the extract of the adrenal gland and in the extract of the urine makes the possibility of it being a metabolic product of one of the above hormones very remote.

The fact that the adrenal gland plays an important role in the regulation of the renal excretion of both H^+ and NH_4^+ in metabolic acidosis seems to be well-established. However, the issue of whether or not one

or more specific steroids, or other factors, are the mediator(s) of this effect is very difficult to substantiate from a review of the literature. The issue of the role that THB, THE, or THF may play in the regulation of acid-base balance is raised by the report of Fazekas and Fazekas (43). First, this work needs to be repeated. The fact that these workers were unable to detect any of these three tetrahydrosteroids in the blood of control animals, and then detected such high concentrations of all three of them in each of the 5 months of the treatment certainly indicates that there is either a change in the metabolism of their parent steroids or an active secretion of them by the adrenal gland during a metabolic acidosis.

The metabolic pathways of B, E, and F are quite diverse. The putative concept that THB, THE, and THF are only metabolic products is questioned in two different articles by Fukishima *et al.* (46, 47). They have reported studies on the metabolism of cortisol using isotopes and specific activities of metabolites. They state, "The assumptions implicit in estimations of production rates have been discussed in detail. . . . The results described in this report establish that at least for some subjects . . . one or more of the assumptions is not valid." Then, in their 1969 report they state, "The adrenal cortex has the enzymes to make these[2] and related metabolites and circumstances could exist where they might contribute to the adrenal secretion to distort the specific activity of selective cortisol metabolites."

Evidence for Acute Changes

In this section, literature that specifically indicates early increases in the excretion of NH_4^+, H^+, or both is reviewed. Arbitrarily, we have defined early increases as occurring within a 6-hr time period of initiating the administration of NH_4Cl or other acidifying substance.

Sartorius *et al.* (2) wrote, "It is usually stated that the kidney responds to an increased acid load by excreting acid in free titratable form, and only after some delay, by excreting increased quantities of ammonia." They go on to state that in their experiments on two men, they observed early increases in both H^+ and NH^+ excretion in the first 120 min after ingesting NH_4Cl. Sartorius *et al.* (12) reported marked increases in excretion of both ions during the first 60 min after an acute load of NH_4Cl in 16 rats. They also reported the following as evidence that the early increase in excretion of these ions is associated with activity of the adrenal gland. Adrenalectomized animals (both 2 weeks and 2 months, postoperatively) were subjected to NH_4Cl loads, and showed some increases in both NH_4^+ and H^+ excretion, but the values at 5 hr were only 55% and 33%, respectively, of the rates in unoperated controls with identical loads. In the same paper, the authors reported a decrease in the cholesterol and ascorbic acid content of the adrenal gland 2 hr after the administration of the NH_4Cl. This is evidence of activity of the adrenal cortex. No determinations of cholesterol and ascorbic acid content were made between 0 and 2 hr.

The evidence then supports this statement: After the administration of an acid load, the adrenal cortex excretes one or more factors that contribute to the initial increase in excretion of both ammonia and an acid urine. These adrenal cortical substances are not the only mechanism by which the kidney responds, but they are a major factor.

Welbourne (51) reported that rats, during a 4-hr period after intubation with an NH_4Cl solution, showed more than a 3.5-fold increase in renal ammonia excretion and practically a 100% increase in the plasma corticosterone concentration at the end of the 4 hr.

Yoshimura *et al.* (52) studied 30 normal dogs, 10 adrenalectomized dogs, and 5 adrenalectomized dogs treated with deoxycorticosterone acetate. Urinary excretion of NH_4^+ and of titratable acidity (TA) were determined in all animals before and during an HCl infusion. The animals were under barbiturate anesthesia.

We will focus primarily upon the time course of the urinary response in the 30 normal animals. Urine samples were collected frequently and TA and $[NH_4^+]$ were determined. The authors present, in graphic form, the data from only one of the 30 normal dogs, which they state is typical for all of the 30 in the series. In the first hour, five urine samples were obtained. The TA was more than double that of the control in the second sample, and continued to rise for 2 hr, then was maintained at this high level for the duration of the 4-hr period reported. Similarly, $[NH_4^+]$ and the rate of excretion rose during the 4-hr period, although the rise was not as prompt as that of the TA. In the adrenalectomized animal (again, graphic presentation of one typical animal), there was virtually no increase in TA nor in NH_4^+ excretion in the 4-hr period after onset of a matched HCl infusion.

Perez *et al.* (48) measured both cortisol and aldosterone (Ald) levels in humans during this acute acidosis period, although they did not report any data on excretion of TA or of NH_4^+. They measured plasma levels in their patients 3 hr after the onset (9:00 a.m.) of a 2-hr loading period. There was a marked increase in the concentration of aldosterone at 12:00 noon, which contrasted with a decrease in $[Ald]_p$ at 12:00 noon in control observations. The cortisol levels were unchanged. On the other hand, there was a slight increase in $[K^+]_p$ produced by the acidosis. This is a factor known to stimulate aldosterone secretion, so it could be argued that the change in plasma pH as the stimulus is not proven.

In another paper, Perez *et al.* (49) studied metabolic

[2] THB, THE, THF, and others.

acidosis in 19 mongrel dogs. They produced acidosis both by NH_4Cl infusion in one experimental group, and by HCl infusion in another experimental group of animals. They measured the plasma concentrations of cortisol, renin, and aldosterone three times during the equilibration period before starting the infusions, and then every 15 min for 1 hr during the infusions. The animals were under barbiturate anesthesia. The major finding was a 2.5-fold increase in $[Ald]_p$ in response to HCl. The $[Ald]_p$ increased from the pretreatment levels in the NH_4Cl-infused animals, but the interpretation of this fact is clouded because the pretreatment $[Ald]_p$ of control animals was about five times the pretreatment $[Ald]_p$ of the NH_4Cl animals. The $[Ald]_p$ of the NH_4Cl animals was less than that of the control group throughout the 1-hr period of infusion. In some NH_4Cl animals studied at 2 and 3 hr, the $[Ald]_p$ did rise above that of the controls.

Are the Ammoniuretic and Protonuretic Factors Identical or Different?

In this section, work suggesting that there are separate ammoniuretic and protonuretic factors mediating the response of the kidney to an acidosis are presented. Two different studies indicate that the ammoniuretic and protonuretic effects might be due to two different factors. Melton (53) reported numerous studies on both urine extracts and plasma preparations. He assayed his preparations on toad bladders mounted between chambers. He frequently compared preparations from animals in metabolic acidosis with control preparations from the same animals in either a normal acid-base state or metabolic alkalosis. Positive results were found only on preparations of either urine or plasma from animals in metabolic acidosis.

Melton studied extracts of human urine, extracts of both dog and human plasma, and some dialyzed samples of plasma of both species. In some experiments, both the ammoniuretic and the protonuretic effects were produced by the same preparation. However, frequently he found only the ammoniuretic effect without the protonuretic effect.

Earlier, Frazier and Vanatta (30) reported studies on the effects of various plasma preparations on excretion by toad bladders mounted between chambers. In one study, they noted only an ammoniuretic effect from one fraction of plasma from acidotic toads and only a protonuretic effect from another fraction of the same plasma. This would clearly support the idea that the ammoniuretic and protonuretic factors are different substances. They were unable to show an effect of preparations of plasma from toads in normal acid-base balance on either NH_4^+ or H^+ excretion by the bladders.

Discussion and Future Directions

The adaptive response to acidosis in terms of changes in NH_4^+ excretion has been studied extensively over the last 40 years. The preponderance of evidence indicates that the adrenal cortex plays an important role in mediating the response of the excretory system to acute and chronic metabolic acidosis. This review is not an attempt to evaluate the renal mechanism by which such a response has been brought about. Welbourne (51) presents a review of some of these studies.

This review emphasizes that there is a very prompt response of the renal system to an acidosis. Four papers report on a marked increase in NH_4^+ and/or TA excretion in the first few hours of the acidosis in three species: two men, 30 dogs, and a total of 22 rats. Three of these reports specifically indicate that an intact adrenal cortex is necessary for a maximum early response. Two other works report on changes in $[Ald]_p$ during the acute period, but do not include any observations on the excretion of electrolytes. We have not read any reports of an absence of a response of the renal system during these earlier periods. It is true that the magnitude of this early response is much less than the magnitude of the response observed 3 to 4 days after the onset of the acidosis.

There is evidence in the literature that corticosterone may be at least one of the hormones stimulating the excretory system to excrete NH_4^+ and H^+ early in an acidosis. Other papers indicate that aldosterone may play an important role. However, there is much conflicting evidence regarding corticosterone and aldosterone being the only adrenal steroids involved in both the acute and chronic phases of a metabolic acidosis. The changes in concentration of many hormones reported by Fazekas and Fazekas (43) is one such study. Also, the studies of an ammoniuretic factor found in adrenal cortical extracts and in extracts of urine from human beings in metabolic acidosis cannot be explained on the basis of corticosterone or aldosterone. The work of Alleyne and Roobol (28) suggests still another factor.

The following areas of research are suggested as a result of this survey of the literature: (i) there should be further work to document the role of the pituitary gland in this adaptive process. If the pituitary does drive the adrenal cortex to release a specific hormone, then the stimulus to the pituitary, the receptor involved, and other such details of this mechanism should be explored; (ii) there should be a wider search for one or more specific compounds that are produced and released from the adrenal cortex which then stimulate the kidney. In such a search, the following possibilities should be kept in mind: (a) there may be different adrenal compounds producing the acute response and the chronic responses noted in renal function; and (b) there is evidence suggesting there may be separate ammoniuretic and protonuretic compounds. Finally, (iii) the unknown factor(s) found in rat, toad, and dog plasma, human urine, and adrenal extract (29–32, 41)

should be purified and characterized, and a time course for activity should be determined.

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