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ABSTRACT

While it is generally accepted that adrenalectomy is associated with impairment of ammonium and/or titratable acid excretion by the kidney, it remains unclear whether renal net acid excretion is similarly affected and whether the absence of the adrenal gland per se, or other concomitants of adrenal insufficiency mediate such changes. For example, dramatic increases in NH_4^+ excretion can accompany the normalization of potassium status. Less directly, there is also evidence that reduced rates of acid excretion can result from changes in food intake, urine flow rate, urine pH or distal sodium delivery rates. In this context our studies were undertaken to isolate the chronic effects of adrenalectomy on renal net acid excretion rates in the unanaesthetized rat. Pilot studies showed adrenalectomized (ADX) animals are successfully maintained without steroid replacement if drinking water is replaced by saline. Results showed these animals develop hyperkalemia without concommittant metabolic acidosis when compared to sham operated animals (sham rats).

The capacity of ADX rat to maintain acid balance after acid loading was assessed by giving pair-fed ADX and sham operated rats equal amounts of NH_4Cl by gavage (10mEg/Kg twice daily BID) for 3 days. Hyperkalemia was avoided by dietary K^+ restriction. Results showed ADX rats developed metabolic acidosis while pair treated sham animals maintained a normal acid-base balance.

To document chronic changes in net acid excretion during adrenal insufficiency ADX and sham operated animals were placed in metabolic balance cages for 24 hour urine collections uninterrupted for a 5-8 day

control period followed by a 5 day acid loading period during which time animals received 10mEq/Kg NH_4Cl on day one and 20mEq/Kg in 2 equal dosages on day 4 and day 5. For these experiments animals were paired by weight from among litterimates and paired for food, drink and NH_4Cl intake. Additional dietary changes in K^+ and Na^+ intake were made so that urine flow rates, urine pH, and distal Na^+ delivery rates were controlled.

Under these conditions the mean control period net acid excretion rate for each ADX rat was less than that for each pair treated sham operated rat. In response to NH_4Cl loading, 10mEq/Kg BID, the increase in net acid excretion rate from the mean control period value for each ADX rat was again less in ADX rats when compared to the pair treated sham animals.

In a final protocol, we demonstrated that the severe metabolic acidosis in ADX rats seen after NH_4Cl loading was prevented with steroid replacement (1μg dexamethasone, 2μg aldosterone BID).

Accordingly we conclude that the adrenal gland is essential for normal renal acid excretion.

CHAPTER I

Introduction

It is well known that the adrenal gland greatly influences the physiology of the kidney. This has been appreciated since 1933, when adrenal insufficient animals and man were known to develop metabolic acidosis, hyperkalemia, hyponatremia, and sodium deficiency (1, 2, 3, 4). These changes in blood chemistry had also been correlated with inappropriately high rates of sodium excretion (2) and low rates of sulfate excretion (3). It had been amply demonstrated that without the adrenal gland, animals become moribund and die in vascular collapse. Since these pioneering observations, the regulatory influence of the adrenal gland on the sodium and potassium balance has been well established (5) while its role in the excretion of acid continues to intrique scientists.

There is no doubt that in normal animals and man the reabsorption of filtered bicarbonate and the formation of titratable acid and ammonium all contribute to the excretion of acid (6,7,8,9). Central to these processes is the production of hydrogen ions by renal tubular cells. These protons decompose bicarbonate in tubular fluid to carbon dioxide and water which effectively prevents the loss of alkali in urine. Gradually this process reduces the pH of tubular fluid and titrates filtered acid anions. The capacity of these anions to participate in acid excretion depends on the amount and the pK value of each. In general the pK values are too low for the excretion of equimolar amounts of hydrogen ions in urine containing the

maximal concentrations of protons against which the tubular cells can secrete. To avoid this physiologic limit on proton secretion and therefore acid excretion, renal tubular cells produce ammonia, which, by diffusing into tubular fluid, buffers the tubular pH above this critical value. As a result, the content and rate of excretion of acid in urine can equal the amount of acid necessary to maintain the acid balance (6,7,8,9). In adrenal insufficient animals and man several of these processes are impaired.

In the ADX rat, rates of ammonium and titratable acid excretion are reduced under basal conditions and in response to metabolic acidosis (10,11,12,13,14,15) while marked improvements are seen after mineralocorticoid and glucocorticoid replacement (11,12,15). These observations are confirmed in the dog and man (16,17,18,19). The suggestion that the adrenal gland plays a role in the normalization of the acid-base balance in HCl acidotic dogs after chronic hypotonic extracellular fluid volume expansion (ECFV), is supported by the fact that, Sham animals respond but ADX animals do not. Interestingly, associated with this response are dramatic increases in the plasma aldosterone concentration and rates of urinary aldosterone excretion (20). Similarly, NH_4Cl induced metabolic acidosis in man also stimulates a rise in the plasma aldosterone concentration (21). Since adrenalectomy leads to a reduced capacity for acid excretion when compared to intact or steroid replaced animals, the adrenal gland appears to have a beneficial effect on acid excretion.

Several explanations for the above observations are possible. Firstly, adrenal steroids, particularly aldosterone, are essential for the maintenance of potassium balance. Several lines of evidence indicate that this effect is important for normal rates of acid

excretion. A classic experiment by Szylman et al (18) strongly supports this notion. By normalizing the potassium balance in a patient with reduced rates of ammonium excretion, and hyperkalemic metabolic acidosis, the acid-base disturbance disappeared. This effect was documented while the patient continued to suffer from a deficiency of aldosterone. The authors proposed that potassium retention and hyperkalemia effectively interfered with the excretion of ammonia. Such an effect had already been documented in paired studies on normal subjects given potassium supplements in their diets. The results show that potassium chloride reduces the capacity for ammonium and net acid excretion after ammonium chloride loading (22). Normal rats receiving potassium chloride supplements either chronically or acutely are equally sensitive to these suppressive effects (23,24,25) while hyperkalemic ADX dogs exhibit a reduction in renal acid excretion which, at least in part, is attributable to potassium retention (16).

A number of mechanisms have been suggested to explain how potassium exerts its effects. Tannen et al (25) working on kidney tissue slices showed that potassium reduced the production of ammonia. In addition, because potassium increases the renal blood flow (RBF)(26) ammonia is diverted from renal tubular cells to peritubular blood rather than to the tubular fluid and ultimately into the urine. This would result in a fall in the rate of ammonium excretion. Counteracting such an effect is the resultant increase in glutamine delivery (27) for ammonia synthesis. As yet, the relative importance of these two effects are unresolved.

Potassium may also influence urinary acidification by competing with hydrogen ions for secretion from renal tubular cells to

tubular fluid (28). This view is supported by numerous experiments in which the administration of potassium salts was demonstrated to enhance the secretion of potassium while inhibiting the secretion of hydrogen ions (29,30,31,32).

It seems likely that both the lack of ammonia as a buffer and the diminished rate of hydrogen ion secretion contribute to reduced rates of acid excretion. In view of the fact that adrenal insufficiency leads to potassium retention and hyperkalemia and steroid replacement promotes potassium excretion, an increase in acid excretions is expected secondary to their kaluretic effect (12,19).

Secondly, several lines of evidence indicate that changes in sodium metabolism lead to important changes in hydrogen ion secretion and consequently urinary acidification. It is therefore a possibility that the effects of adrenal steroids on acid excretion are secondary to the maintenance of sodium balance. This is emphasized in the following studies.

After adrenalectomy Ditella et al (33) were able to restore normal rates of ammonium and net acid excretion and prevent metabolic acidosis by simply salt loading the animals to prevent ECFV contraction and a large reduction in GFR. The authors concluded that "ammonium excretion rates were affected by changing distal sodium delivery rates rather than by an action of mineralcorticoids per se.
That ADX rats can be maintained in acid-base balance with salt supplements has been supported by other authors (34,35). Analdosterone human subjects are maintained without an acid-base disturbance if an adequate sodium intake is preserved (36).

Under different experimental conditions, changes in the distal nephron sodium delivery rates have been examined. Its importance in contributing to

the capacity for net acid excretion has been supported in studies designed to correct metabolic acidosis by ECFV expansion (20,37,38) or to increase acid excretion by maintaining subjects on a sodium free diet. In the latter case the induced increase in sodium reabsorption led to intensely acidic urine with increased rates of ammonium and net acid excretion (39). As mentioned above, chronic hypotonic ECFV expansion corrects HCl metabolic acidosis in intact dogs but not in ADX animals (20). The difference is accounted for by a mineralocorticoid effect on the distal nephron avidity for sodium reabsorption (20). However, when ADX dogs were subjected to an acute isometric expansion of the ECFV, increased rates of acid excretion followed (40). The different results obtained may be related to the difference in distal nephron sodium delivery rates. Since the proximal tubule function is sensitive to ECFV expansion, increased sodium delivery to the distal nephron would enhance the transtubular potential and facilitate the secretion of hydrogen ions (41). In hypotonically expanded ADX animals it is possible that their filtered load of sodium was too low for a sufficient increase in the distal nephron sodium delivery rates. As a consequence, the excretion of acid would not be increased (41,42). By increasing rates of sodium delivery to the distal nephron with sodium phosphate infusions, titratable acid excretion is also increased in ADX dogs albeit less than in animals receiving mineralocorticoids (16). Consequently, rates of net acid excretion are affected by the distal nephron sodium delivery rate and its avidity for sodium.

In considering the importance of the adrenal gland in contributing to a normal capacity for acid excretion, the direct effects of steroids are of interst. There is evidence that adrenal steroids

facilitate the secretion of hydrogen ions and the production and excretion of ammonium. In experiments on ADX dogs, after controlling for both sodium and potassium balance a defect in acid excretion persisted (43). While infusing ADX dogs with sodium phosphate, animals replaced with mineralocorticoids were able to increase the rate of acid excretion more than non-replaced animals, despite equivalent distal tubular delivery rates (16). In other experiments on ADX dogs, increased rates of acid excretion are seen by combining potassium depletion with mineralocorticoid replacement. When amiloride is given, it prevents this increase by specifically blocking the reabsorption of sodium and the subsequent generation of a lumen negative potential difference. Accordingly, the increased rate of acid excretion is dependant on an effect of the mineralocorticoid (44). Aldosterone also promotes hydrogen secretion in the absence of sodium reabsorption. This is shown by the fact that even after blocking the aldosterone mediated sodium reabsorption with actinomycin D hydrogen ion excretion persists (45). In patients suffering from metabolic acidosis and hyporeninemic hypoaldosteronism, mineralocorticoid replacement augments renal hydrogen ion secretion. This improvement occurs even in the absence of pre-existing potassium retention and reduced rates of ammonium excretion (19).

Considerable attention is being given to the effects of adrenal steroids on ammonia production and excretion. Invariably, mineralocorticoid replacement in ADX animals increases the rate of ammonium excretion (11,12). These observations have been extended by the finding that aldosterone replacement leads to increases in ammonium excretion without inducing kaluresis or hypokalemia (46). In vitro studies using isolated kidneys taken from aldosterone treated rats confirm these preliminary observations. Glucocorticoids were also shown to normalize

the rates of ammonia production in kidneys taken from ADX rats treated with triamcinolone when compared to intact rats. Later a mechanism was proposed to explain these results. It is believed steroids increase the permeability of the mitochondrial membrane to glutamine thereby supplying glutaminase I with a substrate for ammonia production. Ultimately this ammonia diffuses into tubular fluid to buffer secreted protons (47).

Circumstantial evidence supporting a role for aldosterone in renal acid excretion comes from work by Burnell et al (48). After dogs are potassium depleted, a dramatic decrease in the rate of ammonium and net acid excretion is seen associated with a fall in blood pH and plasma bicarbonate concentration. A possible mechanism, to explain these reduced rates of acid excretion involves the effects of aldosterone deficiency secondary to potassium depletion (49). This explanation is particularly attractive in view of the apparent dependancy of adrenalectomized animals and man on mineralocorticoids for a normal capacity for acid excretion (11,12,16,17,19).

Recently, a number of investigators have begun to examine the effect of mineralocorticoids on hydrogen ion secretion. Some believe aldosterone promotes acid excretion by stimulating the reabsorption of sodium, thereby generating a lumen negative potential difference in collecting ducts for proton secretion (50). However, aldosterone stimulated secretion can occur in the absence of sodium reabsorption. As mentioned above, after sodium reabsorption is blocked by Actinomycin D, proton secretion persists (45). These observations have been extended by Qais Al-Awqati et al working with the turtle urinary bladder (51). Their study revealed that aldosterone does stimulate hydrogen ion transport independent of sodium transport. Further, they showed that aldosterone does not increase the force of the proton pump ie. maximum proton gradient.

against which it can pump, but it does facilitate the flow of protons through the active transport pathways. In view of these findings, without invoking secondary effects of the adrenal gland, aldosterone may, at least in part, contribute to the excretion of acid by a direct mechanism.

In addition to the effects of sodium, potassium, and steroids, the interpretation of reduced rates of acid excretion requires some knowledge of the effects of urine pH, urine flow rates, food intake altered glomerular filtration rate and the contribution of extrarenal mechanisms in the defense against acidosis. Variations in the pH and flow of urine primarily affect the excretion rate of ammonium. The explanation for this effect follows from the belief that ammonia synthesized in renal tubular cells diffuses down its concentration gradient into either the peritubular environment, or into the tubular fluid. A certain portion of this ammonia, depending on the pH of the new environment, becomes ammonium . Appropriately, this mechanism for ammonium excretion is referred to as the "Theory of non-ionic diffusion" (52). Because ammonia becomes ionized, the effective concentration of ammonia is decreased. Consequently, further diffusion of ammonia down its concentration gradient is promoted. The effects of such a mechanism are demonstrated in experiments by Balagura et al (53). After injecting ammonium and creatinine into chronically acidotic dogs, ammonia appears in the urine before creatinine indicating that ammonia must diffuse across the tubular cells to be found in the urine before filtered creatinine. When the experiment is repeated in acutely alkalotic dogs only trace amounts of ammonia appear in the urine indicating that even filtered ammonia must diffuse back to blood from the tubular fluid. In conclusion, the net diffusion of ammonia in the dog appears to be determined by the hydrogen ion gradient. Predictably the rate of ammonium excretion is decreased in the rat, when its

urine pH is increased following the administration of a carbonic anhydrase inhibitor or after injecting sodium bicarbonate (54,55). In contrast to data available on the dog and in man, ammonium excretion in the rat appears to be more dependant on systemic factors rather than on the pH of urine per se (55). For example, a marked increase in ammonium excretion is seen in ammonium chloride acidotic rats while a decrease in the rate of ammonium excretion is produced by sodium bicarbonate infusion. When diamox is given intravenously to acidotic rats a rise in urine pH is seen without a change in the pH of ECF. Contrary to what is expected, ammonium excretion does not fall signifiantly despite an increase in urine pH similar to that seen after sodium bicarbonate infusion. Similarly when a fall in urine pH is induced without a change in blood pH using sodium sulphate intravenously, only a small increase in ammonium excretion is found. This is in sharp contrast to the effects of HCL infusion on ammonium excretion in normal rats. Here, although the pH of urine decreases less, a much greater rise in ammonium excretion results. In the interest of this study,, although systemic factors appear to be more important, the effect of urine pH albeit smaller than that seen in the dog and man, warrants consideration.

Urine flow rates also influence the excretion of ammonium. Increases in urine flow rates are thought to dilute the ammonia in the tubular fluid and by decreasing its concentration, facilitate the diffusion of ammonia from the tubular cells. Such a mechanism is consistent with changes in ammonium excretion rates seen with increased urine flow rates in normal human subjects (56). The authors stipulated that ammonium excretion varied directly with increases in urine flow rates when the urine pH was within the alkaline range. Ammonium

excretion proves to be independent of urine flow rate when the pH of urine is between 4.8 and 6.0. In the dog, rates of ammonium excretion is similarly unaffected by urine flow rates when the pH is less than 5.5 (57). Tannen (58) extended these observations to show that urine flow not only increases the excretion of ammonium but of net acid. Of historic interest, Eggleton in 1947, had already suspected that, by varying the urine flow rate, the ammonium output is changed (59).

Given the finding that adrenalectomized rats excrete less acid than intact animals, alternative explanations to that of a renal impairment are possible. Since net acid excretion normally reflects the rate of the endogenous acid production (60,61), it is possible that a reduction in food intake per se could alter the rate of acid excretion independent of other changes. Alternatively, in view of the fact that the filtered load of bicarbonate, sulfate and phosphate are in part dependant on the glomerular filtration rate, and to an important extent influence renal acid handling, its changes in adrenalectomy are important. The glomerular filtration rate is markedly reduced in adrenalectomized rats (62,63,33). However, several authors have shown that this is not an issue when animals are maintained with saline. Such animals exhibit glomerular filtration rates indistinguishable from that of intact rats (64,35,33).

In addition to tissue buffering, several investigators have given evidence for extrarenal mechanisms contributing to the disposition of unexcreted acid. Continuous positive acid balances are seen among normal subjects during periods of chronic stable ammonium chloride acidosis (65). A contribution by bone alkali is suggested

by changes in bone structure seen in rats made chronically acidotic with ammonium chloride (66, 67). It is found that such animals develop osteoporosis as both the reabsorption of bone mineral and matrix increase. Finally, in man, it has been calculated, the amount of acid retained during ammonium chloride induced acidosis is matched on an equivalent basis by a negative calcium balance (68).

There are basically two mechanisms by which bone could affect the excretion of acid in adrenalectomized rats. Firstly, as a consequence of metabolic acidosis, bone resorption would lead to a decrease in the rate of acid excreted by an amount equal to that buffered by released alkali. Such a decrease in acid excretion would not represent an impairment although the cause of the metabolic acidosis would still require separate consideration. A second or indirect mechanism should also be considered. For example, if adrenalectomy were to stimulate the production of parathyroid hormone or if ionized calcium Ion were to decrease, one could postulate a parathyroid hormone (PTH)-induced acidosis(69). Available evidence suggests that PTH would be decreased secondary to the retention of calcium known to occur in the adrenal insufficient man (70). Consequently, a contribution of bone to acid regulation would not rule out a defect in renal acidification arising from the absence of adrenal hormones.

The following experiments were designed to isolate the effect of the adrenal gland per se on rates of net acid excretion. Therefore, by using dietary manipulations, attention was given to controlling non-adrenal variables known to impair urinary acidification in ADX animals. The variables successfully controlled were hyperkalemia,

sodium depletion, changes in food intake, urine pH and urine flow rate. The results indicate that under these controlled conditions reduced rates of net acid excretion persisted in ADX animals both with and without ammonium chloride loading. Further, adrenal steroid replacement prevented the metabolic acidosis seen in ADX animals maintained in sodium balance without hyperkalemia. Consequently, it is concluded that the adrenal gland is essential for normal rates of net acid excretion.

CHAPTER 11

Methods

A. Experimental Rationale

These experiments were carried out on 87 male Sprague-Dawley rats bred and raised in a climate-controlled animal facility at the University of Ottawa. Over 90 animals were used in preliminary experiments to develop a satisfactory experimental design in which the potassium status as well as food and sodium intake were controlled.

Pilot studies were designed to evaluate whether or not ADX rats developed metabolic acidosis. Since ADX animals maintained with saline did not become acidotic, NH_4Cl loading was introduced to further challenge these animals. A final protocol in this section included a comparison of the systemic acid-base status between ADX and sham rats paired for food, saline and NH_4Cl intake.

In experimental protocols the propensity of adrenalectomized rats to retain potassium was circumvented by inducing potassium depletion by dietary potassium restriction (Groups 1A, 11A - Table 1). Because this, in turn, led to polydypsia and polyuria, the sodium concentration in the drinking water of sham rats eating a normal diet was doubled to equalize their sodium intake (Group 11B). Potassium chloride was added to the drinking solution of control animals to achieve a less negative potassium balance. As a result of changing the dietary intake of potassium and sodium, and pair feeding, we were able to appropriately manipulate potassium stores, sodium ingestion and excretion, and food intake between paired groups.

Results of early experiments showed that potassium-depleted adrenalectomized rats excreted less net acid than control rats eating a normal potassium diet. A subsequent protocol giving ADX rats a normal potassium diet (Group III - Table I) was carried out to determine if reducing potassium stores per se, in adrenalectomized rats, interfered with the renal excretion of acid.

All animals were studied under control conditions with and without NH_4Cl loading. In Groups 1, 11 and 111 the five-day acid period consisted of a single bolus of 10 mEq NH_4Cl/Kg on day one, followed by a two-day observation period. This enabled us to observe the response of our animals to a mild acid challenge as well as serving as an induction for ammoniagenesis for the subsequent large acid load. It consisted of 10 mEq NH_4Cl/Kg B.I.D. on days four and five and represented a severe acid challenge which clearly separated the responses of ADX and sham-operated animals.

Our results showed considerable baseline variation among rats during the control period. To avoid the effect of baseline variation, we expressed our data as a change from the mean control values for each rat. Consequently, this approach required that each animal be studied to the end of the acid period. Since muscle potassium content analysis required that we sacrifice the animals, another group of rats, Groups IV A-D, were treated identically to those of Groups 1, 11, and 111. This enabled us to obtain systemic acid-base and electrolyte values corresponding to the end of the control period and gave us some indication of the effects of control period treatments and condition of the animals before NH_4Cl loading.

Finally, because profound metabolic acidosis was observed to be associated with a defect in net acid excretion, and because this defect was

hypothesized to be due to the absence of adrenal steroids, rather than due to changes in other factors altering urinary acidification, a steroid replacement protocol was undertaken (Groups V A-C, Table 1). To be certain that adrenalectomized rats did not have higher potassium stores than adrenalectomized rats, given steroids (VC), these animals were potassium-depleted the (Group V-A, Table 1). To provide an indication of the degree of restoration of acid-base balance induced by the steroids, sham-operated animals were treated similarly to the steroid replacement group (Group V-B, Table 1).

B. Pilot Studies and Experimental Protocols

a) Pilot Studies

i) ADX rats, maintained on .9% saline and Purina Rat Chow.--Sixteen ADX and 16 sham-operated rats were maintained on .9% saline and Purina Rat Chow for 13 days. On the fourteenth day all rats were anaesthetized and blood and tissue samples were taken.

ii) Potassium-depleted ADX and Sham rats given NH_4Cl containing diet ad libitum.--Twenty ADX and 20 sham-operated rats were given free access to a low-potassium diet and .9% saline for 7 days. On days 8, 9, and 10 all rats received 10% NH_4Cl in their low potassium diet and .45% saline containing 75 mEq/l NH_4Cl. On day 11 the animals were anaesthetized and blood and tissue samples were taken.

iii) Potassium-depleted ADX and sham rats paired for NH_4Cl, food and drink intake.--Each sham rat was given the equivalent weight of food and drink consumed by its ADX pair in the previous 24-hour period. Both groups received a low potassium diet and drank .9% saline for the duration of the experiment. On days 6, 7, and 8 all rats received NH_4Cl 10 mEq/kg B.I.D. On day 9 all rats were anaesthetized and blood and tissue samples were taken.

b) Experimental Protocols

i) Group 1A-ADX and 1B-sham.--Five animals were adrenalectomized, maintained on a low-potassium diet and given .9% NaCl to drink. These animals constituted Group IA. They were placed in metabolic balance cages immediately after adrenalectomy with the first day of the control period commencing three days after surgery. The control period was eight days in duration and was followed by a five-day period in which a small and large acid load was given. The small acid load consisted of two molar NH_4Cl solution gavaged in the amount of 10 mEq/Kg body weight on day one. After a two-day period of observation four more gavages were given B.I.D. for two days constituting the large acid load. The amount of food and saline drunk by this group of rats dictated the amount ingested by their sham partners. Group IB consisted of five sham-operated animals eating a low potassium diet and drinking 0.9% NaCl to which .5 mEq/l KCl was added. The control period of these animals was started four days after the sham operative procedure and the control and acid-loading periods were carried out as indicated above in a paired fashion.

ii) Group IIA-ADX and IIB-sham.--Six adrenalectomized rats eating a low-potassium diet and drinking 0.9% NaCl constituted Group IIA. They were paired with six sham-operated rats eating a normal potassium diet and drinking 1.8% NaCl to which 1 mEq/l of KCl was added. Group IIA and Group IIB were treated in a fashion identical to that described for Group IA and Group IB except that both groups had free access to their respective drinks.

iii) Group III-ADX.--Five adrenalectomized rats constituted Group III. These animals ate a normal potassium diet and drank 0.9% NaCl. The control period and acid-loading periods were identical to that noted for Groups IA, IB, IIA and IIB. However, there was no paired control for these animals. As

already noted in the experimental rationale and Table I, these animals were compared with Group IA adrenalectomized rats on a low-potassium diet for the purposes of exploring the effects of potassium depletion in adrenalectomy.

iv) Systemic values for control period groups IV; A, B, C, and D.--Six adrenalectomized animals eating a low-potassium diet, drinking 0.9% NaCl constituted Group IVA. Six sham-operated animals eating normal potassium food and drinking 0.9% NaCl to which 0.5 mEq/l KCl was added constituted Group IVB. Six sham-operated animals eating normal potassium food and drinking 1.8% NaCl, to which 1 mEq/l KCl was added, constituted Group IVC. Six adrenalectomized animals eating a normal potassium diet and drinking 0.9% NaCl constituted Group IVD. These four sub-groups of animals were treated as Group I, Group II and Group III above, up to the end of the control period. No acid loading was undertaken in these animals. Rather, at the end of the control period, these animals were sacrificed and systemic blood was taken for acid-base and electrolyte values. Kidneys were then removed for histological examination for lesions of potassium depletion, and muscle samples were taken to determine potassium content.

v) Hormone replacement, Groups V; A, B, and C.--Twelve adrenalectomized animals eating a low-potassium diet, drinking 0.9% NaCl constituted Group VA. Twelve sham-operated animals eating normal potassium food, drinking 0.9% NaCl constituted Group VB. Twelve adrenalectomized animals eating a normal potassium diet, drinking 0.9% NaCl constituted Group VC. This protocol was 18 days in duration. The additional five days (when compared with Groups I, II, and III above) were devoted to the administration of two microgrammes aldosterone and one microgramme dexamethasone B.I.D. to Group VC while Group VA received oil injections. On the morning of the nineteenth day, all animals were sacrificed and blood and tissue samples were taken.

TABLE I

EXPERIMENTAL PROTOCOLS: AN OVERVIEW

Group	No.	Diet*	Treatment	Comment
IA-ADX	5	Low K^+	8 days, control period. 5 days, small and large acid loading.	Pair-fed, both groups were K^+ depleted; ADX rats excreted less net acid.
IB-Sham	5	Low K^+ 0.9% NaCl + 0.5 mEq/l KCl		
IIA-ADX	6	Low K^+	8 days, control period. 5 days, small and large acid loading.	Pair-fed, sham rats have normal K^+stores; ADX rats still excrete less net acid.
IIB-Sham	6	Normal K^+ 1.8% NaCl + 1 mEq/l KCl		
III-ADX	5	Normal K^+	5 days, control period. 5 days, small and large acid loading.	Group IA vs III, same rate of net acid excretion while eating less food; indicates impairment in net acid excretion of ADX rats was not caused by K^+ depletion.
IVA-ADX	6	Low K^+	8 days, control period.	Provides systemic acid-base and electrolyte data as well as kidney histology and muscle K^+ content values corresponding to post-control period state of Groups I, II and III.
IVB-Sham	6	Low K^+ 0.9% NaCl + 0.5 mEq/l KCl		
IVC-Sham	6	Normal K^+ 1.8% NaCl 1 mEq/l KCl		
IVD-ADX	6	Normal K^+		
VA-ADX	12	Low K^+	10 days post-operatively steroid replacement begun and continued for 8 days-- large acid load given on days 16, 17, 18.	Systemic analysis after acid loading shows profound metabolic acidosis in ADX rats of Group VA which was prevented by steroid replacement in ADX rats of Group VC.
VB-Sham	12	Normal K^+		
VC-ADX + steroids	12	Normal K^+		

*All animals received 0.9% NaCl to drink unless otherwise specified.

C. Surgery

Male Sprague-Dawley rats, bred in our colony and housed in climate-controlled rooms were used in all experiments. Rats were anaesthetized with 10 mg Nembutal i.p. and a three-inch wide dorsolumbar area was shaved. A unilateral one-inch incision through the outer skin layers was made beginning one half inch from the animal's vertebral column and running a transverse course one half inch below the costal margin. At the end of the incision a small opening was made into the abdominal cavity by gently separating muscle fibers of the abdominal wall. A hemostat was inserted and the abdominal wall was pressed along the incision line. Using small scissors, it was cut along the hemostatic line to avoid sanguination. Using cotton-tipped probes, the adrenal gland was localized and manipulated into clear view. A small clamp was applied onto the supporting tissues holding the adrenal gland. Along the surface of the clamp, opposite the side of the adrenal gland, a single cut through the supporting elements released it in one piece. Intact adrenal glands taken from rats during any given operative session were placed into labelled, formalin containing vials. Subsequently they were examined to ensure no pieces or whole glands were missed. Complete removal of the adrenal glands was confirmed by inspection at the end of the experiment and by radioimmunoassay for aldosterone in plasma samples from each rat. The muscle and skin layers were sutured separately and the procedure was repeated for the contralateral side. Sham-operated rats underwent identical operative procedures except for the removal of the adrenal glands.

D. Balance Technique

a) Metabolism Units

The metabolism unit was used so that food and drink intake could be accurately determined while 24-hour urine collections were being made.

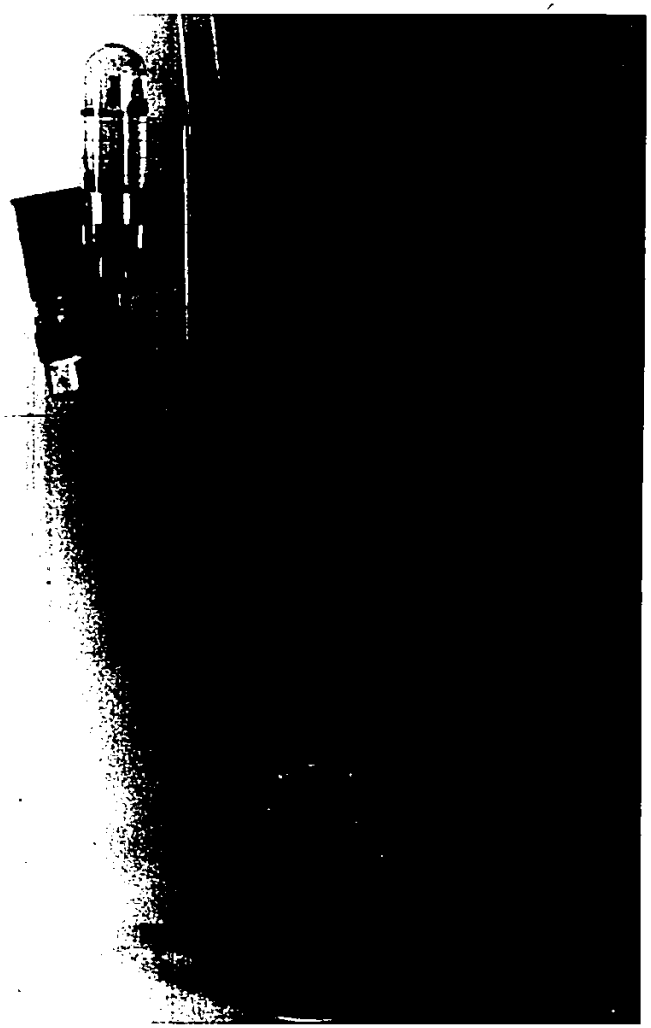
Each unit consisted of a cage having a wire floor placed over a urine-feces separator (see Figure 1). A food jar was placed at the end of a feeding tunnel and a drinking source was accessible through a small opening in the front wall of the cage. Any food or drink not consumed was recovered from the source or from catch trays placed in such a position as to prevent any losses by spillage. Through adhesion and cohesion urine flowed to the tail of the separator and into the urine bottles while fecal pellets rolled forward by gravity and trajection ahead of the tail and urine bottles. The tendency for fecal adhesion and subsequent blockage of urine flow was greatly reduced by coating the walls of the separator with teflon. In the laboratory, recovery rates were estimated at 98% by collecting 42 milliliters of water over a ten-minute period.

b) Daily Balance Routine

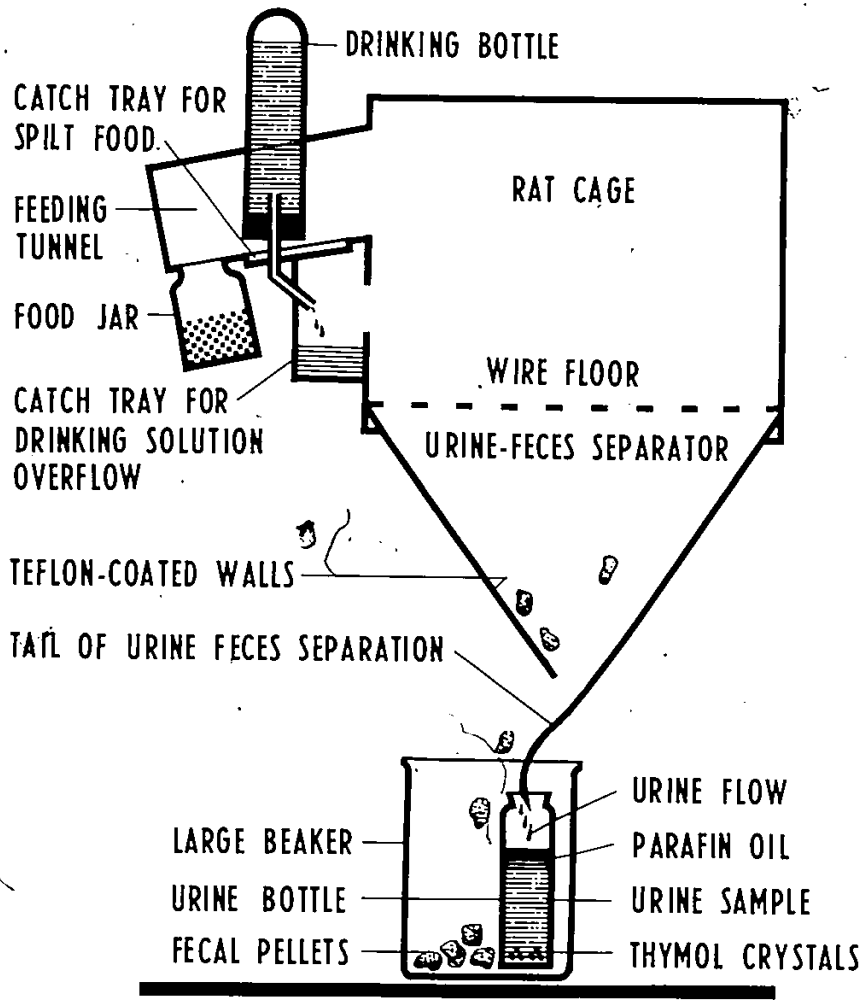
The chronological order of activities carried out each day to determine the weight of food and drink consumed by each rat as well as the weight of urine excreted per 24-hour period is given as follows. At approximately 09:15 hours all food jars and drinking bottles were removed from the metabolism units. Any spilled food was added to the food jar while spilled drink was transferred from the catch tray to pre-weighed plastic flame cups. At a later date, after dessication, they were re-weighed in order to determine the weight of the salt remaining by difference. These values were used to correct electrolyte intake data, thereby avoiding falsely positive electrolyte balances due to spilled drinking solutions. Clean urine bottles containing thymol and paraffin oil were weighed. Each rat was briefly held above its cage (so that initial urine losses as a result of handling, fell into the urine bottles), then transferred to a pre-weighed container, carried to a scale, weighed, and returned to its cage. Urine losses into the container during this procedure were measured by difference. The weight of food and drink consumed by each

Fig. 1

Metabolism Unit.



METABOLISM UNIT



animal was calculated by subtracting the final weight of each food jar and drinking bottle from its respective initial weight recorded the previous morning. After all jars and bottles were emptied, the calculated weight of food and drink consumed by each ADX rat was placed into the jars and bottles of their respective sham-operated pairs (when paired for food and drink intake). Bottles and jars to be given to ADX animals were filled with more drink and food than they could eat. All initial weights were recorded. Each urine feces separator was removed, washed with warm water, dried, and returned to the original cage. If the rat excreted urine while the separator was removed, it was caught by pre-weighed paper towels placed under the cage. The weight of their urine was determined by difference. Each urine bottle was then removed and replaced under each separator with the pre-weighed clean ones containing thymol and oil. Ammonium chloride was given to each rat using a gavage needle and syringe as the rat was being held over its cage. The volume of acid given was determined by weighing the syringe plus gavage needle before and after the gavage procedure. All food jars and drinking bottles were returned to the metabolism units. The weights of the urine bottles containing thymol, paraffin oil and the 24-hour urine samples were measured and recorded. By subtracting the initial weight of each bottle from its final weight the 24-hour urine output for each rat was calculated. The balance routine was completed by approximately 12:00 hours.

c) Storage of Urine Samples

After urinary pCO_2 and pH measurements were completed (they were done promptly after the balance routine), each sample was divided into two aliquots and frozen with thymol under oil in sealed vacutainer tubes. Urine samples were taken from one tube for titratable acid and electrolyte determinations while from the other, samples were taken for ammonia concentration measurements.

E. Measurements of Net Acid Excretion Rates

a) Introduction and Definition

The net acid excretion rate is defined as the sum of ammonium and titratable acid excretion rates minus the bicarbonate excretion rates. Ammonium, titratable acid and bicarbonate excretion rates are calculated by multiplying the concentration of each in urine by the urine flow rate. The methods for determining the concentrations of each component of net acid excretion are described below.

b) Ammonia Concentration Determinations

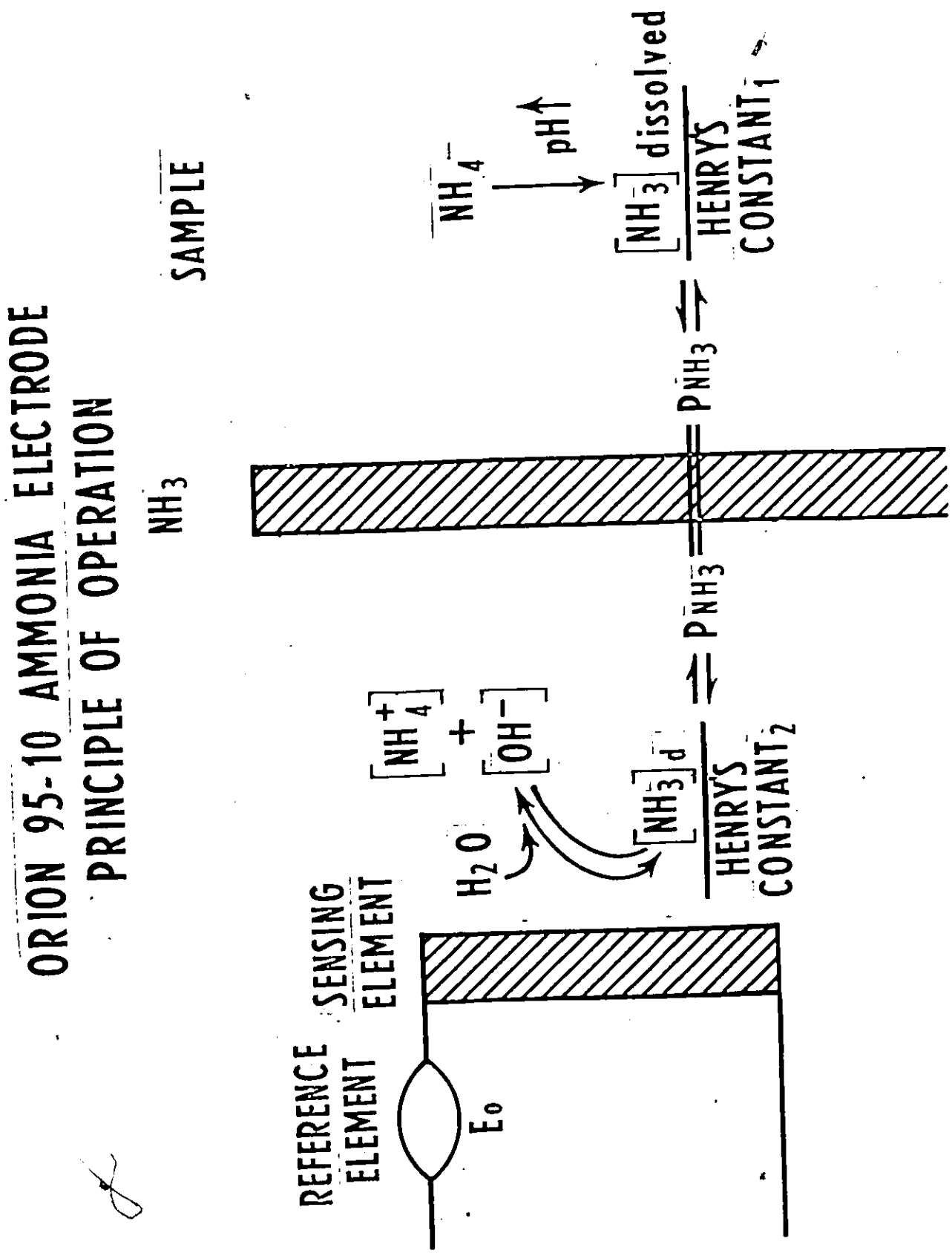
i) Chemical events in ammonia measurements (71).--The Orion 95-10 ammonia electrode was used to determine the concentration of ammonia in urine (Figure 2). The electrode consists of an internal reference element which responds to the fixed concentration of chloride in the electrode solution, a sensing element which responds to changes in the hydroxide ion concentration in the electrode solution and an ammonia permeable membrane which separates the electrode solution from the sample to be measured.

Dissolved ammonia gas, in a given sample diffuses across the ammonia membrane into the electrode solution until the partial pressure of ammonia is equal on both sides. A certain amount of gas, directly proportional to the pressure of the gas (Henry's Law) will dissolve in the electrode solution, react with water, and form ammonium and hydroxide ions. The potential of the electrode sensing element with respect to the inner reference element varies in a Nernstian manner with these changes in the hydroxide ion concentration. Since changes in the hydroxide ion concentration are proportional to the changes in the ammonia concentration, the potential of the electrode also varies in a Nernstian manner with changes in the dissolved ammonia concentration in the electrode solution. The resulting electrode potential is

Fig. 2

Orion 95-10 ammonia electrode (principle of operation).

FIGURE 2


ORION 95-10 AMMONIA ELECTRODE
PRINCIPLE OF OPERATION
SAMPLE
NH3
NH4+
pH↑
[NH3] dissolved
HENRY'S CONSTANT1
PNH3
PNH3
[NH4+] + [OH−]
H2O
[NH3]d
HENRY'S CONSTANT2
SENSING ELEMENT
REFERENCE ELEMENT
E0

described by the Nernst equation.

$$E = E'_0 - S \log [NH_3]$$

where E is the ammonia electrode potential in MV and E'_0 is determined by the internal reference element and S is the electrode slope.

ii) Electrode response: physiochemical consideration.--The ammonia electrode responds to the free base form of ammonia in a given sample. However, in acid pH urine, ammonium represents at least 99% of total ammonia (ammonia plus ammonium) in the solution. The relative amounts of ammonia and ammonium in such a solution are determined by pH, ionic strength and temperature. The relationship between pH and the ammonia forms, under varying conditions of temperature and ionic strength is described by the Henderson-Hasselbalch equation:

$$\text{Antilog } (pH - pK_1') = \frac{[NH_3]}{[NH_4^+]}$$

The value for pK' varies with changes in ionic strength and temperation and represents the equilibrium constant for ammonia, pH represents the free hydrogen ion concentration in the reaction medium while $[NH_3]$ and $[NH_4^+]$ represent the molar concentration values for ammonia and ammonium in the same medium. By substituting a urine pH of 6.00 and a pK' of 9.20 into the above equation, the relative concentrations of ammonia and ammonium can be calculated:

$$\text{Antilog } (6.0\text{-}9.2) = \frac{[NH_3]}{[NH_4^+]} = .00063$$

This means that more than 99% of the total ammonia in the sample is in the ionized form. Since the electrode only responds to the free base form of

ammonia, and the relative concentrations of ammonia and ammonium are pH dependent, the samples were alkalinized so that the free base form represented the total ammonia concentration in the samples. The addition of one milliliter of ten normal sodium hydroxide to 50 milliliters of sample increased the hydroxide concentration to approximately 200 mEq/1 which is equal to a pOH of .69 or a pH of 13.31. Then by substituting 13.31 into the Henderson-Hasselbalch equation, and solving for the ratio of $[NH_3]$ to $[NH^+_4]$, the calculation shows that more than 99% of the ammonium was converted to the free base form enabling it to be measured with the ammonia electrode.

As has been stated, the value for the equilibrium constant pK varies with temperature and ionic strength (71). Published reports indicate that by varying the temperature of a dilute ammonia solution from 5°C to 37°C the pK' values change from 9.87 to 9.22 (71). This means that after adjusting sample pH to approximately 13, such differences in temperature would increase the free base form from 99.94% of total ammonia to 99.99%. Similarly, over the range of ionic strength normally found in urine the pK' values vary from 9.05 to 8.95 (71). This means that changes in urine ionic strength per se could increase the proportion of free base in a given solution from 99.989% to 99.991%. In view of the fact that variations in temperature and ionic strength on the amount of free base in sample ions is much less than 1%, it was concluded that the concentration of free ammonia buffer after alkalinization was a good representation of the concentration of total ammonia in measured samples despite potentially large variations in the sample temperature and ionic strength.

The first event leading to an electrode response to a solution of ammonia is the diffusion of the free base form across the ammonia membrane

into the electrode solution which determines the magnitude of the change in the electrode potential. Ammonia diffusion ceases and the electrode potential stabilizes when the partial pressure of ammonia is the same on both sides of the membrane. At this point the concentration of dissolved ammonia in the electrode solution will be determined by the concentration of ammonia in the sample and the values for Henry's constant in the electrode solution and the sample being measured. Since the electrode response is used as a measure of the concentration of ammonia in a given sample, changes in the amount of ammonia dissolved for a given NH_3 gas tension resulting from variations in Henry's constant in the two compartments would represent a source of analytical error. Since Henry's constant varies with the temperature and the level of dissolved species, attention was given to identifying existing variations in these factors in my measuring system.

The electrode solution was examined first. The value for Henry's constant did not vary because its composition was not altered and it was stored and used at room temperature. In contrast however, relatively large differences in osmolality and therefore the value for Henry's constant among samples were possible. Published mean urine osmolality values for groups of rats comparable to those studies here were 374 ± 51 milliosmoles (n = 11) and 1070 ± 60 milliosmoles (n = 12) (72). To reduce these differences in osmolality, 2 milliliter aliquots of urine samples were diluted with 48 ml of distilled water. Assuming the urine osmolalities noted above were comparable to those reported here, dilution would have reduced the values to approximately 15 and 42 milliosmoles respectively. Then, by alkalinizing the samples with 1 milliliter of 10 N NaOH per 50 milliliters of diluted urine, the range of osmolalities among samples was reduced as a

percent of the mean value to 415 and 442 respectively. Accordingly, significant differences in Henry's constant among groups of rats as a result of large differences in osmolality would not have been seen. Differences in temperature among samples was not expected since all samples were diluted with distilled water, stored at room temperature and subsequent measurements were also made at room temperature.

iii) Ammonia concentration determinations.--The method of measuring the concentration of ammonia buffer in the urine with the ammonia electrode involves making a standard curve, diluting samples, measuring the electrode potential for each sample and calculating the ammonia concentration (71).

The standard curve is a graph representing the relationship between millivolts and ammonia concentration for a given electrode at the time of measurement. The curve is made by recording the electrode millivolt response to each of six samples having a known ammonia concentration. The results are plotted as MV versus log $[NH_3]$. This gives a straight line. Then, by using the millivolt response of the electrode to a sample of unknown ammonia concentration, its value can be interpolated from the calibration line.

Calibration curve samples were prepared by adding estimated amounts of NH_4Cl directly into pre-weighed 100 ml volumetric flasks and then measuring the exact amount by difference using the analytical balance. Each sample was made up to mark with .1 N $NaNO_3$ solution and sealed until measured. Each flask was emptied into 100 ml beakers placed on a magnetic stirrer. A teflon-coated stirring rod was added and the stirring rate was set at 3.5. The electrode was rinsed, blot-dried in position A, and placed into position B so that its tip was immersed in the diluted sample at a 20^{o}

Fig. 3

Ammonia concentration determination (samples and apparatus).

AMMONIA CONCENTRATION DETERMINATION (SAMPLES AND APPARATUS)

Figure 3

CALIBRATION CURVE

SAMPLES AND STANDARDS

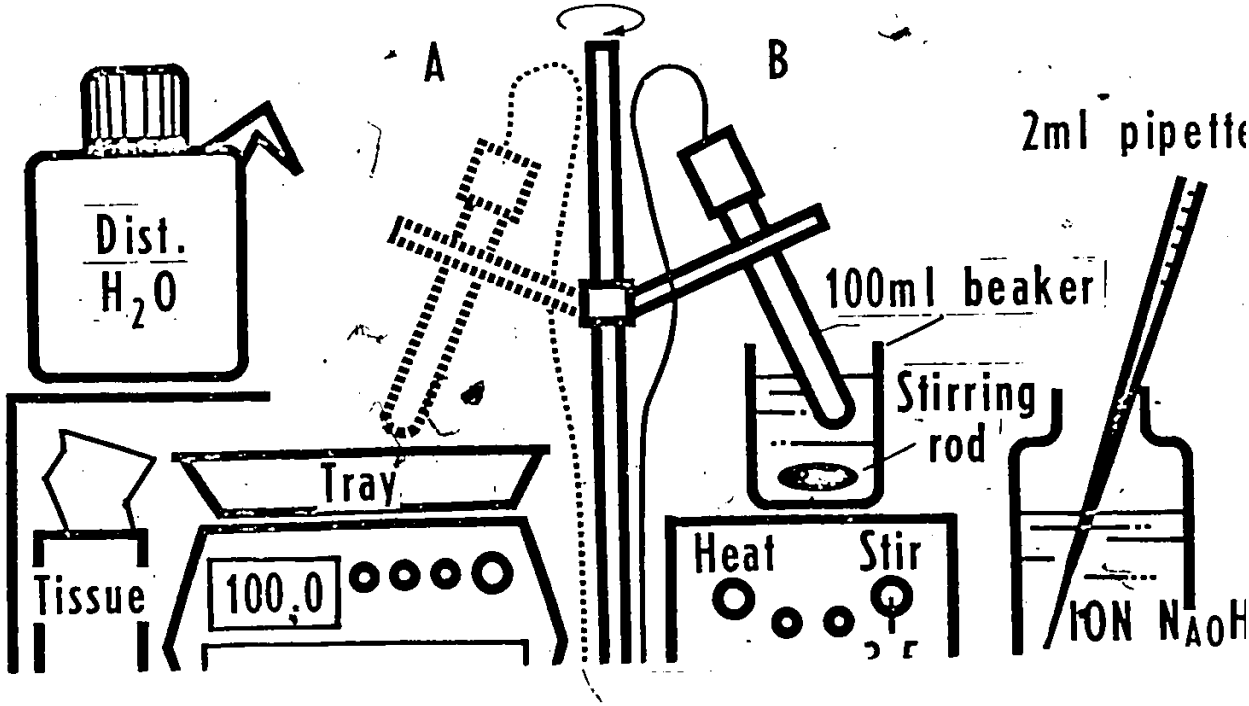
1 2 3 4 5 6

7 8 9 10 11 12 13 14 15 16 17 18

19 20 21 22 23 24 25 26 27 28 29 30

31 32 33 34 35 36 37 38 39 40 41 42 43 44

1-44 SIGNIFY ORDER OF MEASUREMENTS
1-6 SAMPLES FOR CALIBRATION CURVE
7 ,8,19,20,31,32,43,44 URINE AND NH_4Cl STANDARDS
9-18, 21-30, 33-42 SAMPLE SOLUTIONS, ALTERNATING ADX AND SHAM RAT SAMPLES



angle. While stirring, 1 milliliter 10 N NaOH per 50 milliliter sample was added and MV readings were recorded every 15 seconds for approximately 2 minutes. The electrode was then removed from the solution and replaced into position A, rinsed with distilled water and blot-dried. The stirring rod was removed, rinsed, dried and the procedure was repeated for each of the remaining solutions. Diluted urine samples from ADX and sham-operated rats were alternatively measured. After a stable electrode potential was recorded for each solution, the calibration line was drawn. To avoid errors of judgment in drawing and reading the calibration line, the equation of the regression line of least squares fit was calculated for (x, y) where x is the electrode potential in millivolts and y is the log of the corresponding ammonia concentration and m is the slope of the line, by substituting into:

$$m = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad b = \frac{\Sigma y}{n} - \frac{m\Sigma x}{n} \qquad \text{ie } b = \bar{y} - m\bar{x}$$

$$b = \frac{\Sigma y}{n} - \frac{m\Sigma x}{n}$$

$$\text{cor. coef.} \quad r^2 = \frac{\left[\Sigma xy - \frac{\Sigma x \Sigma y}{n}\right]^2}{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}$$

The calculated values m and b were substituted into $y = mx + b$ so that the NH_3 concentration in diluted samples y could be calculated by substituting the electrode potential for a given sample x into the equation.

iv) Accuracy and precision of NH_3 measurements. In order to get some indication of how close our measured numbers were to the true values of our samples, an estimate of accuracy was made by comparing carefully prepared standards for comparison. A measure of precision or reproducibility was obtained by measuring numerous samples from a given standard source both during a given measuring session and among samples measured on different days. Reproducibility was expressed as a coeficient of variation and is defined as $\frac{\sqrt{SD}}{n}$.

Table II shows data from an experiment designed to measure electrode accuracy. Ten different NH_4Cl samples were made by placing an approximated weight of NH_4Cl granules directly into pre-weighed 100 ml volumetric flasks and determining the exact weight by difference. The calculated values for

TABLE II

NH_3 Electrode: Accuracy and Precision

	Calculated Conc.	Measured Conc.	Absol. Error	Rel. Error
1. NH_4Cl sol.	(15 - 110 mEq/l)	55.4 ± 10.4	.13 ± 75	-1.5% ± 1.1
n = 10	55.3 ± 9.8			
2. Urine + NH_4Cl	15.7 ± 5.2	15.4 ± 4.8	-.3 ± .5	1.2% ± 1.5
n = 10				
3. Precision	$\bar{x}$ = 2.72 SD = .08	Coef. of Var. 3.0%		
n = 9				
Remeasure, 6 days freezing				
n = 4	$\bar{x}$ = 2.69 SD = .08	paired t = .08		

NH_4Cl concentrations after the addition of distilled water up to mark were consistent with the measured concentrations of chloride and therefore were used as the accepted values for the solution.

The ammonia concentration of each sample used was then measured with the electrode. The results showed that the values for NH_3 concentration for the 10 samples, on the average, were within 2% of the accepted value when measured with the NH_3 electrode. In order to determine the accuracy of the NH_3 measurements in diluted urine samples, NH_4Cl granules were added directly into pre-weighed 100 ml flasks and the exact weight, determined by difference, was used to calculate the concentration when made up to mark. Ten milliliters of pre-measured urine and 90 ml of distilled water were subsequently added and the final mixture was measured. The results showed that, on the average, the accuracy of the measured values were within 2% of the calculated values.

Table II shows data from experiments designed to evaluate the precision of NH_3 measurements when prepared and measured as described in Methods. The coefficient of error for nine consecutive measurements of nine samples independently prepared from a single source was 3.0%. Four samples were measured after 6 days to see if freezing per se had an effect on ammonia concentration determinations. A paired t-test revealed the ammonia concentration was not different after 6 days of freezing.

v) Balance studies: sample handling and NH_3 measurements.-- Reproducibility and the effects of long periods of freezing were the two most important issues associated with ammonia measurements for the balance studies. To address the issue of urine reproducibility, standards were interposed between sample measurements. The values for these standards served as a measure of reproducibility during each measuring session and

among sessions completed on different days. To prepare urine standards 24-hour urine collections from several rats were pooled, mixed and subsequently divided into 10 samples, and frozen in separate bottles with thymol under oil. When sample containers were removed from the freezer for analysis, one of these bottles was also removed and used to prepare the urine standards as described above. For the three groups of samples measured, three different sets of urine standards were prepared. Table III shows the mean coefficient of variation, calculated from the mean values for the urine standard for each measuring session for all days on which samples were measured by group. The average values for the coefficient of variation for Groups I, II, and III were 9.9%, 5.6%, and 4.2% respectively. The data indicate that measurements made over consecutive days were reproducible. In order to determine the effects of one to two months of freezing and storage on sample ammonia concentrations several groups of samples were remeasured after two to four months of storage. Results in table III show significant changes in measured ammonia concentration did not occur. Therefore, several months of freezing per se was not a source of significant analytical error.

The accuracy of ammonia measurements was evaluated by comparing the measured ammonia concentration with the calculated values for NH_4Cl solutions measured with Group II and Group III samples. The results show that (Table III) the average measured value was within 1.5% for Group II. It should be noted that even if the concentrations of NH_3 were inaccurate, the differences between ADX and sham rats would not change since these samples were alternately measured.

Since the calibration line represents the changes in electrode potential with varying concentrations of ammonia and is used for determining

TABLE III

Ammonia Measurements: Quality Control

		Group I 7 Days	Group II 8 Days	Group III 10 Days
I	Precision	9.9%	5.6%	4.2%
II	Accuracy	-	1.5%	6.0%
III	Standard Curves			
	Slope	57.0±3	58.4±.5	58.7±.8
	Cor. Coef.	.9997	.9996	.9991
	Drift MV	-.1±.6 MV	-.1±.4 MV	+.7±.5
IV	Three Months Freezing	n = 14		
	April mEq/l	56.49±3.16		
	July mEq/l	56.99±3.24	NS	
	Change mEq/l	-.49±1.10		
	% Change	.96%±2.01		

NS is not significantly different after six days of freezing using a pair t-test. $p < .05$

the concentration of ammonia in sample solutions, care was taken to ensure its quality and reproducibility. Both the slope of the calibration line and the correlation coefficient of NH_3 concentration vs electrode MV were used as parameters for defining the quality and reproducibility of the curve. Table III shows the correlation coefficients and slopes for the calibration lines used during measurements of samples from Groups I, II, and III were comparable and high, indicating the calibration curve per se did not limit the accuracy and precision of these measurements.

Over the course of a measuring session, the baseline value or the MV reading for a given sample tends to change. This instability is termed "electrode drift" and represents a systematic source of error. Table III shows the average electrode drift for NH_3 measurement sessions for Groups I, II, and III were $-.1 \pm .6$, $-.1 \pm .4$ and $+.7 \pm .5$ MV respectively. This data means that the electrode drift was small and comparable for all groups. The +.7 MV drift seen in Group III represents an increase in NH_3 concentration for any given sample measured at the beginning and end of a session of 3.2%.

Since urine was collected and stored with thymol under paraffin oil, their effects on the measured value for NH_3 concentration in urine samples was studied. The addition of thymol to one member of duplicate urine samples had no apparent effect on the value for the concentration of ammonia when compared to the thymol-free sample. In contrast, traces of oil on diluted urine samples interfered with the normal electrode response by reducing the slope and electrode potential response while increasing the response time required to achieve a stable reading. These effects were consistently accompanied by the appearance of black dots on the ammonia membrane. By using a syringe and needle instead of a pipette for withdrawing urine samples from under oil in storage, samples were transferred free of oil to volumetric flasks and the symptoms of electrode

malfunction did not appear.

The possibility that substances normally found in urine interfered with NH_3 measurements made with the NH_3 electrode was examined by Cunarro et al (73). They found PAH, creatinine, urea, uric acid, amino acids, proteins, NaCl, KCl and $CaCl_2$ did not affect the validity of their measurements. In contrast, however, volatile amines such as methylamine and ethylamine do interfere with NH_3 measurements (74). In view of the fact that the level of these substances in urine is several orders of magnitude below NH_3 concentrations found in urine (75), significant interference would not be expected.

c) Titratable Acid Determinations

i) Definition and principle of measurement.--Titratable acid refers to the amount of acid excreted in urine in combination with filtered buffers in excess of that which was filtered. It is generated as acid added to the urine by the kidney combines with filtered buffers. The principle used to quantitate the amount of titratable acid in urine is based on the fact that the addition of acid reduces the pH of urine in proportion to the amount of acid which was added. Therefore, the amount of alkali which must be added to the sample to restore the pH to plasma values is equal to the titratable acid. Accordingly, to quantitate the titratable acid in urine, each sample was titrated with sodium hydroxide to an end-point pH approximately equal to the pH of filtered plasma. The titratable acid was determined by calculating the number of mEq of sodium hydroxide that was consumed in the titration using the normality and volume of titrant used.

ii) Instrumentation.--The radiometer automatic titration system was used for acid titrations. The system was designed to deliver sodium hydroxide (ABU 11 Autoburette) to a sample vial (TTA 31 Titration Assembly)

at a controlled rate (TTT 60 Automatic Titrator) to an end-point of pH 7.40 (Radiometer PHM 64 Research pH meter). The volume of sodium hydroxide consumed during each titration was registered on a digital read-out on the automatic burette.

iii) The titration.--Unmodified, two milliliter urine samples, exposed to air, were titrated at room temperature with .1 NaOH to an end-point of 7.40. Urine samples were transferred from storage containers by syringe and needle to sample vessels. After the tip of the pH electrode was submerged in the sample, the titration process started automatically by the push of a button. The titrant was continuously delivered until a pre-selected pH value was reached. At this point, the titrant was added in steadily decreasing increments interrupted by increasing time intervals as the end-point was being approached. The addition of sodium hydroxide stopped when the end-point of titration was reached in the time interval after the last increment of sodium hydroxide sufficient to complete the titration to a pH of 7.40. The titrant was standardized by measuring the volume of prepared .1 N sodium hydroxide solution used for titrating 15 milliliters of .1 N oxalic acid in the presence of phenolphthaleine. The end-point of titration was indicated by the appearance of a pinkish hue to the oxalic acid solution.

iv) Effects of ionic strength and temperature.--Ideally the *in vitro* alkaline titration of urine to the pH of plasma should be the exact reverse of *in vivo* acid titration of filtered buffers. Since the method depends on restoring the pH to plasma values while assuming the pH decrease was solely the result of the renal addition of acid, factors other than the addition of acid or alkali which affect pH would represent a source of error. In view of this possibility attention was given to the effects of a difference in ionic strength and temperature between plasma and urine samples on TA

determinations.

Firstly, attention was given to the effects of ionic strength. The pH of urine decreases with the renal addition of acid and with increases in the ionic strength by an effect on the pK values. Therefore to restore urine pH to plasma pH, alkali must be added to increase the pH decreased by both the ionic strength and the renal addition of acid. The amount of alkali consumed for the former was quantitatively equal to the amount that the TA was over-estimated. However, since the ionic strength decreased urine pH by decreasing the pK while the alkali used for titrating urine increased urine pH by increasing the base to acid ratio of urine, at the end point of titration this ratio was higher than in plasma with the same pH. Therefore the values for TA of urine samples were over-titrated.

Secondly, attention was given to the effects of temperature on T.A. determinations on our method of measurement. Temperature differences were due to the fact that titratable acid was generated in the kidney at approximately 37°C but titratable acid measurements were made by titrating urine samples between two pH values at 22°C. Since the equilibrium constant for the dissociation of acids and therefore the pH is temperature dependent, the temperature effects on the value for titratable acid measured by titrating urine at a pH different from 37°C was examined. The pH of urine at 22°C was found to be approximately .12 pH units higher than at 37°C. It should be pointed out that although this represents the net effect of temperature on pK values for all acids and bases in the urine sample, for the titration of monobasic phosphate from the higher pH, it represents in practical terms, a pK change for phosphate. Therefore, by titrating urine to a pH of 7.40 at 22°C, the pK would be 6.92 when compared to 6.8 at 37°C. In effect, the result is an incomplete titration of monobasic phosphate to the equivalent pH of 7.28 at 37°C. This means that when

urine at room temperature is titrated to a pH of 7.40, the ratio is only that which would be expected at a pH of 7.28 in plasma (considering only temperature effects). As a result, without correcting for temperature, the values for titratable acid would be underestimated.

v) Titratable acid calculations.--Clearly then, errors resulting from differences in ionic strength and temperature between filtered plasma and titrated urine samples are due to changes in the pK and therefore pH which in turn represents a difference in the relationship between the pH and base to acid ratio of urinary buffers. To avoid this, the T.A. was calculated by 1) determining the concentration of dibasic to monobasic phosphate concentration in urine, 2) by calculating the dibasic to monobasic phosphate ratio in plasma calculated in 1) above using the plasma ionic strength and temperature, 3) calculating the amount of alkali which would be required to restore the ratio as calculated in 1) to that which was calculated in 2). The calculation was carried out with the assumption that although phosphate, sulphate and organic anions, chiefly creatinine, together contributed as filtered titratable acids participating in TA excretion, by treating the titratable acids as though they were all phosphate, appreciable errors were unlikely. This assumption derives support from the fact that the pK of phosphate is closest to the pH of urine in these experiments while phosphate excretion represents the largest proportion of TA acids excreted. Having said that, the relative amounts of dibasic and monobasic phosphate in samples before titration is described by the Henderson-Hasselbalch equation:

$$pH = pKa + \log \frac{D}{M} \quad (1)$$

where pH and pKa represent the pH measured at room temperature and for pKa recalculated and corrected for the ionic strength of urine. D and M are the

molar concentrations of dibasic and monobasic phosphate respectively. The principle used to quantitate the relative amounts of dibasic and monobasic phosphate samples is based on the fact that the amount of base required to change the pH of a solution is determined by the amount of acid contained in that solution. After the addition of alkali, the relative amounts of dibasic and monobasic phosphate is described by:

$$7.40 = pKa + \log \frac{D + t}{M - t} \quad (2)$$

where 7.40 and pKa are the values for pH and pKa corrected for the ionic strength in titrated urine. t is the amount of titrant (NaOH) consumed in raising the sample pH to 7.40 by titrating monobasic phosphate to dibasic phosphate. To solve for M, the monobasic phosphate concentration, equation (1) was rearranged in terms of M and substituted for D in equation (2) giving the following expression:

$$M = \frac{t(1 + \text{Antilog } [7.40 - pKa])}{\text{Antilog } (7.40 - pKa) - \text{Antilog } [pH - pKa]} \quad (3)$$

To solve for D, the calculated value for M was substituted for M in equation (1), (D = M Antilog (pH - pKa)). A sample calculation is shown in the appendix. It should be pointed out that because the temperature and ionic strength over the range of titration was approximately equal, these factors did not contribute to the amount of alkali required for the measured pH change. In summary, the above calculations gave us the values for D and M, the dibasic and monobasic phosphate concentrations in urine before titration.

The second part of the calculations was carried out to determine the ratio of dibasic to monobasic phosphate in plasma using the total amount of phosphate as D + M in urine calculated from the above determined values for each. The ratio of dibasic to monobasic phosphate in plasma was determined using the following formula:

$$\text{Ratio} = \frac{\text{D plasma}}{\text{M plasma}} = \text{Antilog } (7.40 - 6.8)$$

where 7.40 and 6.8 are the values for pH and pK_1' in plasma. D plasma and M plasma are the values for the molar concentration of dibasic and monobasic phosphate in plasma. Using the total amount of phosphate M + D, the relative amount of each in plasma, was calculated using the plasma pH and pK values. To determine the titratable acid in a given sample the amount of alkali required to titrate the D / M buffer ratio in urine to that of plasma was calculated.

Samples were titrated to a pH of 7.40 since this value was assumed to be an approximation of rat blood pH based on previous records of rat blood pH under normal conditions. In contrast however, after NH_4Cl loading I continued to titrate samples to a pH of 7.40 while keeping in mind that the actual blood pH's were probably significantly lower than this value. Nevertheless, this method did not lead to misleading results since the blood pH's of ADX rats after NH_4Cl loading were significantly lower than sham rats. Therefore by titrating to a blood pH of 7.40, the values for titratable acid for the ADX rats was overestimated and represented an error which worked against the thesis that ADX rats have a reduced capacity for net acid excretion.

vi) The accuracy and precision of titration.--The accuracy and precision of data obtained with the automatic titration system was evaluated by studying the titration results for standard phosphate solutions measured among samples. The results are shown in Table IV. Standards were prepared with .025 moles of Na_2HPO_4 and .025 moles of KH_2PO_4 made up to 1 liter with distilled water. The pH of standards was approximately 6.87, a value in agreement with the pK value corrected for ionic strength and temperature. End-point titration to a pH of 7.40 of 2 milliliter aliquots of standard

TABLE IV

T.A. Measurements: Accuracy, Precision and Quality Control

	n = 11	n = 12	n = 19
Measured $\bar{X}$ Moles/l	.0280 ± .0004	.0286 ± .0001	.0287 ± .0003
Calculated Moles/l	.0276	.0280	.0280
Relative error	+1.2% ± .4	±2.7% ± .4	+2.4% ± .3
Coef. of error	1.4%	1.4%	1.1%
pK' or pH before titration	6.87 ± .01	6.86 ± .02	6.86 ± .01
Calculated pK	6.88	6.88	6.88
Change in pK after titration	-.01	-.01	-.01

solutions consumed an average of .0284 milliequivalents of base. This value was in agreement with the calculated value of .0280 using the measured pK in each group corrected for changes in ionic strength and volume resulting from the addition of the titrant. The results show, the measured values were within 3% of the calculated values. The coefficient of error for these standards measured among samples was 1.4% indicating a high degree of reproducibility for the measurements.

d) Bicarbonate Concentration Determinations

The concentration of bicarbonate in urine was calculated using the Henderson-Hasselbalch equation with substituted values for urine pH and pCO_2.

$$\left[HCO_3^-\right] = \alpha\ pCO_2 \quad \text{Antilog } (pH - pK_1)$$

where $\left[HCO_3^-\right]$ expresses the millimolar concentration of HCO_3^-, pCO_2 expresses the millimolar concentration of dissolved carbon dioxide plus carbonic acid, α used for urine has a value of .0309. pK_1 expressed the equilibrium constant corrected for ionic strength (76).

The pH of urine was measured with the radiometer pH electrode gun and the PHM 72 amplifier. Two-point calibration of the pH electrode was made with radiometer standard buffers with pH values of 7.383±.005 and 6.841±.009 at 37°C. Buffers were frequently remeasured between successive measurements and experimental and control group samples were alternated for measurement.

The pCO_2 of urine was measured with a pCO_2 electrode and BEU blood gas unit connected to a second PHM 72 amplifier. Two point calibration of the electrode was made with 3% and 8% CO_2 gas mixed with N_2. Frequent gas checks were made and samples from experimental and control groups were alternately measured. Reconstituted plasma with known values for CO_2 tension

were measured between successive samples. Measured values were always within 2 mm of the standard value.

Bicarbonate concentration in plasma was determined as described for urine. However, a pK_1 of 6.1 was used for all calculations and for plasma is .0301.

e) Net Acid Excretion Rate Values and Urine Samples Infected with NH_3 Producing Bacteria

It has long been recognized that the determination of urinary NH_3 excretion rates using urine collections infected with NH_3 producing bacteria give falsely high values (77, 78). However, the effects of infection on net acid excretion values were unknown until Mookerjee et al undertook to investigate this problem under controlled, in vitro conditions (77). Their results showed that because the increase in NH_3 concentration was equalled mole by mole with a reduction in titratable acid, bacterial infection did not preclude the accurate determination of net acid excretion rates. Notwithstanding the foregoing, the bacterial infection of urine samples used for net acid determination in these experiments did constitute a major source of error not only for NH_3 excretion rates but also for net acid excretion rates. This is due to the fact that NH_3 and TA and HCO_3^- concentration determinations were done with aliquots of urine collected or stored in 3 different containers at different times. As a result, in the event one or both of the samples was contaminated, it was unlikely that an increase in the NH_3 content of one sample was associated with the mole by mole decrease in TA in the other container. The difference between the increase in NH_3 and decrease in TA represented a source of error for the net acid excretion rate determinations. To avoid these errors, a set of criteria used to identify infected urine was formulated and urine samples described by these criteria were selected by two individuals, independently and excluded from the data. Without corroboration, both persons chose the

same values. The criteria and rationale for each is described below.

Contaminated urine was associated with increased NH_3 concentration, decreased TA concentration and increased pH and pCO_2 values. These parameters were each measured using urine from one of 3 different test tubes, A, B, or C, respectively, stored for different periods of time before analysis. Consequently, all, some or none of the values could have been falsely altered depending on which of the test tubes grew ammonia producing, urea metabolizing bacteria. Therefore an indication of test tube A infection would be a grossly deviant NH_3 concentration without any concomitant changes in food intake or the addition of NH_4Cl loading. An increase in pH and decrease in TA concentration were used as evidence for bacterial contamination in test tube B. Finally, markedly elevated pH and pCO_2 in test tube C were used to identify infected samples. The table on the follow page shows which of the 3 test tubes used for net acid excretion determination was or were contaminated. In Group I from ADX and sham rat data 7.7% and 6.2% of the values were excluded while in Group II 19.0% and 7.1% of the values respectively were not included in the analysis. In Group III 3.3% of the numbers were excluded.

CRITERIA FOR INFECTED URINE

Test tube A. for NH_3 concentration	Test tube B. for TA concentration	Test tube C. for HCO_3^- concentration	Theoretically contaminated test tubes used for net acid determination
No evidence of Contamination	No evidence of Contamination	No evidence of Contamination	A.B.C.
		↑pH ↑pCO_2*	A.B.C.*
	↓TA ↑pH	No evidence of Contamination	A.B.*C.
		↑pH ↑pCO_2*	A.B.*C.*
NH_4	No evidence of Contamination	No evidence of Contamination	A.*B.C.
		↑pH ↑pCO_2*	A.*B.C.*
	↓TA ↑pH	No evidence of Contamination	A.*B.*C.
		↑pH ↑pCO_2*	A.*B.*C.*

* Infected, Possible Combinations;
If evidence for contamination was present in any of the criteria, as described above, the value was excluded.

F. Sodium and Potassium Concentration Measurements

a) Flame Photometry: Principle of Operation

The concentration of sodium and potassium in plasma and urine samples and tissue preparations were measured with the IL 343 digital flame photometer. The principle of operation is based on the fact that atoms of a given element in their excited state are unstable and on returning to the ground state emit light of a characteristic wave length. Since the intensity of this light is directly proportional to the number of atoms undergoing transition, the concentration of atoms of a given element can be determined by measuring the intensity of light emitted with a wavelength characteristic of that element. Therefore, the flame photometer consists of a chamber designed to atomize a given sample diluted 1 + 200 with 15 mEq/l lithium chloride solution by mixing it with air and propane fuel. Subsequently, it is forced into a flame where the combustion of propane provides enough energy to excite alkali atoms. The light emitted from atoms returning to their ground state pass through interference filters onto 3 photodetectors each permitting the passage of a specific wavelength of light (in this case 589 nM for sodium, 766 nM for potassium and 671 nM for lithium). Behind each photodetector is a phototube which responds to varying intensities of light by transducing the light into electrical current directly proportional to the intensity of light, striking it. The photometer was calibrated with standard solutions containing 140 mEq/l sodium and 5.0 mEq/l of potassium.

b) Urine and Plasma Sample Preparation

Ten microliters of urine or plasma to be analyzed were diluted with two milliliters of 15 mEq/l lithium chloride. Lithium chloride was used as a diluent in order to eliminate that portion of the instruments'

output which would have reflected emission signal noise caused by changes in flame temperature, sample aspiration rate or various chemical interferences. The rationale for its use follows. The emission signals for sodium, potassium and lithium vary with the concentration of their respective atoms and with emission signal noise. The concentration and noise factors are reflected in the emission signal for sodium and potassium while only the noise factor is reflected in signal variations for lithium since it is added to all samples at the same concentration. Therefore, by expressing the concentration of sodium and potassium and a ratio of their respective output signals to the lithium output signal the position of the emission caused by noise will not be reported. In this manner stable and accurate read-outs were obtained.

c) Muscle Sample Preparations

Frozen muscle samples were prepared for potassium content analysis using the following method: Tissue samples were transferred from storage vials to preweighed grinding tubes and reweighed. Samples then were ground to a paste, dried for 20 hours at 110°C, and cooled in a desiccator. The dried sample and grinding tube were then reweighed. Petroleum ether was added to the tubes and samples were ground to a fine slurry. After 24 hours, they were centrifuged at 20,000 rpm for six minutes and the supernatant was discarded. The procedure was repeated and the resulting fat-free, dried sample was reweighed. Samples were then mixed with HNO_3 and left to stand for 24 hours. The tissue digest was then transferred to a 10 milliliter volumetric flask and made up to mark with HNO_3. A ten-microliter aliquot of this solution was used for potassium determination as described for plasma and urine above. The results were expressed as mEq of potassium per 100 grams of fat-free, dry tissue solid. A sample calculation follows:

weight of grinding tube = 46.647 grams

weight of grinding tube and
weight fat-free, dry solid = 46.944 grams

weight of fat-free, dry solid = .297 grams

photometer reading = 11.46 mEq/liter = .12 mEq in 10 ml

Therefore .12 mEq of potassium were contained in .297 grams of fat-free, dried muscle, which equals 38.58 mEq of K^+/100 gm.

G. Steroid Dosages, Preparations and Measurements

a) Steroid Dosages

Steroid dosages were chosen so that the amounts of steroid given were both physiological and effective for maintaining the acid-base balance in adrenalectomized rats after NH_4Cl loading.

A physiologic dose for aldosterone was chosen after considering dosages reported to be effective for acutely decreasing the urinary Na^+/K^+ ratio in ADX rats (79, 80, 81) and endogenous secretion rates (81, 82). In pilot studies where the plasma bicarbonate concentration and blood pH values of ADX and steroid-replaced ADX rats were compared after NH_4Cl loading, 2 mg aldosterone B.I.D. exerted a significant protective effect. This dose is the approximate daily equivalent for the dosages adequate for decreasing the sodium to potassium ratio in ADX rats, while its lower than the aldosterone equivalent for replacement DOCA dosages used by other investigators (11,35).

A physiologic dose of dexamethasone was calculated after considering endogenous secretion rates for corticosterone (82, 83) and selecting a low normal value (83). The cortisone equivalent based on liver glycogen deposition tests was calculated using .5 as the relative potency (84). The

dexamethasone equivalent was calculated using the cortisone equivalent for corticosterone secretion rates and a dexamethasone to cortisol relative potency of 265 (85, 86) giving the dexamethasone equivalent of approximately 2.0 micrograms per day. Evidence for a glucocorticoid effect was taken from micropuncture studies on adrenalectomized rats receiving 2 mg of dexamethasone per day prior to the studies. Animals maintained on this dose of dexamethasone were able to maintain their blood pressures above 100 mm more consistently than ADX rats receiving no hormone. Dosages above two micrograms per day were avoided since they tended to mask the effects of aldosterone infusions on urinary sodium to potassium ratios in ADX rats.

b) Steroid Preparation for Injection

Five milligrams of aldosterone or dexamethasone was weighed out in a plastic flame cup using the analytical balance. The steroid was dissolved in alcohol and transformed to a 5 milliliter volumetric flask. The steroid solution was made up to mark with alcohol washings from the flame cup and five hundred microliters of this solution was pipetted into a vial containing ten milliliters of sesame oil. It was then gently heated until the alcohol was evaporated, leaving a steroid oil preparation containing 1 microgram of hormone per 20 microliters. Each vial was stoppered, labelled and stored at five degrees celsius until one half hour before use.

c) Radioimmunoassay for Aldosterone

i) Introduction and principle of measurement.--The concentration of aldosterone in rat plasma was determined using radioimmunoassay. The technique is based on the fact that the binding of aldosterone molecules to their antibodies is competitive. Therefore, by determining the relationship between labelled aldosterone bound in a given system and the amounts of

unlabelled aldosterone added one can determine the unknown by measuring the labelled aldosterone bound and interpolating the standard curve. The details of the radioimmunoassay method used for aldosterone measurements in these studies was described by T. Ito (87).

ii) Sample preparation.--Approximately 2.0 milliliters of rat plasma was used for aldosterone assays in these experiments. Four thousand counts per minute (cpm) of tritiated aldosterone in alcohol was added to each sample for recovery determinations. The aldosterone was then extracted from these samples twice with 8.0 milliliters of methylene chloride methanol 98:2. The extraction phase was separated by centrifugation and dried under nitrogen in a water bath kept at 45°C. The solute was then redissolved in .2 milliliters of methylene chloride for application to the top of the sephadex LH-20 column chromatograph for purification. The sample was rinsed twice with .2 milliliter portions of the solvent. Eletion patterns determined prior to measurements determined the volume of eluate containing most aldosterone. After collecting this volume it was dried over a water bath at approximately 45°C. The sediment was redissolved in 1.0 milliliters of alcohol. A .2 milliliter aliquot was added directly to 10 milliliters of scintillation fluid and counted in order to determine the recovery rate for the labelled aldosterone added to the plasma before purification. One fifth milliliter aliquots were taken in duplicate from the remaining sample and dried under nitrogen at 45°C. These samples were then redissolved in .1 milliliters of .1 M phosphate buffer. To this solution was added the assay system.

iii) The standard curve.--The points for the standard curve was determined by adding the assay system to vials containing 0, 10, 25, 50, 100, and 200 pg/ml of unlabelled aldosterone. The bound fraction was separated with charcoal as described above and transferred to scintillation vials

containing 10 milliliters of acquasol for counting. Total counts of the assay system were measured by adding the whole system directly into the scintillation fluid without charcoal treatment. Blanks were included in the counting procedure for background determinations. The cpm values for blanks were subtracted from each sample reading and duplicates were averaged. Typical values for the calibration curve varied between 8,000 and 3,000 c.p.m. for 50% to 14% labelled aldosterone bound corresponding to the addition of 0 to 200 pg/nl unlabelled aldosterone to the assay system.

iv) Specificity, sensitivity, and precision.--Radioimmunoassay for rat plasma aldosterone was used to corroborate the completeness of ADX and to detect differences in the concentration of aldosterone between two groups of rats treated differently. In order for the conclusions based on the results to be valid, firstly the method must specifically measure aldosterone, secondly the method must be sensitive enough so that if no aldosterone is detected, it can be said none was there and thirdly, the method must be reproducible and in itself not add variation to data thereby reducing the significance of differences between sample means. To determine to what extent these criteria are satisfied, attention was given to the specificity, sensitivity, and precision of the methods as determined by the in vitro laboratory at the Ottawa General Hospital.

Selectivity in radioimmunoassay depends on the selectivity of the Ab used and the purity of the sample measured. Cross-reactivity for the antiserum is used as a measure of selectivity, and was reported by N.I.H. to be less than 1% for other steroids as compared to aldosterone. To increase the purity of samples measured, aldosterone was extracted from plasma samples with the sephadex LH-20 column chromatograph. Preliminary studies showed aldosterone was included in a separate fraction, which

effectively reduced the presence of potentially interfering steroids to insignificant levels.

Recovery experiments with small amounts of aldosterone have shown the method is sensitive enough to detect 5 pg/ml in given samples. Since the normal range of aldosterone in rat plasma ranges from 80 to 400 pg/ml depending on normal dietary variables, a reading of 0 with the method would support the successful removal of the adrenal glands.

Repeated measurements of samples from a common source showed a coefficient of variation ranging from 5.6 to 15%.

H. Urinary Organic Anion Concentration Determinations

Urinary organic anion concentrations were measured by the Chan method (88). This first required the precipitation of phosphate and carbonate ions with calcium hydroxide. Following centrifugation, 2 ml aliquots were titrated to a pH of 2.7 with hydrochloric acid. Subsequently, each sample was retitrated to a pH of 7.4 with sodium hydroxide and the concentration of organic anions in mEq/L was calculated using the following formula: $\frac{\text{ml of NaOH used}}{\text{2 ml sample}}$ X normality of NaOH X 1000. Twenty-four hour excretion rates were calculated by multiplying the concentration of organic anions in mEq/L times the twenty-four hour urinary flow rates.

I. Balance Study Calculations & Statistics: For each rat, the mean daily rate of net acid excretion in the control period was calculated and compared with its pair treated littermate using a paired t-test. For non-paired comparisions the mean rate of net acid excretion for each group was used for unpaired t-test analysis. Analogous calculations for other parameters were made. During the acid loading period the change from the mean control period values for each rat for each day was calculated. Days 4 and 5 were averaged and used

for comparisons. Other parameters were not expressed as a change from control period unless indicated.

Throughout this thesis, all numbers are expressed as the mean ± standard error of the mean and levels for significance are at least $p < .05$ unless otherwise indicated.

CHAPTER III

Results

It should be recalled that the purpose of these studies was to determine whether or not a normal capacity to maintain acid-base balance and excrete net acid in the rat is dependent on the presence of adrenal steroids. Accordingly, the effects of ADX on acid-base balance and net acid excretion as compared with sham rats was examined. However, since ADX is associated with the retention of potassium, an element which itself has been shown to impair urinary acidification, effects of ADX in the absence of potassium retention was examined. The results are presented in two sections documenting the effects of ADX on a) systemic acid-base balance, b) net acid excretion.

a) Results of Pilot Studies

i) ADX rats maintained on .9% saline and Purina Rat Chow.--To study the effects of adrenal insufficiency on the acid-base balance of rats, a group of rats was adrenalectomized and after a period of 13 days, their acid-base status was evaluated and compared with that of sham-operated rats. Table V shows the acid-base and potassium data for 16 ADX and 16 sham-operated rats after 13 days of .9% saline and Purina Rat Chow containing 1% potassium by weight. ADX rats maintained normal plasma HCO_3^- concentrations and blood pH (24.7 ± .4, mEq/l, 7.41 ± .01). These values were not significantly different from those seen in sham-operated rats (25.9 ± .4, mEq/l, 7.40 ± .01). No significant differences in muscle potassium content (53.3 ± .4 vs 53.6 ± .4, mEq/100 grams of fat-free dry solids) were detected, while plasma potassium concentrations were significantly elevated in ADX rats

TABLE V

Pilot Study: ADX Rats Maintained on .9% Saline and Purina Chow

	ADX n = 16 $\bar{X}$	±SE	Sham n = 16 $\bar{X}$	±SE	
1. Acid-base Status					
Plasma HCO_3^- mEq/l	24.7	.4	25.9	.4	NS
pCO_2 mm of Hg	40.0	2.2	42.6	1.1	NS
pH	7.41	.01	7.40	.01	NS
2. Potassium Status					
Plasma K^+ mEq/l	4.4	.4	3.9	.1*	
†Muscle K^+	53.3	.4	53.6	.4	NS

† mEq/100 gm fat-free dry solid.

* $p<0.05$ when compared with ADX animals

NS = not significant at .05 level

(4.4 ± .4 vs 3.9 ± .1, mEq/l).

ii) Potassium-depleted ADX and sham rats given NH_4Cl containing diet ad libitum.--Since no acid-base disturbance was demonstrated in ADX rats maintained on .9% saline, it was of interest to see if ADX rats were able to maintain their acid-base balance after ingesting larger amounts of acid. In view of the fact ADX rats had significantly higher plasma potassium concentrations than sham-operated rats, all animals were depleted of potassium prior to the addition of NH_4Cl to their diets in order to avoid supranormal potassium stores in the ADX rats.

Table VI shows acid-base and potassium data for 17 ADX and 20 sham-operated rats after 7 days of a diet containing no potassium and 3 days of same diet but for the addition of 10% NH_4Cl. ADX and sham-operated rats had metabolic acidosis (7.23 ± .03, 16.7 ± 1.0, mEq/l vs 7.25 ± .03, 15.4 ± 1.0, mEq/l). These values were not significantly different. The pCO_2 was significantly higher in the ADX group (40.8 ± 2.3 vs 35.4 ± 1.2, mm of Hg). Further, muscle K^+ content was significantly higher in the ADX group (42.8 ± 5.5 vs 38.6 ± 2.6 mEq/100 grams fat-free dry solids).

iii) Potassium-depleted ADX and sham rats paired for NH_4Cl, food and drink intake.--While ADX rats do not become more acidotic than sham-operated rats when their diets are supplemented with NH_4Cl, the possibility remains that the lack of differences may be related to differences in NH_4Cl intake. To control NH_4Cl intake, potassium-depleted ADX and sham rats were gavaged with measured and equal amounts of NH_4Cl.

Table VII shows the acid-base and plasma potassium concentration of 3 ADX and 3 sham-operated rats paired for NH_4Cl, food and drink intake. The results show, in contrast to the NH_4Cl ad libitum protocol, ADX rats developed metabolic acidosis while sham-operated rats maintained a normal acid-base balance. Plasma HCO_3^- concentration and pH were significantly lower in ADX rats when compared with sham-operated rats (7.22 ± .08, 13.9 ± .9, mEq/l vs 7.40 ± .01, 23.8 ± 1.5, mEq/l). In both groups of animals

TABLE VI

Pilot Study: Potassium-Depleted ADX and Sham rats given NH_4Cl containing diet ad libitum

	ADX n = 17 $\bar{X}$	± SE	Sham n = 20 $\bar{X}$	± SE	
1. Acid-base Status					
Plasma HCO_3^- mEq/l	16.7	1.0	15.4	1.0	NS
pCO_2 mm of Hg	40.8	2.3	25.4	1.2	*
pH	7.23	.03	7.25	.03	NS
2. Potassium Status					
Plasma K^+ mEq/l	3.4	.2	2.3	.1	*
†Muscle K^+	42.8	5.5	38.6	2.6	*

† mEq/100 gm fat-free dry solids.

$p < 0.05$ when compared with ADX animals

NS = not significant at .05 level

TABLE VII

Pilot Study: Potassium-Depleted ADX Rats and Sham Rats Paired for NH_4Cl, Food and Drink Intake

	ADX ± SE	Sham ± SE
Plasma HCO_3^- pr #1	12.2	22.5
mEq/l pr #2	15.1	22.1
pr #3	14.4	26.8
	13.9 ± .9	23.8 ± 1.5*
Blood pH	7.22 ± .08	7.40 ± .01*
Plasma K^+	2.7 ± .4	1.8 ± .3*
mEq/l		

* $p < 0.05$ when compared with ADX animals

plasma potassium concentrations were in a potassium-depleted range although the values for ADX animals were significantly greater ($2.7 \pm .4$ vs $1.8 \pm .3$ mEq/1).

b) Results of Experimental Protocols

Figure 4 shows daily net acid excretion during control and acid administration periods in rats from four representative protocols. It can be seen that during the control period, acid excretion is reasonably constant over the eight days of observation. Although there were differences between the various groups (see below), a major increase in acid excretion was observed in each animal in response to acid loading.

i) Control period.--Table VIII and Figures 5 and 6 summarize the balance data for Groups IA, IB, IIA and IIB. Results obtained at the end of the control period on acid-base status, tissue and plasma potassium stores, and plasma aldosterone levels are given in Table IX. Rats subjected to potassium restriction invariably showed potassium depletion nephropathy. It is noteworthy that, despite comparable muscle potassium stores and the presence of potassium depletion nephropathy, ADX rats had higher plasma potassium concentrations than sham animals (Group IVA vs Group IVB, 2.6 ± 0.1 vs 2.1 ± 0.1 mEq/1, $p < .05$). However, all parameters of potassium stores were significantly lower in potassium-depleted ADX rats when compared with pair-fed sham rats receiving food with normal potassium content (Group IVA vs Group IVC, Table X, and Group IIA vs Group IIB, Table VIII).

The main finding is that net acid excretion is significantly less in potassium-restricted adrenalectomized rats when compared with either pair-fed controls, subjected to potassium restricted diets (Table VIII, Group IA vs Group IB) or pair-fed controls allowed access to normal dietary potassium (Table VIII, Group IIA vs Group IIB). In both pair-fed groups, no differences were observed in the amount of food eaten

Fig. 4

Rates of net acid exretion during control and acid loading periods: Groups IA-ADX; IB-Sham; IIB-Sham; III-ADX. Daily rates of net acid excretion are shown over the control and acid loading periods for Groups IA-ADX; —— IB-Sham;- - - -IIB-Sham;····· III-ADX.—·—·—·—·

N.B.1 In contrast to the method used for calculating data presented in table VIII (see methods section I), for this figure, rates of net acid excretion for all rats in each group for each day were averaged to get the points shown.

N.B.2 Post surgery all animals were given free access to saline and food for a 2-3 day recovery period before 24 hour urine collections were started.

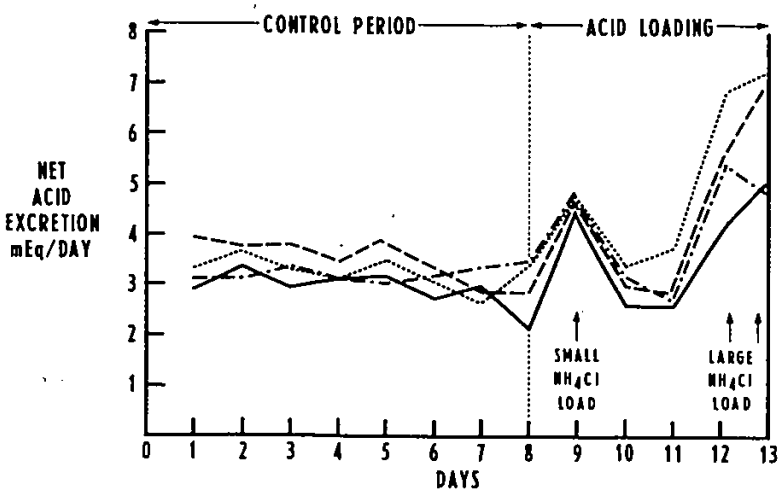

CONTROL PERIOD
ACID LOADING
NET ACID EXCRETION mEq/DAY
8
7
6
5
4
3
2
1
SMALL NH4Cl LOAD
LARGE NH4Cl LOAD
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DAYS

TABLE VIII

BALANCE DATA FOR PAIR-FED ADRENALECTOMIZED AND CONTROL RATS DURING THE EIGHT-DAY CONTROL PERIOD

	Group IA ADX (Low K^+ Diet)	Group IB Sham (Low K^+ Diet)	Group IIA ADX (Low K^+ Diet)	Group IIB Sham (Normal K^+ Diet)
	N = 5	N = 5	N = 6	N = 6
NH_4^+ exc. mEq/day	2.74±0.16	3.16±0.19*	2.61±0.11	2.05±0.10‡
T.A. exc. mEq/day	0.25±0.01	0.27±0.02	0.32±0.03	0.89±0.05‡
HCO_3^- exc. mEq/day	0.25±0.04	0.28±0.02	0.27±0.02	0.02±0.00‡
Net acid exc. mEq/day	2.74±0.13	3.15±0.19*	2.66±0.13	2.92±0.14‡
Daily K^+ bal. mEq/day	-0.07±0.01	-0.04±0.00*	-0.05±0.00	.20±0.03‡
K^+ excretion mEq/day	0.07±0.01	0.06±0.00	0.05±0.00	3.21±0.14‡
Cum. K^+ bal. mEq/8 days	-0.62±0.04	-0.33±0.03*	-0.41±0.02	1.62±0.28‡
Urine pH	6.74±0.07	6.62±0.02	6.64±0.03	5.71±0.04‡
Urine flow ml/day	50.0±5.2	48.7±4.9	49.8±5.8	18.2±1.4‡
Na^+ exc. mEq/day	7.84±0.78	7.60±0.68	7.51±0.80	5.79±0.53

* $p<0.05$ when compared with Group IA

‡ $p<0.05$ when compared with Group IIA

Fig. 5

Rates of net acid excretion and cumulative potassium balance over 8 day control period: Groups IA-ADX; IB-Sham. Rates of net acid excretion and cumulative potassium balance over the eight-day control period for Groups IA-ADX; "O" vs IB-Sham; "●" are shown. Lines connect pair-treated animals.

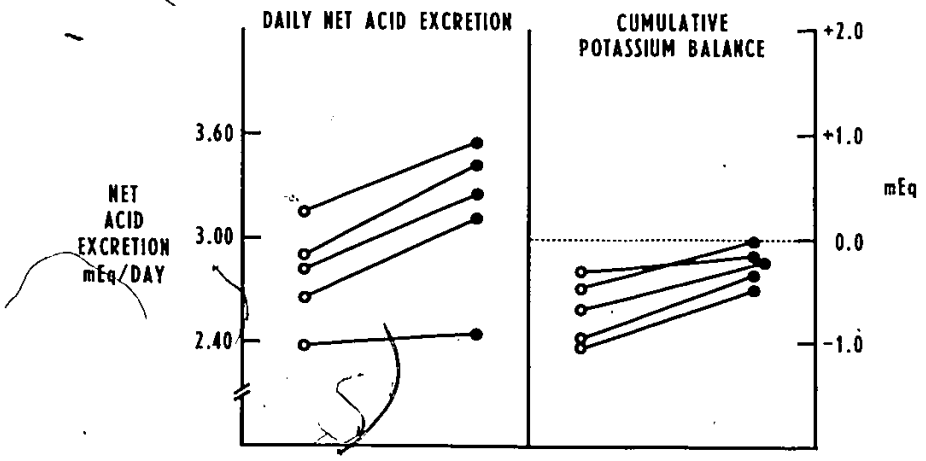
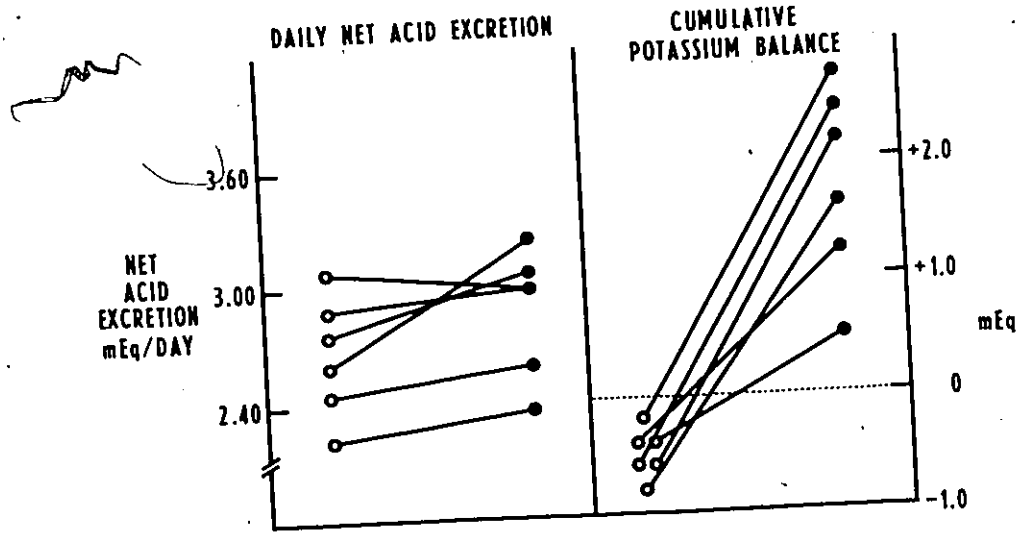

DAILY NET ACID EXCRETION
CUMULATIVE POTASSIUM BALANCE
NET ACID EXCRETION mEq/DAY
3.60
3.00
2.40
+2.0
+1.0
0.0
-1.0
mEq

Fig. 6

Rates of net acid excretion and cumulative potassium over the 8 day control period: Groups IIA-ADX; IIB-Sham. Rates of net acid excretion and cumulative potassium balance over the eight-day control period for Groups IIA-ADX; "o" vs IIB-Sham; "●" are shown. Lines connect pair-treated animals.


DAILY NET ACID EXCRETION
CUMULATIVE POTASSIUM BALANCE
NET ACID EXCRETION mEq/DAY
3.60
3.00
2.40
+2.0
+1.0
0
-1.0
mEq

or changes in body weight. Heigher rates of organic anion excretion were also not seen in the ADX animals (.81 ± .04 mEq/day) when compared with potassium restricted sham animals (.87 ± .05 mEq/day) or the sham group receiving the normal potassium diet (.72 ± .05 mEq/day). As shown in Table VIII, when Groups IA and IB are compared, urine flow rate, sodium excretion rates and urine pH were also constant. It is noteworthy that the sham-operated rats with access to potassium still excreted more net acid than their pair-fed adrenalectomized partners despite the significant reduction in urinary ammonium excretion (Table VIII), and lower urine flow rates.

As noted in Methods, it was of interest to determine whether in adrenalectomized rats dietary potassium restriction and associated higher urine pH could account for an impairment in net acid excretion independent of the effects of adrenalectomy.

For this reason, five adrenalectomized rats (Group III) were studied over the control period but with access to normal dietary potassium. They were then compared with the potassium-restricted rats of Group IA. Urine pH was significantly lower, urine flow was greater, and both the sodium excretion rate and food intake were higher. Despite these differences which tend to favour a lower rate of net acid excretion in the potassium-restricted animals, no significant reduction in net acid excretion was noted. When compared with Group IA animals, Group III rats had expectedly higher rates of potassium excretion higher cumulative potassium balances and higher daily K^+ balances.

	GpIII ADX N diet	GpI A ADX no K diet	
Urine pH	5.95 ± .05	6.74 ± 0.07	$p < .00$
Urine flow ml/day	71.4 ± 2.0	50.0 ± 5.2	$p < .01$
Na exc. mEq/day	11.18 ± 0.29	7.84 ± 0.78	$p < .01$
Food intake gm/day	25.5 ± 0.5	22.6 ± 1.1	$p < .05$
Net acid mEq/day	2.74 ± 0.13	2.91 ± 0.08	$p < 0.05$
K^+ exc. mEq/day	3.51 ± 1.0	.07 ± .01	$p < 0.001$
Cumulative K^+ bal.	+7.97 ± .53 mEq/13days	-.62 ± .04	mEq/8days
Mean daily K^+ bal.	mEq/day +.60 ± 0.07	-.07 ± 0.01	$p < 0.001$

ii) Response to acid loading.--Table IX and X show various results obtained before and after acid loading. In addition to the documentation of the adrenalectomy state, it is clear that only sham-operated animals fed a normal potassium diet, did not develop significant metabolic acidosis

TABLE IX

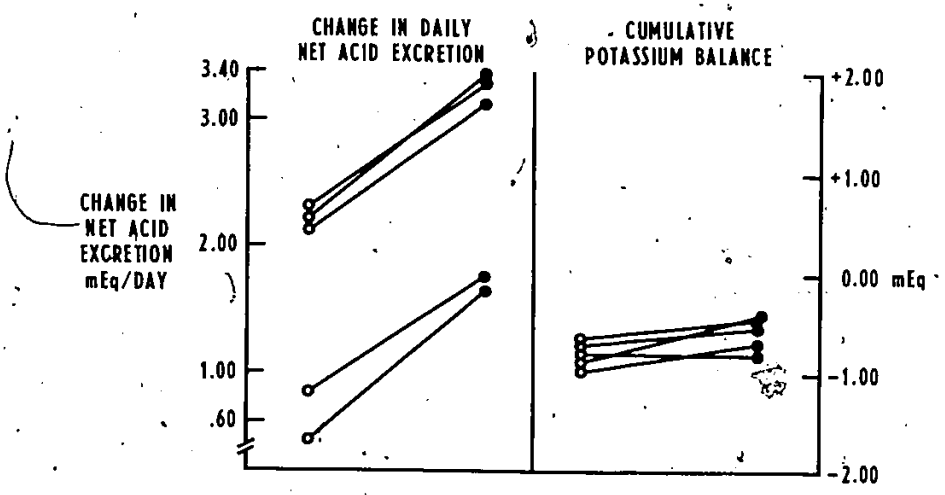
DELTA NET ACID EXCRETION AND FACTORS AFFECTING ACID EXCRETION FOR GROUPS IA AND IB DURING NH_4Cl LOADING

	Group IA ADX ($Low K^+$ Diet) N = 5	Group IB Sham ($Low K^+$ Diet) N = 5
NH_4^+ exc. mEq/day	+1.18±.45	+2.24±.41*
T.A. exc. mEq/day	+.01±.07	+.17±.04
HCO_3^- exc. mEq/day	−.21±.04	−.23±.03
Net acid exc. mEq/day	+1.59±.39	+2.64±.39*
K^+ exc. mEq/day	.08±.01	.12±.02
Cumulative K^+ Bal. mEq to end of study	−.86±.06	−.60±.06*
Urine pH	6.02±.11	6.03±.07
Urine flow ml/day	32.2±14.4	29.8±8.9
Plasma K^+ mEq/l	3.6±.4	2.2±.2*
Muscle K^+ content mEq/100 gm FFD wt.	42.3±0.8	40.4±0.5
K^+ depletion nephropathy	present	present
Food intake gm/day	8.0±1.5	8.0±1.5
Na^+ exc. mEq/day	4.82±1.17	4.29±1.25*
Aldosterone pg/ml	0	166 ± 55*

*$p < 0.5$ when compared with Group IA, no asterisk means comparisons are not significant.

Fig. 7

Change in net acid excretion and cumulative potassium balance over the two-day large acid regimen for Groups IA-ADX; "○" vs IB-Sham; "●" are shown. Lines connect pair-treated animals.


CHANGE IN DAILY
NET ACID EXCRETION
CUMULATIVE
POTASSIUM BALANCE
CHANGE IN
NET ACID
EXCRETION
mEq/DAY
3.40
3.00
2.00
1.00
.60
+2.00
+1.00
0.00 mEq
-1.00
-2.00

after gavage with NH_4Cl.

Thus, the enhanced net acid excretion (described below) was concomitant with the generation of metabolic acidosis in the three other groups of rats.

In response to the small acid load, no differences among groups were noted.

As seen in Figure 7, Groups 1A and 1B, rats increased their net acid excretion rates in response to the large acid load 10 mEq/Kg NH_4Cl B.I.D. for two days. The change in net acid excretion was greater in the sham-operated animals ($2.64 \pm .39$ vs 1.59 ± 0.39 mEq/day, $p < 0.05$). Cumulative potassium balance was significantly more negative in the adrenalectomized animals ($-.86 \pm 0.06$ vs $-.60 \pm 0.06$ mEq, $p < .05$). Muscle potassium stores, and potassium excretion rates were not different. Both groups of animals showed severe degrees of potassium depletion. However, plasma potassium concentration was significantly higher in adrenalectomized rats both before and after the acid loading period (Tables IX and X). As already noted after the control period, the serum potassium in ADX animals was significantly higher than those of the sham-operated animals (2.6 ± 0.1 mEq/1 vs 2.1 ± 0.1 mEq/1, $p < .05$). After acid gavage, the plasma potassium was also expectedly higher (3.6 ± 0.4 mEq/1 vs 2.2 ± 0.2 mEq/1, $p < .05$). Finally, it is clear from Table IX that differences in food intake and urine flow rate in the two groups of animals were not present, while ADX animals had higher rates of sodium excretion.

Because of the possibility that differences in serum potassium concentration could modify renal acid excretion independent of the presence of adrenal glands, it was of interest to compare sham-operated animals with access to potassium, Group IIB, with Group IA potassium restricted adrenalectomized animals. The results show that before acid loading, sham animals

TABLE X

SYSTEMIC ACID-BASE AND ELECTROLYTE DATA BEFORE AND AFTER NH_4Cl LOADING

		ADX Rats Low K^+ Diet[1]	Sham Rats Low K^+ Diet[2]	Sham Rats Normal Diet[3]	ADX Rats Normal Diet[4]
Blood pH	Control	7.39±0.04	7.46±0.02	7.42±0.01	7.41±0.02
	Acid Loading	7.18±0.08	7.38±0.02	7.46±0.03*	7.00±0.17
Blood pCO_2 (mm of Hg)	Control	43.5±4.1	41.3±1.1	35.3±0.8	36.0±2.9
	Acid Loading	27.4±2.8	34.2±2.0	31.7±4.8	32.4±6.1
Plasma HCO_3^- mEq/l	Control	24.8±0.6	28.7±1.0*	22.1±0.6*	21.8±1.3*
	Acid Loading	10.7±2.3	19.4±0.7*	21.5±1.6*	8.8±2.8
Plasma K^+ mEq/l	Control	2.6±0.1	2.1±0.1*	3.5±0.2*	4.2±0.1*
	Acid Loading	3.6±0.4	2.2±0.2*	3.0±0.2	3.8±0.4
Muscle K^+ mEq/100 gm/FFDS	Control	39.9±1.0	37.0±1.1	48.1±0.9*	50.2±0.4*
	Acid Loading	42.3±0.8	40.4±0.5	44.5±0.7	48.0±0.7*
Potassium depletion nephropathy	Control	present	present	absent	absent
	Acid Loading	present	present	absent	absent
Aldosterone pg/ml	Control	0	8±6*	83±32*	0
	Acid Loading	0	166±55*	169±58*	0
Hct	Control	42.9±0.8	46.6±1.0*	43.0±2.1	38.7±2.0
	Acid Loading	53.1±0.2	49.1±0.8	46.9±2.4	52.5±4.7

* $p<.05$ when compared with ADX rats, low K^+ diet[1], no asterisk means comparisons are not significant.
Control data from groups: 1. IVA, N=6; 2. IVB, N=6; 3. IVC, N=6; 4. IVD, N=6.
Acid loading data from groups: 1. IA, N=5; 2. IB, N=5; 3. IIB, N=4; 4. III, N=4.

receiving a normal potassium diet had significantly higher serum potassium values when compared with potassium-restricted ADX animals (Table X, Group IVC vs Group IVA, 3.5 ± 0.2 vs 2.6 ± 0.1 mEq/l, $p < .05$).

Despite the significantly higher cumulative potassium balance ($-.86 \pm .06$ vs 0.64 ± 0.46 mEq over 8 days, $p < .05$) and the absence of potassium depletion nephropathy in the sham-operated group, the rate of delta net acid excretion was significantly less in Group IA ADX ($1.59 \pm .039$ vs 3.60 ± 0.69 mEq/day, $p < 0.05$).

It is important to note that in association with the higher potassium stores, the urine pH was significantly more acid (5.53 ± 0.12 vs 6.2 ± 0.11, $p < .05$ in Group IIB rats vs Group IA rats). As a result, it is possible that the sham-operated rats excreted more acid as ammonium due to pH facilitated ionic trapping of ammonia in urine rather than due to the presence of the adrenal glands. Therefore, to isolate the effects of higher potassium stores and low urine pH from those of adrenalectomy, a comparison of adrenalectomized rats eating potassium (Group III) with adrenalectomized animals subject to potassium restriction (Group IA) is particularly pertinent. Group III ADX rats had significantly lower urine pH values (5.68 ± 0.06) when compared with Group IA ADX rats (6.02 ± 0.11, $p < .05$). Yet, net acid excretion rates were not significantly different: $1.96 \pm .49$ vs $1.59 \pm .39$ N.S.

Therefore it is reasonable to conclude that the higher potassium stores and lower urine pH (Group IIB) were not responsible for the higher delta net acid excretion rates seen in Group IIB.

Throughout the study great care was taken to pair feed animals so that differences in food intake were not responsible for measured rates of net acid excretion. The success of this manoever was assessed, in part, by following rat weights daily. The following table shows the mean rat weight during the control period and the change from control values after Day 1 of the NH_4Cl loading period (10 mEq/kg) and the average of the

changes in rat weight over Day 4 and Day 5 i.e. 10 mEq/kg BID for two days.

Changes in Rat Weight During The NH_4Cl Loading Period

	Control Period Mean Rat Weight	Day 1 10 mEq/kg	Day 4 and 5 10 mEq/kg BID
Group IA - ADX	247 ± 5	11 ± 4	-8 ± 6
	NS	NS	NS
Group IB - Sham	246 ± 6	5 ± 2	-11 ± 4
Group IIA - ADX	253 ± 7	3 ± 3	
	NS	*	
Group IIB - Sham	255 ± 4	-4 ± 4	-5 ± 5
Group III - ADX	239 ± 4	5 ± 1	3 ± 4

NS not significant
* $p < .05$
NB Differences in net acid excretion were demonstrated without differences in rat weights between paired groups.

iii) Effect of steroid replacement in response to acid loading.--

Table XI show acid-base and potassium data on 12 adrenalectomized rats replaced with adrenocortical steroids (1 microgramme dexamethasone and 2 microgrammes aldosterone B.I.D.), 10 adrenalectomized rats and 12 sham-operated animals. All animals received 10 mEq/Kg of NH_4Cl twice daily for a period of three days. In order to be certain that the animals receiving steroids did not have lower body potassium stores than the non-replaced adrenalectomized rats, the latter were maintained on a potassium deficient diet. The sham animals ate a normal diet. It is obvious from Table XI that despite higher potassium stores, steroid replacement was associated with a dramatic restoration of the plasma bicarbonate concentration (21.5 ± 0.7 mEq/l vs 9.0 ± 1.8 mEq/l, $p < 0.05$). When compared with sham-operated animals, a small but significant difference in plasma bicarbonate concentration persists, indicating that steroid replacement did not induce a complete recovery. The severe acidosis in the non-replaced adrenalectomized animals cannot be attributed to higher potassium stores because plasma potassium concentration was not different when compared with the replaced animals and muscle potassium stores were significantly lower (39.7 ± 0.7 mEq/100 gm fat-free dry solids vs 47.6 ± 0.4 mEq/100 gm fat-free dry solids, $p < .05$).

TABLE XI

EFFECT OF ADRENOCORTICAL STEROIDS ON THE SYSTEMIC ACID-BASE RESPONSE TO NH_4Cl LOADING

	Group VA ADX Low K^+ Diet	Group VB Sham Normal Diet	Group VC ADX + Steroids Normal Diet
	N=10	N=12	N=12
Blood pH	7.09±0.05*	7.43±0.01*	7.37±0.06
Blood pCO_2 mm of Hg	28.0±3.7*	41.1±2.0	39.0±2.4
Plasma HCO_3^- mEq/l	9.0±1.8*	25.8±0.8*	21.5±0.7
Plasma K^+ mEq/l	4.4±0.5	3.3±0.3*	4.2±0.1
Muscle K^+ mEq/100 gm FFDS	39.7±0.7*	46.2±0.6	47.6±0.4

* $p<0.05$ when compared with steroid replaced ADX rats (Group VC), no asterisk means comparisons are not significantly different.

CHAPTER IV

Discussion

Although steroid replacement studies have demonstrated that steroid hormones can normalize reduced rates of acid excretion in the adrenal insufficient rat (11, 12), dog (16), and man (17, 18, 19), their role remains uncertain. Recently it has been suggested from experiments on human subjects that potassium retention leads to reduced rates of ammonium excretion and the effect of steroids on acid excretion is via the restoration of potassium balance (18, 19). Nevertheless, evidence that steroids have a more direct effect on acid excretion was reported recently by Hulter et al (43). After controlling plasma potassium concentration and the sodium balance in their dogs, a defect in acid excretion persisted. Subcellular studies have similarly shown that both potassium and adrenal steroids can alter the rate of ammoniagenesis although the details of these effects have not been described (46, 25). On the other hand Ditella et al reported that adequate sodium availability prevented urinary acidification abnormalities in mineralocorticoid deficient rats (33). In order to rule out potassium retention, an important variable in these observations, we undertook to study net acid excretion rates in adrenal insufficiency in the absence of potassium retention. We further considered other factors reported to affect acid excretion which we found altered after adrenalectomy in our studies and developed protocols to control food intake (60, 61), urine flow rate (56, 58, 59), urine pH (59, 57, 53, 56) and rates of distal sodium delivery (41, 42, 39). Our results showed that in

adrenal-insufficient rat net acid excretion rates are reduced as a result of the absence of the adrenal gland.

a) Potassium Status

In our studies, several parameters of potassium status were used to assess body potassium stores. Muscle potassium content was useful since it was representative of potassium content in this major body potassium depot. Plasma potassium was interpreted as being indicative of potassium concentration in renal plasma, and hence of the peritubular environment. Potassium excretion rates give some indication of the potassium passing the luminal aspect of cells. Potassium balances were measured over control and acid period days but were applied with reservation because of the limitation arising from the fact that at highest rates of potassium excretion, during the post-operative recovery period, a potassium tally was not kept. The examination of kidneys taken from animals eating K^+restriced diets showed evidence of K^+depletion nephropathy while such findings were not seen in animals eating normal food. Together these parameters served as a formidable measure of potassium status in our animals and convincingly enabled us to identify any possibility that potassium was an important interfering variable in our studies.

Supranormal potassium stores were prevented in our adrenalectomized animals by dietary potassium restriction. Their control partners were identically treated except for the addition of 0.5 mEq/l KCl to their drinking solution. This served to provide us with less negative potassium balances in these animals over the period of observation. According to the above-mentioned indicators of potassium balance, the adrenalectomized animals were potassium-depleted and had potassium stores comparable to their controls with one exception.

Their plasma potassium concentration values were significantly greater, albeit in a low potassium range, both after the control period and after acid loading (Table X, Groups IA vs IB). For this reason, the experiment was repeated, giving the control animals a normal potassium diet thereby obviating potassium depletion in that group. The results showed that for all but one of our parameters of potassium status, ADX animals had significantly lower potassium stores during both control and acid periods. The exception was the plasma potassium concentration after NH_4Cl loading, since it was not significantly different between Groups IA and IB. However, it is our belief that this is more a reflection of the severe metabolic acidosis and the consequent efflux of muscle potassium rather than the cause of the acidosis itself. At the kidney cell level, the adrenalectomized animals showed lesions characteristic of potassium depletion nephropathy, while sham counterparts showed no such lesions.

A third protocol was undertaken to determine whether potassium depletion per se could have reduced net acid excretion in our adrenalectomized animals. This was of particular interest since the effects of potassium depletion in the adrenalectomized rat have not been studied, while its reported effects in the intact dog, rat and man have been variable (34, 89, 72, 48). To this end, ADX animals were treated identically to those of Group IA potassium depleted ADX animals, with the substitution of their diet with one of normal potassium content. Their potassium stores clearly differed while rates of net acid excretion were unchanged (Table X, and see Results). In summary, the above protocols enabled us to exclude potassium as an occult variable in our experiments.

b) Food Intake

Attention was also given to the amount of food eaten by our

animals. Concern for this variable arose from pilot studies, which showed potassium depleted ADX animals ate 30% less food than their intact control partners. In view of the reports that food acid intake is reflected in rates of net acid excretion (60, 61), care was taken to avoid such differences by pair-feeding our animals. This was done by restricting the intake of food by sham animals to only that amount eaten by their adrenalectomized partners. Pairing in this fashion eliminated differences in food intake.

c) Urine pH, Urine Flow and Sodium Excretion

Differences in urine pH, urine flow and sodium excretion resulted from both the adrenalectomy state and potassium depletion. As a result of potassium depletion, animals developed polydypsia and consequently increased their intake of sodium and water which, in turn, led to increased rates of urine flow, sodium excretion and urine pH. All three responses to potassium depletion have been well described in intact animals.

Although high urine flow rates at a high urine pH as well as increased rates of sodium excretion have both been reported to enhance acid excretion (58, 56, 41, 42, 39), their effects in comparisons between Group IA potassium depleted ADX animals and IB potassium depleted sham animals were of no importance since both groups were potassium depleted and significant differences in these variables were not found. This was true (Table VIII and IX) during both control and acid loading periods. However, when comparisons were made between Groups IA or IIA with Group IIB (Table VIII and text in Results under large acid loading), the effect of potassium depletion became critical. Specifically, during the control period, a lower urine pH could have facilitated a greater excretion of net acid in sham animals by titrating dibasic

phosphate, while after acid loading the greater increase in ammonium and net acid excretion could have resulted from low pH facilitated ionic trapping of ammonia (58, 57, 55, 53, 56) in tubular fluid. For this reason, Group III ADX animals eating normal potassium content food, served to demonstrate that low urine pH during the control period or during acid loading did not significantly increase the rates of net acid excretion in adrenalectomized animals. As a result, we conclude the observed differences were due to the presence of the adrenal gland rather than low urine pH or potassium status.

d) Pilot Studies In our laboratory ADX rats maintained on saline did not develop metabolic acidosis (Table V), despite the general belief that ADX rats have a reduced capacity for acid excretion. To further challenge these animals with acid, NH_4Cl was added to their food and served ad libitum. Once again, although both groups of animals developed a moderate metabolic acidosis (Table VI) no difference in the plasma HCO_3^- concentration was seen between them. To rule out the possibility that these results were a consequence of differences in NH_4Cl intake, pairing for food, drink and NH_4Cl intake was introduced. The results showed (Table VII) that ADX rats in the absence of supranormal potassium levels developed a severe metabolic acidosis while sham rats were able to maintain their systemic acid-base balance. This was unexpected since the sham animals maintained their acid-base status after the introduction of NH_4Cl gavage. This suggested that, when NH_4Cl was given ad libitum sham animals ate more acid than normal animals can tolerate or, that perhaps, the adrenal gland requires a bolus of acid as a stimulus for its response. This mechanism would still not exclude the possibility that ADX animals ate less food than normal animals and therefore did not appear to be more impaired than sham animals. In order to associate the inability to maintain the acid-base balance, with a reduced capacity for net acid excretion, ADX and sham rats were studied in metabolic cages where, 24-hour urine collections were made for net acid excretion determinations.

e) Experimental Protocols

i) Control period.--After accounting for possible variables which could contribute to reduced rates of net acid excretion in adrenalectomy, the rather lengthy control period protocol enabled us to convincingly demonstrate that higher rates of net acid excretion are associated with the presence of the adrenal gland. The interpretation of these rigorously controlled studies is less limited than in previously reported work, where the above-mentioned factors were less well controlled (10, 11, 12, 33, 13, 14). Hulter et al (43) presented data showing dogs with improved plasma potassium concentration, albeit significantly higher than values seen in mineralocorticoid repleted animals. In one protocol where potassium was normalized by increasing the intake of sodium chloride the animals developed a positive sodium balance, hypernatremia and increased glomerular filtration rates. Since increased sodium intake is associated with increased rates of calcium excretions (90, 91) and these animals were given a constant intake of calcium it is possible the steady plasma bicarbonate concentrations and reduced rates of net acid excretion were a consequence of increased parathyroid hormone action on bone and renal bicarbonate handling rather than an effect of mineralocorticoid deficiency (69, 8). In our protocols there was no reason to suspect an increase in parathyroid hormone and indeed, lack of corticosteroids have been associated with increased levels of blood calcium which would then decrease the stimulus for parathyroid hormone secretion (70, 92). Finally, in the last of 4 "groups" of animals only one dog was studied, and although they successfully controlled all factors, uncertainty as to its significance remained in view of the large scatter of values seen from a similar protocol with a larger number of observations.

In our studies, it is of interest that during the control period potassium or steroids did not appear to maintain rates of net acid

excretion by an effect on ammonium excretion. This is in contrast to observations reported by Szylman et al (18), where the restoration of potassium status in adrenal insufficient man led to the restoration of acid-base balance by increasing the rate of ammonium excretion. This disparity may be due to species differences or experimental design. In our results, higher rates of net acid excretion were seen in sham animals with higher or lower rates of ammonium excretion indicating that lower ammonium excretion rates did not preclude a greater rate in net acid excretion. It appears, therefore, that the adrenal gland may have a more direct effect on hydrogen ion secretion rates while potassium may affect the availability of ammonia, thereby determining the allocation of protons to buffers. Nevertheless, still another mechanism for the maintenance of net acid excretion rates would be required to explain why a dramatic decrease in urinary ammonium excretion found in ADX animals eating a normal potassium diet was not associated with a significant change in the excretion of net acid. Although our studies show a consistent decrease in net acid excretion in adrenalectomized rats, metabolic acidosis was not obvious. Since there can be no doubt that food intake was well controlled, it is worth considering whether the adrenalectomy state per se can alter endogenous acid production. Lack of major differences in the urinary excretion of organic acids in the control period suggests that either our method for measuring organic acids was not sensitive enough to detect significant changes or that mild but significant degrees of metabolic acidosis can be assumed to exist and would have been manifest if the control period had been carried out for a longer period of time. The possibility that adrenal steroids can alter endogenous acid production and renal net acid excretion without a change in acid-base status is supported by the recent preliminary report of

Hulter et al (93).

ii) Acid loading.--After the control period, each group of animals was subjected to an acid loading regimen consisting of 10 mEq/Kg NH_4Cl and two days of observation followed by 10 mEq/Kg NH_4Cl B.I.D. for two days. The smaller acid load served to induce ammoniagenesis and enabled us to examine the responsiveness of our animals to a less severe challenge of acid. The results showed that all animals excreted the same amount of net acid within 24 hours of receiving it by predominantly increasing the excretion of ammonium. Although we detected no difference in the increase in rates of net acid excretion, our data cannot rule out the possibility that the ADX animals excreted the gavaged acid load less briskly than their controls within the subsequent 24-hour period. Alternatively, in response to acid loading, the mechanism for increasing the excretion of net acid may be different and unimpaired under these conditions when compared to the control period. Nevertheless, these observations are in sharp contrast to the control period results, where increased rates of net acid excretion were not regularly associated with commensurate changes in ammonium excretion.

All animals returned to their repective control period values by day two of the acid period. The large acid period clearly separated the response of adrenalectomized and sham-operated rats. Under these conditions, increased rates of ammonium excretion represented the greater contributor to increased rates of net acid excretion and also appeared to be reduced in animals deficient in net acid excretion. Control-period base line excretion rates did not appear to be related to the level of response after acid loading. After NH_4Cl, 10 mEq/Kg BID it appears the adrenal gland may modulate the increased rate of net acid by an effect on ammonium excretion. These observations more clearly coincide

with the numerous uncontrolled steroid replacement studies with rats, where ammonium excretion had consistently been shown to increase (11, 12). These results would still not rule out the possibility that the increase in ammonium excretion could be dependent on a mechanism which still could affect potassium levels closer to the site of ammoniagenesis. Nevertheless, our results have unequivocally demonstrated that the intact adrenal gland is essential for the normal capacity to excrete net acid and to maintain the systemic balance of acid.

iii) Possible Explanations for Rates of Net Acid Excretion in these Experiments.---These studies were not designed to uncover mechanisms for the rate of net acid excretion. Neverthless the data may contribute some characteristics to possible mechanisms involved.

During the control period sham animals with or without potassium depletion excreted significantly more net acid than pair treated ADX animals. Possible events leading to these results include; a) steroid dependant increase in NH_3 production and diffusion into tubular fluid which secondarily increased proton secretion; b) steroid dependant increase in proton secretion per se.

The results under control period condition are most consistent with the latter possibility. This is supported by the observation that rates of net acid excretion were significantly higher in sham animals with significantly higher or lower rates of NH_3 excretion when compared to ADX animals. Consequently net acid excretion remained constant in sham animals despite large changes in ammonia, titratable acid and bicarbonate excretion. Further, the lower urine pH and lower rate of NH_3 excretion in Group IIB as compared to Group IB

suggests that ammonia production was actually decreased since the rate of diffusion into low pH tubular fluid is production limited (9). Potassium depletion and retention have been shown to stimulate and suppress ammoniagenesis or excretion respectively (18,22,23,24,25,89). Interestingly our results showed large differences in ammonia excretion determines the buffer profile of excreted net acid but does not necessarily dictate changes in net acid excretion.

In response to acid loading with 10 mEq/Kg x 1 day, the increase in ammonia and net acid excretion was not different among the groups studied. Possible mechanisms fall into two categories; a) ADX animals have an undetected impairment; b) ADX animals were not impaired under these conditions. Specifically, the rate of net acid excretion may have been more brisk in sham animals but over the twenty-four hour post gavage period the ADX animals were able to excrete a comparable amount of acid at a slower rate. Alternatively, the mechanism for acid excretion post gavage could have involved a different mechanism from that operating during the control period. For example, at a given rate of proton secretion by the nephron, acute metabolic acidosis reduces the pH and filtered bicarbonate load. Both events enable a larger portion of secreted protons to participate in buffer titration and acid excretion rather than in the reabsorption of filtered bicarbonate. Finally a steroid independant cytoplasmic mechanism of ammoniagenesis suggested by Welbourne et. al. (4, 15) may have been adequate for a normal response in ADX animals.

After NH_4Cl loading with 10 mEq/Kg BID x 2 days sham animals increased the excretion of ammonia and net acid more when compared to pair

treated ADX animals. An increase in TA excretion seen in sham animals was not present in the ADX groups despite comparable pre and post acid loading urine pH values. Our results suggest that the adrenal gland is essential for these events. Welbourne et. al. described a steroid dependant transport system which increases the availability of intramitochondrial glutamine for ammoniagenesis (15,46,47) which results in a higher NH_3 produced/glutamine delivered ratio. Since sham animals also increased the excretion of TA more than ADX animals as noted above, this suggests that, an increased amount of titratable acid became available for titration and a greater rate of proton secretion occurred.

In summary, it appears that, under control conditions the adrenal gland primarily regulates the rate of hydrogen ion secretion while in response to NH_4Cl loading stimulatory effects on both hydrogen ion secretion and ammonia production are likely.

iv) Effects of replacement of adrenocortical hormones.-- The defect in renal net acid excretion seen during adrenalectomy was presumed to be due to the absence of adrenocortical hormones since in all protocols, we documented no aldosterone activity in plasma samples. This supposition was confirmed by an additional group of experiments in which adrenalectomized animals were given replacement doses of dexamethasone and aldosterone. These animals did not develop the profound metabolic acidosis which was observed in adrenalectomized animals not given hormone replacement but offered the benefit of potassium depletion. Thus, it appears likely that the metabolic acidosis observed in association with the impaired renal net acid excretion during adrenalectomy is attributable to the absence of adrenocortical hormones.

Concluding Remarks

These experiments demonstrated, over a period of weeks that reduced rates of net acid excretion in the adrenalectomized rat is not necessarily dependent on the presence of hyperkalemia, sodium depletion, hyponatremia, changes in food intake, or alterations in urine pH or urine flow rate. There is ample evidence showing that these factors do contribute to reduced rates of acid excretion or, depending on the experimental conditions, precipitate metabolic acidosis and a significant reduction in the capacity for net acid excretion. This view is supported by the apparent discrepancies among different reports on the adrenal insufficient dog, man and rat showing that reversible impairments in renal net acid excretion result from mineralocorticoid deficiency (43), potassium retention (18) or sodium depletion (33). While our results are consistent with the above quoted dog studies, the effects of potassium and sodium (18, 33) emphasize the importance of controlling these and other concommittants of adrenal insufficiency in isolating the chronic effects of adrenalectomy per se on renal net acid excretion.

APPENDIX

Sample Calculation for Titratable Acid Excretion

Primary Data

#1	urine vol ml/day	23.1
*#2	rat weight gm	236
#3	pH of freshly collected urine	6.36
#4	freshly collected urine pCO_2 mm of Hg	68.2
#5	urine Na^+ concentration Eq/L	.138
#6	urine CL^- concentration Eq/L	.152
#7	urine K^+ concentration Eq/L	.0048
#8	urine NH_3 concentration Eq/L	.096955
#9	urine pH before titration	6.48
#10	urine pH after titration	7.40
#11	vol of titrant used ml	.418
#12	titrant normality	.09868 N

*Used for other calculations in the computer program.

Sample Calculation for Molar Concentration of Monobasic Phosphate (M) in rat urine

$$M = \frac{\left[\frac{(\#12)(\#11)}{2}\right]\left(1+\left(\text{Antilog}\ \#10 - \left\{7.181 - \frac{1.57\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8 - (\#10)(\#12)\right\}}}{1 - 1.49\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8 - (\#10)(\#12)\right\}}}\right\}\right)\right)}{\text{Antilog}\left(\#10 - \left\{7.181 - \frac{1.57\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8 - \frac{(\#10)(\#12)}{2}\right\}}}{1 - 1.49\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8 - \frac{(\#10)(\#12)}{2}\right\}}}\right\}\right) - \text{Antilog}\left(\#9 - \left\{7.181 - \frac{1.57\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8\right\}}}{1 - 1.49\sqrt{\frac{1}{2}\left\{\#5 - \#6 - \#7 - \#8\right\}}}\right\}\right)\right)}$$

$$M = \frac{\left[(.02062)\right]\left(1+\left(\text{Antilog}\ 7.400 - \left\{7.181 - \frac{1.57\sqrt{.2061875}}{1 - 1.49\sqrt{.2061875}}\right\}\right)\right)}{\text{Antilog}\left(7.400 - \left\{7.181 - \frac{1.57\sqrt{.2061875}}{1 - 1.49\sqrt{.2061875}}\right\}\right) - \text{Antilog}\left(6.48 - \left\{7.181 - \frac{1.57\sqrt{\frac{1}{2}(.391755)}}{1 - 1.49\sqrt{\frac{1}{2}(.391755)}}\right\}\right)}$$

$$M = \frac{\left[(.02062)\right]\left(1+\left(\text{Antilog}\ (7.400 - \{6.755767\})\right)\right)}{\text{Antilog}\ (7.400 - \{6.755767\}) - \text{Antilog}\ (6.48 - \{6.7623\})}$$

$M = .02875$ Moles/Liter

Sample Calculation for Molar Concentration of Dibasic Phosphate (M) in rat urine

$$D = M \left(\text{Antilog} \left(\#9 - \left\{7.181 - \frac{1.57 \sqrt{\tfrac{1}{2}\Sigma \#5 - \#6 - \#7 - \#8}}{1 - 1.49 \sqrt{\tfrac{1}{2}\Sigma \#5 - \#6 - \#7 - \#8}}\right\}\right)\right)$$

$$D = (.02875) \left(\text{Antilog} \left(6.48 - \left\{7.181 - \frac{1.57 \sqrt{\tfrac{1}{2}\Sigma .138 - .152 - .0048 - .096955}}{1 - 1.49 \sqrt{\tfrac{1}{2}\Sigma .138 - .152 - .0048 - .096955}}\right\}\right)\right)$$

$$D = (.02875) \left(\text{Antilog} \quad 6.48 - \{6.7623\}\right)$$

$$D = (.02875) \ (.5263)$$

$$D = .1513 \ \text{Moles/Liter}$$

Sample Calculation for Titratable Acid Excretion (mEq/24 hours) (TA exc.)

$$\text{TA exc.} = \frac{\left(\text{Antilog}\left\{\#10 - 6.7950\right\}\right)(M - D)}{1 + \left(\text{Antilog}\left\{\#10 - 6.7950\right\}\right)}\left[\#1\right]$$

$$\text{TA exc.} = \frac{\left(\text{Antilog}\left\{7.400 - 6.7950\right\}\right)\left((.0287446) - (.015128)\right)}{1 + \left(\text{Antilog}\left\{7.400 - 6.7950\right\}\right)}\left[23.1\right]$$

$$\text{TA exc.} = \frac{(4.027)\ (.02875) - (.01513)}{1 + 4.027}\left[23.1\right]$$

TA exc. = .46 mEq/24 hours

References

1. LOEB, RF: Chemical changes in blood in Addison's disease. Science 76:420-421, 1932.

2. LOEB, RF, ATCHLEY, DW, BENEDICT, EM and LELAND, J: Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. J. Exp. Med. 57: 775-792, 1933.

3. SWINGLE, WW and WENNER, WF: Sulfate retention in dogs following bilateral adrenal extirpation. Proc. Soc. Exp.. Biol. Med. 25: 169-171, 1927.

4. HASTINGS, AB and COMPERE, EL: Effect of bilateral suprarenalectomy on certain constituents of the blood of dogs. Proc. Soc. Exp. Biol. Med. 28: 376-378, 1931.

5. HIERHOLZER, K and LANGE, S: The effects of adrenal steroids on renal function. International Review of Physiology, Kidney and Urinary Tract Physiology I, ed, THURAU, K, vol 6:273-333, 1974.

6. MALNIC, G and GIEBISCH, G: Mechanism of renal hydrogen ion secretion. Kid. Int. 1:280-296, 1972.

7. RECTOR, FC, Jr: Acidification of the urine. Handb. Physiol. Section 8. Renal Physiology. 431-454, 1973.

8. MORRIS, RC, Jr, SEBASTIAN, A and MCSHERRY, E: Renal acidosis. Kid. Int. 1:322-340, 1972.

9. GOLDSTEIN, L: Ammonia production and excretion in the mammalian kidney. International Review of Physiology, Kidney and Urinary Tract Physiology II, ed, THURAU, K, vol 11:283-316, 1976.

10. SARTORIUS, OW, CALHOON, D and PITTS, RF: The capacity of the adrenalectomized rat to secrete hydrogen and ammonium ions. Endocrinology 51:444-450, 1952.

11. SARTORIUS, OW, CALHOON, D and PITTS, RF: Studies on the interrelationships of the adrenal cortex and renal ammonia excretion by the rat. Endocrinology 52:256-265, 1953.

12. BORIS, A and GORDON, ES: The effects of adrenal corticoids upon the urinary excretion of ammonia in the adrenalectomized, acidotic rat. Endocrinology 66:630-633, 1960.

13. WILSON, JD and SELDIN, DW: Effect of adrenalectomy on production and excretion of ammonia by the kidneys. Am. J. Physiol. 188:524-528, 1957.

14. DUMM, ME, LESLIE, SH and RALLI, EP: Influence of adrenalectomy and gonadectomy on capacity of rats to excrete an acid load. Proc. Soc. Exp. Biol. Med. 88:592-594, 1955.

15. WELBOURNE, TC: Influence of adrenal glands on pathways of renal glutamine utilization and ammonia production. Am. J. Physiol. 226:555-559, 1974.

16. HULTER, HN, ILNICKI, LP, HARBOTTLE, JA and SEBASTIAN, A: Impaired renal H^+secretion and NH_3 production in mineralocorticoid-deficient glucocorticoid-replete dogs. Am. J. Physiol. 232:F136-F146, 1977.

17. PEREZ, GO, OSTER, JR and VAAMONDE, CA: Renal acidification in patients with mineralocorticoid deficiency. Nephron 17: 461-473, 1976.

18. SZYLMAN, P, BETTER, OS, CHAIMOWITZ, C and ROSLER, A: Role of hyperkalemia in the metabolic acidosis of isolated hypoaldosteronism. N. Engl. J. Med. 294:361-365, 1976.

19. SEBASTIAN, A, SCHAMBELAN, M, LINDENFELD, S and MORRIS, RC, Jr: Amelioration of metabolic acidosis with fludrocortisone therapy in hyporeninemic hypoaldosteronism. N. Engl. J. Med. 297:576-583, 1977.

20. COHEN, JJ, HULTER, HN, SMITHLINE, N, MELBY, JC and SCHWARTZ WB: The critical role of the adrenal gland in the renal regulation of acid-base equilibrium during chronic hypotonic expansion. Evidence that chronic hyponatremia is a potent stimulus to aldosterone secretion. J. Clin. Invest. 58:1201-1208, 1976.

21. PEREZ, GO, OSTER, JR, VAAMONDE, CA and KATZ, FH: Effect of NH_4Cl on plasma aldosterone, cortisol and renin activity in supine man. J. Clin. Endocrinol. Metab. 45:762-767, 1977.

22. TANNEN, RL, WEDELL, E and MOORE, R: Renal adaptation to a high potassium intake: the role of the hydrogen ion. J. Clin. Invest. 52:2089-2101, 1973.

23. KAMM, DE: Effect of oral and intravenous (IV) K on urine $[H^+]$. Abstr. Am. Soc. Nephrol. 6:56, 1973.

24. KAMM, DE: Dissociation of urine pH & NH_3 excretion during KCl and NaCl loading. Abstr. Am. Soc. Nephrol. 5:36, 1971.

25. TANNEN, RL and MCGILL, J: Influence of potassium on renal ammonia production. Am. J. Physiol. 231:1178-1184, 1976.

26. HOLLENBERG, NK, WILLIAMS, G, BURGER, B and HOOSHMAND, I: The influence of potassium on the renal vasculature and the adrenal gland, and their responsiveness to angiotensin II in normal man. Clin. Sci. Mol. Med. 49:527-534, 1975.

27. LEMIEUX, G, VINAY, P and CARTIER, P: Renal hemodynamics and ammoniagenesis. Characteristics of the antiluminal site for glutamine extraction. J. Clin. Invest. 53:884-894, 1974.

28. BERLINER, RW, KENNEDY, TJ, Jr and ORLOFF, J: Relationship between acidification of the urine and potassium metabolism. Effect of carbonic anhydrase inhibition on potassium excretion. Am. J. Med. 11:274-282, 1951.

29. BERLINER, RW, KENNEDY, TJ, Jr and HILTON, JG: Renal mechanisms for excretion of potassium. Am. J. Physiol. 162:348-367, 1950.

30. WINKLER, AW and SMITH, PK: Renal excretion of potassium salts. Am. J. Physiol. 138:94-103, 1942.

31. ROBERTS, KE, MAGIDA, MG and PITTS, RF: Relationship between potassium and bicarbonate in blood and urine. Am. J. Physiol. 172:47-54, 1953.

32. RECTOR, FC, Jr; BUTTRAM, H and SELDIN, DW: An analysis of the mechanism of the inhibitory influence of K^+ on renal H^+ secretion. J. Clin. Invest. 41:611-617, 1962.

33. DITELLA, P, SODHI, B, MCCREARY, J, ARRUDA, J and KURTZMAN, N: Urinary acidification (UA) in mineralocorticoid deficient (MD) rats. Clin. Res. 25:429A, 1977.

34. LUKE, RG: Effect of adrenalectomy on the renal response to chloride depletion in the rat. J. Clin. Invest. 54:1329-1336, 1974.

35. LUKE, RG, and LEVITIN, H: The renal and electrolyte response to respiratory acidosis in the adrenalectomized rat. Yale J. Biol. Med. 39:27-37, 1966.

36. POSNER, JB and JACOBS, DR: Isolated analdosteronism. Clinical entity, with manifestations of persistent hyperkalemia, periodic paralysis, salt-losing tendency, and acidosis. Metabolism 13:513-521, 1964.

37. COHEN, JJ, LYONS, H, HARRINGTON, JT, and SCHWARTZ, WB: Total correction by Doca of HCl-induced chronic metabolic acidosis. Clin. Res. 21:681, 1973.

38. LOWANCE, DC, GARFINKEL, HB, MATTERN, WD and SCHWARTZ, WB: The effect of chronic hypotonic volume expansion on the renal regulation of acid-base equilibrium. J. Clin. Invest. 51:2928-2940, 1972.

39. SCHWARTZ, WB, JENSON, RL and RELMAN, AS: Acidification of the urine and increased ammonium excretion without change in acid-base equilibrium: sodium reabsorption as a stimulus to the acidifying process. J. Clin. Invest. 34:673-680, 1955.

40. HULTER, HN, ILNICKI, LP, HARBOTTLE, JA and SEBASTIAN, A: Correction of metabolic acidosis by the kidney during isometric expansion of extracellular fluid volume. J. Lab. Clin. Med. 92:602-612, 1978.

41. GIEBISCH, G, MALNIC, G, KLOSE, RM, and WINDHAGER, EE: Effect of ionic substitutions on distal potential differences in rat kidney. Am. J. Physiol. 211:560-568, 1966.

42. OSTER, JR, HOTCHKISS, JL, CARBON, M and VAAMONDE, CA: Abnormal renal acidification in alcoholic liver disease. J. Lab. Clin. Med. 85:987-1000, 1975.

43. HULTER, HN, LICHT, JH, GLYNN, RD and SEBASTIAN, A: Renal acidosis in mineralocorticoid deficiency is not dependent on NaCl depletion or hyperkalemia. Am. J. Physiol. 236:F283-F294, 1979.

44. HULTER, HN, LICHT, JH and SEBASTIAN, A: K^+ deprivation potentiates the renal acid excretory effect of mineralocorticoid: obliteration by amiloride. Am. J. Physiol. 236:F48-F57, 1979.

45. LIFSCHITZ, MD, SCHRIER, RW and EDELMAN, IS: Effect of actinomycin D on aldosterone-mediated changes in electrolyte excretion. Am. J. Physiol. 224:376-380, 1973.

46. WELBOURNE, TC, and FRANCOEUR, D: Influence of aldosterone on renal ammonia production. Am. J. Physiol. 233:E56-E60, 1977.

47. WELBOURNE, TC: Mechanism of renal ammonia production. Adaptation to chronic acidosis. Med. Clin. N. America 59:629-648, 1975.

48. BURNELL, JM, TEUBNER, EJ and SIMPSON, DP: Metabolic acidosis accompanying potassium deprivation. Am. J. Physiol. 227:329-333, 1974.

49. BOYD, J, MULROW, PJ, PALMORE, WP and SILVO, P: Importance of potassium in the regulation of aldosterone production. Supplement I to Circulation Res. XXXII and XXXIII:1-39-1-44, 1973.

50. GROSS, JB and KOKKO, JP: Effects of aldosterone and potassium-sparing diuretics on electrical potential differences across the distal nephron. J. Clin. Invest. 59:82-89, 1977.

51. AL-AWQATI, Q, NORBY, LH, MUELLER, A and STEINMETZ, PR: Characteristics of stimulation of H^+ transport by aldosterone in turtle urinary bladder. J. Clin. Invest. 58:351-358, 1976.

52. PITTS, RF: Production and excretion of ammonia in relation to acid-base regulation. Handb. Physiol. Section 8, Renal Physiology, 455-496, 1973.

53. BALAGURA, S and PITTS, RF: Excretion of ammonia injected into renal artery. Am. J. Physiol. 203:11-14, 1962.

54. FERGUSON, EB, Jr: A study of the regulation of the rate of urinary ammonia excretion in the rat. J. Physiol. 112:420-425, 1951.

55. LEONARD, E and ORLOFF, J: Regulation of ammonia excretion in the rat. Am. J. Physiol. 182:131-138, 1955.

56. MACKNIGHT, ADC, MACKNIGHT, JM and ROBINSON, JR: The effect of urinary output upon the excretion of 'ammonia' in man. J. Physiol. 163:314-323, 1962.

57. ORLOFF, J and BERLINER, RW: The mechanism of the excretion of ammonia in the dog. J. Clin. Invest. 35:223-235, 1956.

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

59. EGGLETON, MG: Some factors affecting the acidity of urine in man. J. Physiol. 106:456-465, 1947.

60. CAMIEN, MN and GONICK, HC: Relationship of renal "net acid" excretion to titratable ash-acidity (Ash-TA) in diet and feces. Proc. Soc. Exp. Biol. Med. 126:45-51, 1967.

61. LENNON, EJ, LEMANN, J, Jr and LITZOW, JR: The effects of diet and stool composition on the net external acid balance of normal subjects. J. Clin. Invest. 45:1601-1607, 1966.

62. CORTNEY, MA: Renal tubular transfer of water and electrolytes in adrenalectomized rats. Am. J. Physiol. 216:589-598, 1969.

63. GIEBISCH, G: Kidney, water and electrolytes. Annu. Rev. Physiol. 24:357-420, 1962.

64. LOTSPEICH, WD: The effect of adrenalectomy on the renal tubular reabsorption of water in the rat. Endocrinology 44:314-316, 1949.

65. LEMANN, J, Jr, LENNON, EJ, GOODMAN, AD, LITZOW, JR and RELMAN, AS: The net balance of acid in subjects given large loads of acid or alkali. J. Clin. Invest. 44:507-517, 1965.

66. BARZEL, U: The effect of excessive acid feeding on bone. Calc. Tiss. Res. 4:94-100, 1969.

67. BARZEL, US and JOWSEY, J: The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin. Sci. 36:517-524, 1969.

68. LEMANN, J, Jr, LITZOW, JR and LENNON, EJ: The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. J. Clin. Invest. 45:1608-1614, 1966.

69. HELLMAN, DE, AU, WYW and BARTTER, FC: Evidence for a direct effect of parathyroid hormone on urinary acidification. Am. J. Physiol. 209:643-650, 1965.

70. LEEKSMA, CHW, DE GRAEFF, J and DE COCK, J: Hypercalcemia in adrenal insufficiency. Acta. Méd. Scandinav. 156:455-458, 1957.

71. ORION RESEARCH INC: Instruction manual of Orion ammonia electrode model 95-10. Cambridge, Mass., 1972.

72. LEVINE, DZ, WALKER, T and NASH, LA: Effects of KCl infusions on proximal tubular function in normal and potassium-depleted rats. Kid. Int. 4:318-325, 1973.

73. CUNARRO, JA and WEINER, MW: A comparison of methods for measuring urinary ammonium. Kid. Int. 5:303-305, 1974.

74. BANWART, WL, TABATABAIL, MA and BREMNER, JM: Determination of ammonium in soil extracts and water samples by an ammonia electrode. Comm. Soil Sci. Plant. Anal. 3:449-458, 1972.

75. BROOKS, JB, CHERRY, WB, THACKER, L and ALLEY, CC: Analysis by gas chromatography of amines and nitrosamines produced in vivo and in vitro by proteus mirabilis. J. Infect. Dis. 126:143-153, 1972.

76. SCHWARTZ, WB, BANK, N and CUTLER, RWP: The influence of urinary ionic strength on phosphate pK'_2 and the determination of titratable acid. J. Clin. Invest. 38:347-356, 1959.

77. MOOKERJEE, BK, ALLEN, AC and DOSSETOR, JB: Validity of net hydrogen ion excretion in the prescence of urinary tract infection. J. Lab. Clin. Med. 71:582-585, 1968.

78. WRONG, O and DAVIES, HEF: The excretion of acid in renal disease. Quart. J. Med. 28:259-313, 1959.

79. KAGAWA, CM, BOUSKA, DJ, ANDERSON, ML and KROL, WF: Pharmacological properties of a mineralocorticoid antagonist (SC-14266). Acta. Int. Pharmacodyn 149:8-22, 1964.

80. KAGAWA, CM, STURTEVANT, FM and VAN ARMAN, CG: Pharmacology of a new steroid that blocks salt activity of aldosterone and desoxycorticosterone. J. Pharm. Exp. Therap. 126:123-130, 1959.

81. PRATT, JH, DALE, SL, GAUNT, R and MELBY, JC: Aldosterone secretion in the enucleated rat adrenal during salt retention. Endocrinology 95:48-54, 1974.

82. SINGER, B and STACH-DUNNE, MP: The secretion of aldosterone and corticosterone by the rat adrenal. J. Endocrinology 12:130-145, 1955.

83. SLUSHER, MA and ROBERTS, S: Fate of adrenal ascorbic acid: relationship to corticosteroid secretion. Endocrinology 61:98-105, 1957.

84. PABST, ML, SHEPPARD, R and KUIZENGA, MH: Comparison of liver-glycogen deposition and work performance tests for the bio-assay of adrenal cortex hormones. Endocrinology 41:55-65, 1947.

85. RINGLER, I and DULIN, WE: Potency relationships of various C-21 steroids as measured by two methods of liver glycogen deposition (27676). Proc. Soc. Exp. Biol. Med. 110:869-872, 1962.

86. RINGLER, I, WEST, K, DULIN, WE and BOLAND, EW: Biological potencies of chemically modified adrenocorticosteroids in rat and man. Metabolism 13:37-44, 1964.

87. ITO, T, WOO, J, HANING, R and HORTON, R: A radioimmunoassay for aldosterone in human peripheral plasma including a comparison of alternative techniques. J. Clin. Endocrinology 34:106-112, 1972.

88. CHAN, JCM: Urinary organic anions: clinical significance and evaluation of a method for determination and preservation. Clin. Biochem. 5:182-185, 1972.

89. TANNEN, RL: The effect of uncomplicated potassium depletion on urine acidification. J. Clin. Invest. 49:813-827, 1970.

90. KING, JS, JACKSON, R and ASHE, B: Relation of sodium intake to urinary calcium excretion. Invest. Urol. 1:555-560, 1964.

91. KLEEMAN, CR, BOHANNAN, J, BERNSTEIN, D, LING, S and MAXWELL, MH: Effect of variations in sodium intake on calcium excretion in normal humans (28821). Proc. Soc. Exp. Biol. 115: 29-32, 1964.

92. MYERS, WPL, ROTHSCHILD, EO and LAWRENCE, W, Jr: Adrenal factors in plasma calcium regulation. Bone and Tooth, ed Blackwood, HJJ, pp 193-206, 1964.

93. HULTER, HN, LICHT, JH and SEBASTIAN, A: Effects of glucocorticoid steroid on renal acid-base equilibrium. Proc. Am. Soc. Nephrology 129A, 1978.