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Urinary Concentrating Defects in Metabolic Alkalosis:
Intrarenal and Neuroendocrine Factors

Submitted by

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in partial requirement for the Degree of Master of Science
(Physiology)

Submitted to

The School of Graduate Studies, The University of Ottawa

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Doreen C. Sztorc, Ottawa, Canada, 1984.

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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

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ABSTRACT

This study was undertaken to establish the factors which contribute to the urine concentrating defect observed in metabolic alkalosis. A rat model of chronic, hypochloremic metabolic alkalosis was produced by the loss of gastric hydrochloric acid (HCl), but without diuretics, mineralocorticoids, nitrate substitution or acute dialysis. Dual gastric fistulae were surgically implanted into rats, which were then allowed a 2 week post-surgical recovery period prior to gastric HCl drainage every 48h (3-4x). Once alkalotic, ingestion of a chloride-free diet maintained the animals in this state. Alkalosis was defined, for the purposes of this study, as whole blood pH \geq 7.42, and plasma bicarbonate \geq 30.0 mEq/L.

The alkalosis produced by this method of gastric HCl drainage is characterized by hypochloremia, hyponatremia, hypokalemia, plasma hypo-osmolality, polyuria, polydipsia, decreased body weight and increased hematocrit. The last three characteristics suggest the presence of extra-cellular fluid volume (ECFV) contraction.

Hypochloremic metabolic alkalosis in this study resulted in the development of a urinary concentrating defect. The defect was not corrected through the administration of potassium by gavage and since the extent of depletion was

not determined on tissue samples prior to the repletion no conclusion can be drawn from this experiment. The defect was also not corrected by restricting the alkalotic animals to the amounts of water ingested by control animals. The defect was partially corrected by the administration of exogenous antidiuretic hormone (ADH) (1 I.U.), but the alkalotic rats were still unable to concentrate their urine to control levels.

One possible explanation is that the chronic plasma hypo-osmolality in alkalosis may have been an important stimulus to prevent the release of ADH and lead to the concentrating defect. Extra-cellular fluid volume contraction, hyponatremia and hypochloremia are known stimuli for renin and angiotensin II (AII) release. This may explain the accompanying polydipsia. The increase in AII levels in alkalosis would also increase ADH secretion. However, I hypothesize that the stimulus of hypo-osmolality in hypochloremic metabolic alkalosis overrides the other stimuli to ADH release. Thus, the urinary concentrating defect appears to depend on both renal and extra-renal factors.

INTRODUCTION

Metabolic Alkalosis: Its Generation and Maintenance.

According to most textbook definitions, metabolic alkalosis is a disturbance in acid-base (A-B) homeostasis in which whole blood pH is increased due to either a loss of acid or a gain in alkali. Metabolic alkalosis is defined in this study as a whole blood pH ≥ 7.42 , and plasma bicarbonate (HCO_3) ≥ 30.0 mEq/L.

Metabolic alkalosis is generated by an excessive loss of acid, through renal or extra-renal mechanisms, or through a gain in alkali (Jacobson and Seldin, 1983; Seldin, 1976; Seldin and Rector, 1972). Renal acid loss may ensue from: 1) increased tubular flow rate/distal delivery of sodium (Na), which can be caused by diuretics; 2) persistent mineralocorticoid excess as found in hyperreninemic states such as extracellular fluid volume (ECFV) contraction, magnesium deficiencies, and Bartter's syndrome, which is characterized by severe hypokalemia and hypermineralocorticoidism (the former leads to an increase in urinary ammonium and total acid excretion which, in conjunction with the latter results in metabolic alkalosis), or primary hypermineralocorticoid states such as Cushing's syndrome and adrenogenital syndrome; 3) increased tubule

lumen negativity due to an increase in non-reabsorbable anions; and possibly, 4) a decrease in parathyroid hormone/increased calcium concentration (Cogan et al., 1983; Seldin and Rector, 1972; Seldin, 1976). Bartter's syndrome patients suffer from severe hypokalemia and as such are not really comparable to the gastric model of metabolic alkalosis. Also, aldosterone levels were not measured in this study, therefore, the relevance of the syndrome to the model can not be discussed in this context.

An extra-renal loss of acid may occur by: 1) loss of acid into gastric juice due to vomiting, suction, or fistulae; 2) acid moving into the cells due to potassium-deficiency or glucose induced alkalosis in fasting; and, 3) loss of acid into the stool - congenital chloridorrhea (Cogan et al., 1983; Nascimento and Calcagno, 1981; Seldin and Rector, 1972; Seldin, 1976). A gain in alkali can come from exogenous administration of: 1) bicarbonate as sodium bicarbonate for therapy of acidosis; 2) carbonate as antacids/milk-alkali syndrome; 3) acetate from hyperalimentation; and, 4) citrate from blood transfusions (Cogan et al., 1983).

Metabolic alkalosis is a complex disturbance with many symptoms. Some symptoms are similar to those seen in hypercalcemia, including mental confusion, a decreased sensitivity to pain, a predisposition to seizures,

paresthesia, muscle cramps and tetany. Symptoms may also include those found in electrolyte disorders, if these accompany the alkalosis, i.e. hypokalemia, hypophosphatemia etc. (Cogan et al., 1983). The metabolic consequences of hypochloremic alkalosis include increased pH, HCO_3^- , pCO_2 , plasma renin activity (PRA), angiotensin I concentration (AI), decreased plasma chloride (Cl), sodium (Na), and potassium (K) levels, decreased urine concentrating ability (Max Uosm), and the development of polydipsia and polyuria (Cogan and Liu, 1983; Galla and Luke, 1977; Kassirer and Schwartz, 1966; Nascimento and Calcagno, 1981; and Schwartz and Cohen, 1978). In many instances metabolic alkalosis in conjunction with K-depletion can produce interstitial nephritis (Cronin and Knochel, 1978) and it has also been shown that hypochloremia alone can lead to nephrocalcinosis (Levine et al., 1974). If metabolic alkalosis is not treated and becomes severe, death may result from renal failure, cardiac arrest (due to hypokalemia), or tetany.

Metabolic alkalosis may be maintained, in the presence of increased arterial HCO_3^- by: 1) increasing absolute proximal HCO_3^- reabsorption; 2) decreasing glomerular filtration rate (GFR) (Cogan and Liu, 1983) ; or, 3) increasing absolute distal HCO_3^- reabsorption. The first two mechanisms are mediated through hypochloremia and hypokalemia. Hypochloremia caused by vomiting or gastric

drainage leads to a contracted ECFV, a decreased GFR, increased plasma renin activity (PRA) and increased plasma aldosterone concentrations. These effects of hypochloremia may lead to increased HCO_3 reabsorption by the kidney. Hypokalemia, caused by the increase in renin and aldosterone by hypochloremia, leads to a decreased GFR possibly due to increased production of the vasoconstrictor agents angiotensin II (AII) and thromboxane B₂. Mechanism 3 might be mediated through the increase in plasma aldosterone levels. (Cogan et al., 1983).

Clinically, metabolic alkalosis may occur as a result of vomiting and gastric suction (Figure 1). This produces alkalosis via a combination of the following factors: 1) loss of HCl in the vomitus; 2) loss of potassium chloride (KCl), with most of the K lost through renal mechanisms due to the ensuing hypochloremia leading to increased renal HCO_3 reabsorption; and, 3) loss of sodium chloride (NaCl), with most of the Na also lost through renal mechanisms leading to a contracted ECFV and also to activation of the renin-angiotensin hormone system. The loss of Cl in this manner necessitates an increase in plasma of another reabsorbable anion. This is accomplished through the increased renal reabsorption of bicarbonate. Pitts et al., (1946) showed that this is a reciprocal relationship. The

Figure 1

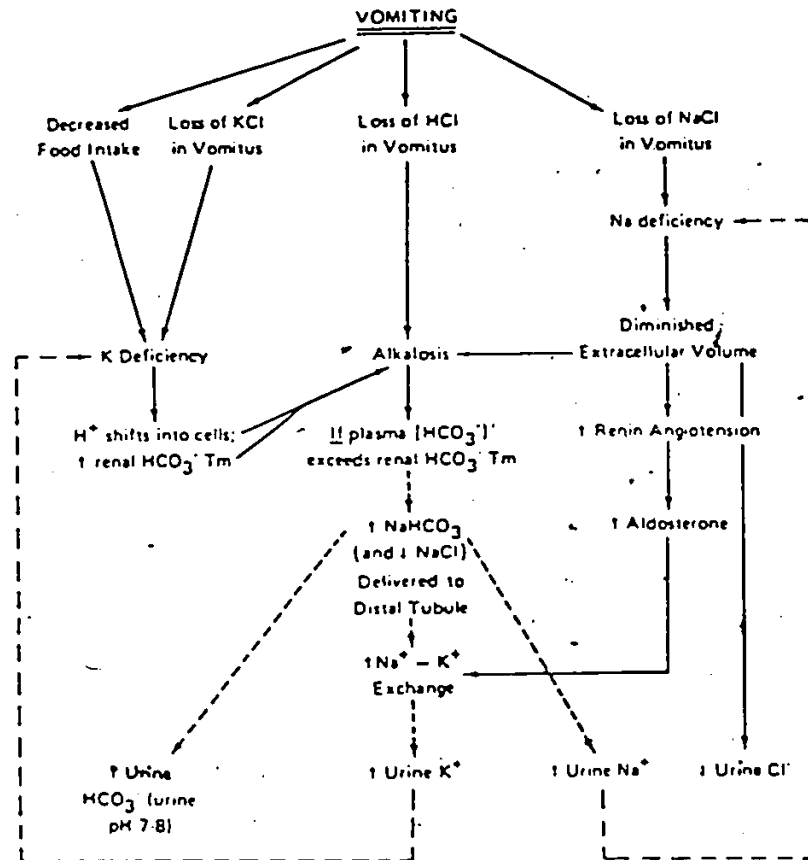


Figure 1: A schematic adapted from Seldin and Rector (1972) illustrating possible factors which may contribute to the initiation of alkalosis associated with vomiting or gastric drainage. It is now believed that the Na-K exchange does not occur; rather, it is thought that the tubule actually secretes K due to the effect of aldosterone and other factors.

increase in plasma bicarbonate as plasma Cl decreases may be responsible for the initial rise in pH leading to alkalosis.

In my study, I wanted to develop a rat model of metabolic alkalosis which closely resembled that caused by vomiting or gastric suction or drainage. This was accomplished by surgical implantation of dual gastric fistulae into the stomach of rats. After post-surgical recovery the animals underwent gastric HCl drainage every second day for a total of three or four times, until metabolic alkalosis could be diagnosed by analyzing cardiac puncture blood samples. Since rats do not possess a natural vomiting reflex, it was necessary to implant chronic gastric fistulae to promote a loss of HCl by gastric drainage. Gastric drainage (suction) mimics vomiting and produces alkalosis by removing the same components of the gastric contents as does vomiting. Gastric drainage, therefore, produces alkalosis as diagrammed in Figure 1. Alkalosis caused by vomiting and/or gastric drainage is hypochloremic in nature due to the loss of Cl as HCl, KCl, and NaCl in the vomitus, and has been produced experimentally in the dog (Needle et al., 1964) and rat (Cooke et al., 1952). The amount of Na lost in gastric juice is slight at high secretion rates and K loss increases as the gastric acid flow rate increases (Makhlouf, 1981). Thus, at high rates the loss of K may contribute to the hypokalemia of gastric

alkalosis. Sodium lost in the gastric juice may be considerable at low flow rates (Makhlouf, 1981); and as such may contribute to the hyponatremia of gastric alkalosis. The flow rates were not measured in this study and food contamination in the gastric juice collected precluded measurement of Na and K.

Hypochloremic alkalosis as described above in the dog and rat can only be repaired by the administration of a salt containing Cl (Atkins et al., 1962; Hulter et al., 1978; and Wallace et al., 1968). Gulyassy et al. (1962) found that Cl was necessary to repair alkalosis due to selective Cl-depletion even when K was not replaced. Similar results were obtained by Kassirer and Schwartz (1966), and Needle et al., (1964). Galla et al., (1983) have proposed that Cl administration decreases plasma renin, leading to a decrease in aldosterone, and causing distal HCO₃ excretion, thus correcting the alkalosis. This occurs regardless of Na balance, hypokalemia, HCO₃ loading and without volume expansion. Chloride-depletion is associated with urine concentrating defects in the rat (Luke et al., 1977) and dog (Rosin et al., 1970). Clearance studies have demonstrated that free water reabsorption is impaired during hypochloremia (Luke et al., 1977; and Wallin et al., 1973).

Urine Concentrating Ability.

Urine concentrating ability is influenced by both neuroendocrine and intrarenal factors. The renal mechanisms for achieving a maximally concentrated urine are complex and not yet fully delineated. First, the collecting duct must respond adequately to ADH to allow water reabsorption to take place. Second, the medullary thick ascending limb of Henle must also respond to ADH to increase NaCl reabsorption, as recently shown by Hall and Varney (1980), Hebert et al., (1981), Greger et al. (1981), Knepper et al., (1983) and Velazquez et al., (1982). These two mechanisms are necessary to enable tubular fluid to equilibrate with the renal interstitium within the cortex and medulla. Third, medullary hypertonicity must be maintained by adequate NaCl delivery to the loops of Henle, normal rates of NaCl transport out of both thick and thin ascending limbs, low medullary blood flow, delivery of adequate amounts of urea to the medulla and its subsequent reabsorption by urea permeable collecting ducts. Finally, by low rates of fluid delivery to the inner medullary region where maximum urine concentration is effected. These aspects of nephron function appear to operate independently for the most part, but there is a great deal of interest in elucidating possible interactions between these intrarenal

factors and the known endocrine factors involved in fluid and electrolyte homeostasis.

More than thirty years ago evidence indicated that in the rat, Cl-depletion may interfere with normal water conservation (Cooke et al., 1952). Since then, numerous studies have evaluated concentrating ability of the rat and dog using nitrate or bicarbonate administration to displace Cl. For example, nitrate infusion in the dog leads to an acute reduction in free-water clearance (Luke et al., 1977; Rosin et al., 1980; and Wallin et al., 1973). In a more chronic preparation, nitrate induced Cl-depletion alkalosis has been associated with a concentrating impairment (Berl et al., 1977). Burg and Green (1973) and Rocha and Kokko, (1973) implicated active Cl transport in the thick ascending limb of Henle (TAL) as the primary driving force in the counter-current multiplication system responsible for urinary concentration and dilution. Berliner (1982) recently critically reviewed the various possible mechanisms of urine concentration. He suggests that evidence to date supports the counter-current multiplication system as the most likely explanation of urine concentrating mechanisms. The TAL appears to transport NaCl actively. Recently, Greger, (1981), Knepper and Burg, (1983), Hebert et al., (1981), and Velazquez et al., (1982) provided evidence that the TAL actually transports Cl actively, secondarily, and in

co-transport with primary Na transport. Greger, (1981) illustrated that this co-transport also involves the K ion. Secondary active Cl transport is driven by the electrochemical gradient for Na from the lumen to the cell interior. This depends on the baso-lateral Na-K-ATPase for removing Na from the cell to keep the gradient intact. The baso-lateral Na-K-ATPase activity is dependent on both K and Na.

Recently, it has been suggested that concentrating defects can occur independently of ECFV changes (Luke et al., 1977). Galla and co-workers also illustrated that hypochloremic metabolic alkalosis can be repaired by the addition of a Cl containing salt without repletion of the ECFV (1983). Luke et al. (1977) found increased urine flow rate, natriuresis, and diminished free-water reabsorption in acutely Cl-depleted rats which were not altered by administration of ADH. It is possible that the dialysis procedure may have altered ADH function or concentration and therefore the conclusions they arrived at may not be accurate. The results of more recent studies (Luke et al., 1978; and Galla et al., 1981) suggest that selective Cl-depletion alkalosis is associated with impaired solute removal by the TAL, leading to the development of a concentrating defect. Gutsche et al. (1982) recently obtained direct evidence in perfused nephrons that nitrate

or bicarbonate substitution for Cl leads to impaired solute removal by the TAL in vivo. These studies are consistent with the view that metabolic alkalosis decreases NaCl reabsorption by the TAL, impairing renal diluting and concentrating ability (Bank et al., 1964). However, factors such as changes in water intake and ADH have not been examined in chronic metabolic alkalosis. The presence of polydipsia or decreased release of ADH could contribute substantially to an impairment in renal concentrating ability.

Galla et al., (1981) stated that TAL function may be an important, if not critical, factor in urine concentration in states of Cl-depletion. They also demonstrated that reduced plasma Cl concentration may serve as the principal stimulus for the renin-angiotensin system, thereby providing a key link between the hormonal mechanisms known to be involved in fluid and electrolyte balance and the various intrarenal effectors controlling urinary concentrating ability.

The Renin-Angiotensin-ADH system.

The roles of renin, angiotensin and ADH in fluid and electrolyte homeostasis have been studied for many years. Verney in the 1940's first demonstrated that ADH release

from the hypothalamo-neurohypophyseal tissue was affected by blood volume as well as osmotic changes. More recently, Robertson (1977) and Vander (1967) argued that ADH, as well as the renin-angiotensin system, has an integral role in body fluid and electrolyte homeostasis. It is well documented that AII is a potent dipsogen (Kucharczyk and Mogenson, 1975; and 1977), therefore playing an important role in ECFV homeostasis. Activation of the renin-angiotensin system by hypovolemia, hypochloremia, or hyponatremia leads to increased secretion of ADH (Abboud et al., 1979, Bonjour and Malvin, 1970, Dunn et al., 1973, and Haack and Mohring, 1978) and aldosterone (Laragh et al., 1972). This, in turn, leads to increased kidney reabsorption of water, Na, and Cl (Laragh et al., 1972, Robertson, 1977, Vander, 1967). Thus it is clear that factors influencing the renin-angiotensin system will also influence renal water and electrolyte metabolism. New evidence of a second site of action of ADH in the kidney by Hall and Varney (1980), Hebert et al., (1981) and Knepper and Burg (1983) increases the importance of ADH in fluid and electrolyte homeostasis. The second site of action is the medullary TAL. Antidiuretic hormone functions to increase NaCl reabsorption from this portion of the nephron (Hall and Varney, 1980; Hebert et al., 1981; and Knepper and Burg, 1983).

In their study of sodium and water conservation in rats acutely depleted of Cl, Luke et al. (1977) argued that increases in urinary flow rate, diminished free-water reabsorption and natriuresis were not related to several renal factors, including the secretion of ADH, or to an osmotic diuresis. To exclude the possibility that deficient ADH secretion was responsible, intramuscular injections of Pitressin tannate in oil (250 munits) were administered on two occasions before the start of the experiment. However, while this study controlled for deficient ADH secretion, it did not control for a possible damping of ADH's effect at the level of the renal tubule. Subsequently, Luke and his colleagues made several other observations consistent with the view that selective Cl-depletion alkalosis is associated with impaired TAL solute removal, thus leading to a concentrating defect, and that Cl-depletion per se may stimulate renin release as well (Galla et al., 1981).

Although considerable effort has been devoted to understanding the factors influencing ADH-angiotensin-aldosterone-interactions in the normal animal, the possible interrelationships between these endocrine mechanisms and the intrarenal determinants of urine volume and composition have not been investigated in the chronic, hypochloremic alkalotic preparation. A further complication of hypochloremic alkalosis is hypokalemia since

it is now well established that K-depletion alone can result in renal concentrating defects (Berl et al., 1977; and Peterson, 1980).

K-depletion.

Numerous investigations have attempted to determine the extent to which hormonal or intrarenal factors may be responsible for the urine concentrating defects associated with acute and chronic disturbances of electrolyte and acid-base balance. In K-depletion, for example, altered renal hemodynamics, medullary solute concentration, and ADH release or target epithelium action have all been postulated as possible mediators of the concentrating defect (Berl, 1980). Medullary blood flow, however, has been shown to be decreased (Whinnery and Kunaw, 1979) and could not effect a "washout" of medullary solute. Antidiuretic hormone release also appears to be normal in K-depletion (Berl, 1980), and the diminution in urinary concentrating ability seems unrelated to circulating levels of ADH (Berl et al., 1977). It remains possible that the collecting duct epithelium may be insensitive to ADH (Carney et al., 1976). Even if ADH was normally active, a driving force for water movement in the form of high medullary solute concentrations must still

be present to maximally concentrate the urine. This is clearly not the case in K-depletion (Berl, 1980).

Based on data from clearance studies in K-depleted rats, Eknoyan et al., (1970) proposed, that both impaired concentrating and diluting ability are the result of reduced Na transport by the TAL into the medullary interstitium. Gutsche et al. (1982) demonstrated a striking impairment in TAL function in K-depleted rats by single nephron perfusion, and by conducting measurements of TAL fluid presented to the early distal site. This impairment was rapidly reversed when K stores were repleted. These studies further suggest that augmented NaCl backflux, rather than active NaCl transport impairment in the TAL (Greger, 1981; and Greger et al., 1981), may explain the inability of K-depleted rats to excrete maximally concentrated urine.

Cl-depletion.

There is also some evidence that Cl transport in the TAL may provide the signal for renin release by the macula densa of the kidney (Abboud et al., 1979; Galla et al., 1979; Kirchner et al., 1978; and Kotchen et al., 1978). In their studies of acute and chronic NaCl loading in the rat, Galla et al. (1979) found that greater Cl reabsorption in the TAL


was associated with suppression of renin release and vice versa. Indeed, recent in vitro studies suggest that Cl depletion may inhibit the stimulatory action of renin-angiotensin on ADH and aldosterone release. This occurs by reducing the level of activity of angiotensin converting enzyme (Yang and Neff, 1972), or by reducing intracellular (Laragh et al., 1972) or plasma (Abe et al., 1972) renin activity or concentration.

In the studies referred to above, as well as others concerned with the effects of anions on urine concentrating mechanisms (Galla and Luke, 1979; Luke et al., 1977; Rocha and Kokko, 1973; and Wallin et al., 1973), no observations were made on chronic Cl-depleted animals with reference to maximum urine concentrating ability, simultaneous plasma osmolality measurements, plasma ADH concentrations, effects of water restriction, or effects of replacement of ADH. Since an alteration in renin-angiotensin levels by Cl-depletion might be expected to alter ADH release (Bonjour and Malvin, 1970, Haack and Mohring, 1978, Robertson, 1977, Verney, 1946), measurement of such hormonal changes would appear to be required to determine the possible role the renin-angiotensin-ADH axis plays in the concentrating defect seen in Cl-depletion alkalosis. Nor have there been any studies of the function of the TAL in which the various volume, osmotic, and ionic factors that impinge on this

segment of the nephron during chronic Cl-depletion have been systematically examined.

Purpose.

Therefore, my primary interests in undertaking these investigations were: 1) To determine if gastric HCl drainage in rats will produce a chronic, hypochloremic metabolic alkalosis; 2) To determine if a renal concentrating defect develops in chronic, hypochloremic metabolic alkalosis; 3) To determine the involvement of Cl, Na and K in the concentrating defect; and, 4) To determine the role of the renin-angiotensin-ADH system in the concentrating defect.



METHODS

(i) Animals, Care and Diet.

The 92 female and 10 male Sprague-Dawley rats (Charles River, Montreal, P.Q.) were housed in individual, wire mesh, metabolic cages. They were allowed ad libitum access to either Purina Rat Chow (Ralston-Purina) or a synthetic Cl₂-free diet (Levine et al, 1974), and either tap water or distilled water.

Except for 10 non-surgical controls, all animals received either dual gastric cannulae, implanted using sterile surgical techniques, or a sham operation. They were allowed a minimum of one week for post-surgical recovery. Food and water intakes and urine outputs (± 1.0 g), as well as body weights (± 1.0 g), were measured at the same time each day. The study began when body weights were equal to or surpassed pre-surgical levels.

At the start of the study, animals were randomly divided into one of three groups: 1) chow; 2) control; and, 3) alkalotic. Chow animals were fed Purina Rat Chow and tap water ad libitum and did not undergo gastric drainage. Control rats were fed the synthetic Cl₂-free diet and distilled water ad libitum. These animals underwent all surgical procedures but did not undergo gastric hydrochloric

acid drainage. Alkalotic rats were fed the synthetic Cl-free diet and distilled water ad libitum. These animals also underwent all surgical procedures, but unlike the other two groups the alkalotic rats underwent gastric HCl drainage as described in Section (iii).

The synthetic Cl-free diet contained the following ingredients expressed as g/5.0 kg of diet: (g% dry wt)

Sodium Carbonate (Na_2CO_3) 5.75 (0.115)

Magnesium Carbonate n Hydrate
($\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot n\text{H}_2\text{O}$) 7.95 (0.159)

Potassium Carbonate (K_2CO_3) 8.70 (0.174)

Potassium Sulphate (K_2SO_4) 9.05 (0.181)

Basal Diet (TD 78093, formerly 72105,
Teklad Electrolyte Diet, Teklad Test Diets,
Madison, Wisc.) 4968.50 (99.37)

Distilled Water (dH_2O) 3.0 l.

The levels of Na, K, and sulphate in the diet surpass the minimum recommended levels set by the National Research

Council (1962). The level of Cl in the diet was undetectable using a CMT 10 Chloride Titrator (Radiometer, Copenhagen) (< 0.001 mg/kg).

(ii) Surgical Procedures

The method of implantation of dual gastric cannulae was modified from Borella and Herr (1971). This surgical procedure was undertaken to allow drainage of gastric HCl from conscious rats in an attempt to produce metabolic alkalosis without the necessity for dialysis, anaesthesia or drug therapy.

Animals were anaesthetized with pentobarbital sodium at a dose of 25-35 mg/kg (Somnotol 65 mg/ml, MTM, Hamilton, Ont). They were prepared for sterile abdominal surgery by shaving the area and swabbing with alcohol and proviodine. A mid-line abdominal incision extending down 2-3 cm from the tip of the xiphoid process was made. The stomach was carefully externalized, taking care not to stretch the esophagus, and was wrapped in a sterile 0.9% saline soaked swab to prevent the tissue from drying. Another saline soaked swab was placed over the abdominal incision to prevent the abdominal cavity from drying.

The fundus of the stomach was incised for a length of 3.

up along the greater curvature. One cannula (8 ga stainless steel hypodermic tubing, (Small Parts Inc., Miami, Florida), tooled to fit 1/4-40 fillister head screws (Small Parts Inc.) and PE-380 tubing (flanged)) was carefully rotated into the incision until the flanged end was inside the stomach (Fig.2a). The tissue was closed over the flange with a double-inverting purse-string suture (3.0 silk, Craville, Montreal, P.Q.). The procedure was repeated on the antral portion of the stomach. Care was taken not to nick the gastric mesenteric vessels or to let the gastric juices drain into the abdominal cavity.

The stomach was unwrapped and placed back into the abdominal cavity. A trochar was used to produce punch wounds in both the left and right abdominal walls below the ribcage, to allow exteriorization of the cannulae. The cannulae were exteriorized and sutured into place with a double-inverting purse-string suture using 3.0 silk (Fig.2b).

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The abdomen was closed in two layers. First, the muscle layer was closed with 3.0 silk in a continuous stitch and second, the skin was closed with stainless steel wound clips (Fig. 1c). The rat was returned to its cage and allowed at least one week to recover from the procedure.

The gastric cannulae and screws were checked daily and the wound clips were removed one week post-surgically. The



Figure 2A: Insetion of the gastric cannula into a rat stomach. The flange can be seen through the tissue at the arrow.

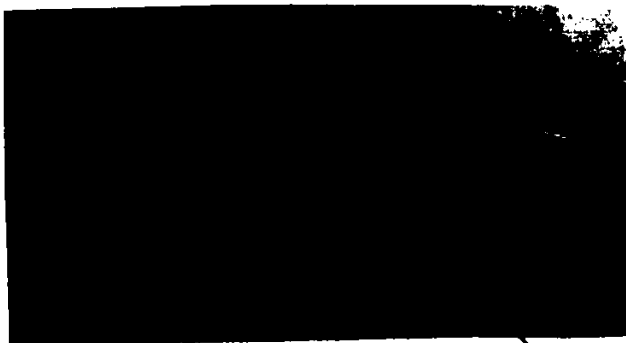


Figure 2B: Exteriorization of cannulae through puncture wounds in the flank.



Figure 2C: Closure of the incision with 3.0 silk and wound clips. A purse-string suture secures the cannula to the skin. A gastric cannula shown actual size is to the left of the photograph.

survival rate in 30 animals was 97%.

A second surgical procedure, inferior vena cava (IVC) cannulation, was performed following the methods of Kaufmann (1930). This cannulation method was carried out to allow for chronic, unstressed blood sampling in conscious animals. Seventy animals and 6 months time were invested in this technique, but it was abandoned due to technical problems. The major difficulty was maintaining the patency of cannulae for the duration of the study. Cardiac punctures had to be carried out as soon as patency was lost. This procedure was therefore abandoned and the cardiac puncture technique was used throughout the study.

G.I. Intake Techniques

Restraint cages, with open sides to allow access to the cannulae, were constructed from threaded, nickel-plated, brass rods (CFR-1420, Small Parts Inc., Miami, Florida), 1/4"-20 bolts and plexiglas (Fig. 3). The cages were placed on a raised platform to allow for collection of the gastric juices into glass tubes by gravity.

Animals were placed in the restraint cages for 2 h every second day for a week to become accustomed to the cages and to having their cannulae manipulated. Once



Figure 3A: Restraint cage with open sides to allow access to the gastric cannulae.

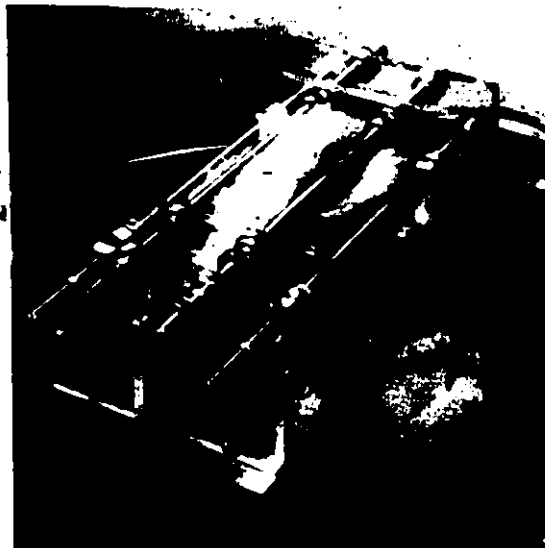


Figure 3B: A rat set up for gastric hydrochloric acid drainage. The tygon tubing leads to a glass collection tube.

trained to remain quiet, the animals were drained of gastric HCl for 3-4 h every second day for a total of 3-5 drainage periods.

The drainage period began with placement of the animals into the restraint cages, removal of the cannulae screws, and placement of tight-fitting tygon tubing into each of the cannulae (Fig.3b). The tubing lead to glass tubes for the collection of the gastric HCl and infusate. Ten ml of distilled water was infused at the rate of 1 ml/min. with a 30 m delay between each infusion. The delay was necessary to allow emptying of the stomach by gravity. Each animal received 50-60 ml of distilled water intragastrically during each session and fluid recovery was approximately 98%. At the end of the session the tygon tubing was removed and washed, the screws were replaced into the cannulae and the animals were returned to their metabolic cages.

(iv) Blood Sampling and Analysis

Cardiac Puncture. All animals were anaesthetized with pentobarbitol sodium (25 - 35 mg/kg) and the heart was located by palpating the chest wall. A heparinized 22 ga needle, attached to a 3 ml heparinized syringe, was inserted into the heart through the chest wall. A 1.5 ml blood

sample was withdrawn for A-B, electrolyte, osmolality, protein and hematocrit analyses. The needle was then removed from the animal and any air present in the sample was forced out of the syringe. The animals were placed into their home cages to recover for a minimum of 4 days before the next procedure.

Analyses. The blood sample was kept on ice until aliquoted for analysis, approximately 30 to 90 min. Whole blood in triplicate 50 ul samples was used to measure pH on a model PHM72 Digital Acid-Base Analyzer (Radiometer, Copenhagen). Another 20 ul of whole blood was used to analyze hematocrit. The blood was placed into a heparinized micro-capillary tube, spun in an IEC micro-capillary centrifuge Model MB, and read on an IEC micro-capillary reader Model CR (IEC, Needham Hts., Mass.). The plasma in the hematocrit tube was used for protein analysis utilizing a Model 10406 Proteinmeter (American Optical, Buffalo, N.Y.). Whole blood was also aliquoted into micro-centrifuge tubes and spun in an Eppendorf Centrifuge 3200 (Brinkmann Instruments, Rexdale, Ontario) to separate the plasma from the cells. The plasma was used for analysis of osmolality, total carbon dioxide (total CO₂) and electrolytes. Total CO₂ was analyzed in duplicate (10 ul) on a Corning 965 CO₂ Analyzer (Corning Medical, Medfield, Ma.). Plasma bicarbonate (HCO₃)

and partial pressure of CO₂ (pCO₂) were calculated using both the total CO₂ and pH values as described in Appendix 1.

Osmolality was measured in 8 ul duplicate plasma samples on a Wescor 5100 CXR Vapour Pressure Osmometer (Johns Scientific). Plasma Na, Cl and K were also measured in duplicate samples. Plasma Cl (10 ul) was analyzed on CMT 10 Chloride Titrator (Radiometer, Copenhagen). Plasma Na and K were measured simultaneously on an IL Model 343 and 443 Flame Photometer (Instrumentation Laboratory Inc., Lexington, Ma.) with lithium as the internal standard. The anion gap was calculated as described in Appendix 1.

Abdominal Aorta - Terminal Sample. All animals were anaesthetized with pentobarbital sodium (25 - 35 mg/kg) and a 6-8 cm mid-line abdominal incision was made. The intestines were reflected to one side and a section of the aorta was isolated. An 18 ga needle, attached to a heparinized 3 ml syringe, was inserted into the abdominal aorta and a 1.5 ml blood sample was withdrawn. The 3 ml syringe was quickly substituted with a 10 ml syringe containing ethylene diamine tetra-acetate (EDTA). The heparinized sample was processed as previously described.

A blood sample was drawn into the 10 ml syringe until breathing ceased (3-7 ml). The sample was transferred to a test tube containing EDTA and spun in a Damon/IEC HN-SII

Centrifuge (Needham, Ma.). The plasma was aliquoted into micro-centrifuge tubes and stored at -20 C until radioimmunoassay analyses for PRA, angiotensin I (AI) concentration and ADH concentration could be carried out. These procedures are described in Section (x) and Appendices 2 and 3.

(v) Study #1 - Preliminary Study on the Suitability of Gastric Drainage as a Model for Producing Metabolic Alkalosis:

Twelve female Sprague-Dawley rats underwent surgery and gastric drainage as described in the previous two sections to test the suitability of the model for producing metabolic alkalosis. Blood samples, for analysis of acid-base status (described in section (iv)), were collected by cardiac puncture under pentobarbital anaesthesia after the third drainage period was complete. Gastric HCl drainage was discontinued if metabolic alkalosis, as defined in the Introduction, was present. Two additional drainage sessions took place if metabolic alkalosis was not present. A second blood sample was taken for analysis as described above and in the following section. All drained animals were alkalotic after the fifth drainage session, while 62% were

alkalotic after the third drainage session. Therefore, the model is suitable for producing metabolic alkalosis and was used to produce alkalosis in all other studies undertaken.

(vi) Study 2 - Urine Concentrating Ability - Max Uosm.

This study was undertaken to elucidate whether metabolic alkalosis of gastric origin would produce a urinary concentrating defect, as has been reported for alkalosis induced by other procedures (see pp. - 5 in the Introduction).

Twenty-five female Sprague-Dawley rats underwent all procedures as described previously in the Methods. Food and water intakes were monitored daily to the nearest 1.0 g except on days when experiments or blood sampling took place. Urine output was also monitored daily to the nearest 1.0 g. The urine was collected under oil to prevent evaporation and thymol was added to prevent bacterial growth.

Renal function was evaluated in all animals by a test of maximum urinary concentrating ability after 18 h of food and water deprivation. This test was performed prior to the onset of alkalosis and at least twice during the period of

stable alkalosis. Intermittent blood samples were taken to confirm that the alkalosis was stable. The test entailed overnight (18 h) food and water deprivation and collection of a 4 h urine sample, under oil, in the morning (0800 - 1200 h). The total volume was measured and aliquots of the sample were analyzed in duplicate to ascertain the maximum osmolality (Wescor 5100 CXR Osmometer) developed by the animals in the dehydrated state. Urine Cl, Na and K concentrations were also analyzed.

The animals were allowed ad libitum access to food and water at the end of the 4 h collection period and throughout the recovery period which was a minimum of 4 days between all tests of Max Uosm and/or blood samples. These tests showed the presence of a defect in concentrating ability in metabolic alkalosis of gastric origin (refer to pp. 48 of the Results section).

(vii) Study 3 - The Second Study of Max Uosm.

This study was undertaken to determine whether the low plasma K levels were responsible, in whole or in part, for the urinary concentrating defect in gastric metabolic alkalosis.

A group of 25 female and 3 male Sprague-Dawley rats

underwent surgical and drainage procedures as described previously. All animals underwent a test of Max Uosm prior to the onset of alkalosis. Two more tests of Max Uosm were performed while the animals were in a state of stable alkalosis. Experimental manipulations were performed after these three initial tests of Max Uosm were performed. Intermittent blood samples were taken to ensure that the alkalosis was stable.

K-repletion was carried out by gavage with a 450 mEq/L solution of potassium phosphate (K_2HPO_4). Each rat received either 1.0 mEq K per 100 g body weight or an equivalent volume of dH₂O as a control for the stress of the gavage procedure. The gavage volumes ranged from 4.5 to 11.6 ml. All animals were deprived of food and water for 18 h (overnight) beginning 8 h post K-repletion. A test of Max Uosm was performed the next morning from 0800 to 1200 h. The urine was analyzed as described earlier.

All animals were allowed a 48 h recovery period prior to sacrifice. A terminal blood sample was obtained and analyzed as previously described. In addition to the blood sample, the left kidney was removed, dissected clean of fat and connective tissue, and placed in 10% buffered formalin for histological analysis.

(viii) Study 4 - The Effect of Exogenous ADH Administration

This study was undertaken to investigate the roles of polydipsia, ADH, and K in the urinary concentrating defect observed in gastric metabolic alkalosis.

A group of 29 female and 7 male Sprague-Dawley rats were prepared as previously described. All animals underwent a test of Max Uosm prior to the onset of alkalosis. At least two more tests of Max Uosm were performed while the animals were in a state of stable alkalosis. Experimental manipulations were performed after the three initial tests of Max Uosm. Intermittent blood samples were taken to ensure that the alkalosis was stable.

Following the preliminary tests of Max Uosm, alkalotic animals were water-restricted for 7 days. The Alkalotic animals' water intake was restricted to that of the controls (on a mls drank per 100 g body weight per 24 h basis). The alkalotic animals remained water restricted throughout the duration of the study, except on days of blood sampling or tests of Max Uosm.

Both groups of animals underwent a test of Max Uosm after the alkalotic animals had been water restricted for at least 7 days. All animals were then allowed ad libitum access to food and water. The alkalotic animals were placed back on the water restriction regime the next morning (i.e.

18 h after ad libitum access to water). The urine was analyzed as described earlier and the effects of water restriction were noted. This test was repeated before further manipulations took place.

A 4 day recovery period was allowed between procedures, after which all animals received 1 I.U. synthetic ADH as Pitressin Tannate in peanut oil (Parke-Davis, Scarborough, Ont). The alkalotic animals were still being water restricted at this time. The pitressin tannate was administered sub-cutaneously (s.c.) on two occasions: 1) 500 mI.U. in 0.1 ml when the food and water were removed for the night; and, 2) 500 mI.U. in 0.1 ml the next morning prior to collection of the 4 h urine sample. The urine sample was analyzed as described previously and the effects of exogenous ADH on the concentrating defect were noted. The animals were allowed a 4 day recovery prior to the next experimental manipulation.

The alkalotic animals were water restricted again when their 18 h of ad libitum access to water, after the urine collection period, was complete. Once the 4 day recovery period had expired the K-repletion study was repeated, this time during water restriction. The repletion procedure was identical to that described earlier. All animals were sacrificed 2 days after recovering from the K-repletion procedure. All blood and tissue samples were collected and

analyzed as described previously and throughout the following sections. A sample of the gastronemius muscle was removed and dissected clean of fat and connective tissue. The sample was weighed and frozen for future analysis of tissue Na and K as described in the next section.

(ix) Muscle Analysis

A portion of the right gastronemius muscle was removed from all rats for analysis of tissue Na and K by flame photometry. The sample of gastronemius muscle was removed from the rats after partial exsanguination. The muscle was dissected clean of obvious fat layers and connective tissues and weighed to the nearest gram on a Mettler 454 Balance. The tissues were dried at 105°C for 24 h and a constant dry weight was determined on the same balance. The dried tissues were ashed at 450°C for 18 h in a muffle furnace (Sybron/Thermolyne, Dubuque, Iowa).

The ash was moistened with water and concentrated nitric acid (HNO₃) was added. This ash solution was refluxed for one hour. The acid digest was evaporated to dryness and re-ashed at 400°C for one hour. The white ash was treated with 1:1 HCl, then heated and diluted with deionized dH₂O for flame photometry. Tissue extracts were

analyzed for Na and K on an IL.951 aa/ae (Atomic Absorption) Spectrophotometer (Instrumentation Laboratory, Inc., Lexington, Ma.) using lithium as the internal standard. The muscle electrolytes were expressed as mEq/100 g dry tissue weight.

(x) Hormonal Analyses

Plasma ADH concentration was measured by RIA using a Diagnostic Systems Laboratories, Inc. (Webster, Tx.) Vasopressin-Arginine RIA kit. Recovery is between 63-95% depending upon the initial concentration. The highest recovery rates occur at concentrations of 12 - 13 pg/ml ADH. The sensitivity of this assay was 0.5 pg/ml ADH. Please refer to Appendix 2 for more information.

Plasma renin activity and Al concentration were measured by RIA using a RIANEN RIA kit from New England Nuclear (North Billerica, Ma.). The sensitivity of this procedure was about 2 pg of Al and the accuracy is within 0.02 ± 0.16 to 0.08 ± 0.13 ng of Al. For more information please refer to Appendix 3. Both RIA kits utilized Iodine¹²⁵ as the radioactive label.

Samples for RIA analyses were collected into EDTA containing tubes as described earlier and were stored at -20°

C until analyzed. Prior to the RIA procedure, the samples were thawed at 4°C and extracted according to the recipe in the respective RIA kit. Once extracted, the appropriate procedure was followed. The completed samples were counted for 10 m on a LKB Wallac 1282 Compugamma Universal Gamma Counter. Detailed description of the RIA procedures are located in Appendices 2 and 3.

(xi) Renal Histology

The left kidney was removed from all animals for histological examination. This was necessary to determine whether the urine concentrating defect was due to renal nephropathy.

The kidneys were dissected clean of fat and connective tissue and were stored in 10% buffered formalin until histological analyses were performed. The kidneys were sectioned in half and set in paraffin. These sections were then cut at 10 µm on a microtome. They were stained with Harris' hematoxylin and eosin (H&E) and examined under a light microscope for evidence of renal pathology, specifically nephrocalcinosis and medullary lesions. The results are presented in Appendix 4.

(xii) Statistical Analyses

All data was analyzed on a Wang 600 Series computer. Un-paired t-tests were used when only two groups were compared and a one-way ANOVA was used when three or more groups were compared. A Scheffé test (see Appendix 1) was used to determine where the statistical significance occurred in the one-way ANOVA. A probability less than 5% ($p < 0.05$) was considered significant.

RESULTS

(i) Preliminary Study on the Suitability of Gastric Drainage as a Model for Producing Metabolic Alkalosis.

The main purpose of Study 1 was to determine whether the gastric acid drained rat was a suitable model for studies of metabolic alkalosis.

Table 1 summarizes results obtained from blood samples taken by cardiac puncture after gastric drainage was complete. Gastric drainage led to an increased whole blood pH, plasma pCO_2 , and plasma HCO_3 . The animals were alkalotic according to the definition stated in the Introduction: $pH \geq 7.42$ and $HCO_3 \geq 30.0$ mEq/L. Animals G-34 and G-45 were included in the control group in figures 4 and 5, since they did not differ significantly from controls.

Table 2 lists the results of the plasma electrolyte analyses in Study 1. Gastric drainage led to a decreased plasma concentration of Na, Cl, and K with the greatest change involving plasma Cl concentrations. The metabolic alkalosis can be described, therefore, as being hypochloremic, hyponatremic, and hypokalemic in nature.

Table 3 lists the results of plasma protein and hematocrit determinations as well as renal histology in

Table 1

Group	Treatment	pH	pCO ₂ (mmHg)	HCO ₃ (mEq/L)
Chow (4)	stock diet no drainage	7.38	47.96	27.48
Control (2)	Cl-free diet no drainage	7.45	37.14	25.03
G-34	Cl-free diet drained 2x	7.42	43.60	27.40
G-45	Cl-free diet drained 2x	7.36	33.40	18.30
G-43*	Cl-free diet drained 5x	7.45	53.80	36.30
G-46*	Cl-free diet drained 5x	7.63	52.10	51.90
G-37*	Cl-free diet drained 6x	7.60	49.20	46.80
G-39*	Cl-free diet drained 6x	7.52	42.50	33.60
G-40*	Cl-free diet drained 6x	7.52	46.10	36.50

Acid-base status in chow, control and individual data for gastric drained rats in the preliminary study on the suitability of gastric drainage as a model for producing metabolic alkalosis (Study 1).

* = alkalotic.

Table 2

Group	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
Chow (4)	142.00	3.35	106.30
Control (2)	142.00	3.35	107.00
G-34	141.50	2.60	99.50
G-45	141.00	3.30	108.00
G-43*	139.50	1.90	94.50
G-46*	126.50	1.60	51.50
G-37*	131.00	2.10	64.50
G-39*	133.00	2.40	90.00
G-40*	140.00	2.10	87.50

Plasma sodium (Na), potassium (K) and chloride (Cl) concentrations in Study 1. * = alkalotic.

Table 3

Group	Protein (mg/dl)	Hematocrit (%RBC)	Histology
Chow (4)	6.85	44.13	normal
Control (2)	7.20	44.50	normal
G-34	8.50	44.50	normal
G-45	7.10	45.50	normal
G-43*	6.80	47.50	normal
G-46*	7.20	52.00	severe nephrocalcinosis
G-37*	8.60	62.00	mild nephrocalcinosis
G-39*	6.40	51.50	normal
G-40*	7.20	51.00	normal

Plasma protein concentration, hematocrit and renal histology in Study 1.
* = alkalotic.

Study 1. Plasma protein was not affected by the gastric drainage, although the hematocrit increased during alkalosis. Renal histology was undertaken to check for the incidence of nephrocalcinosis. It was observed in two animals, but only in one animal was it severe. Data from animals showing histological signs of nephrocalcinosis were not used in Studies 2, 3, or 4.

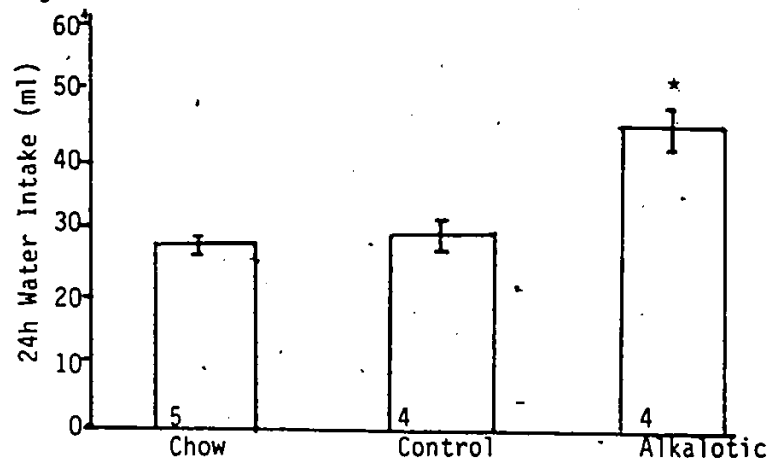
Figure 4 shows 24 h intakes of water in the three groups of animals studied. The alkalotic animals were polydipsic (44.50 ± 2.50 ml) when compared to either the chow (26.90 ± 0.78 ml) or control (28.18 ± 1.92 ml) group ($p < 0.001$). The polydipsia developed with the onset of metabolic alkalosis.

Figure 5 shows 24 h urine output, and it can be seen that the alkalotic group also developed polyuria (18.63 ± 3.33 ml) when compared to the other two groups (6.50 ± 0.75 and 8.10 ± 1.70 ml, chow and control respectively, $p < 0.001$). The polyuria occurred concurrently with the polydipsia.

(ii) Study 2 - Max Uosm.

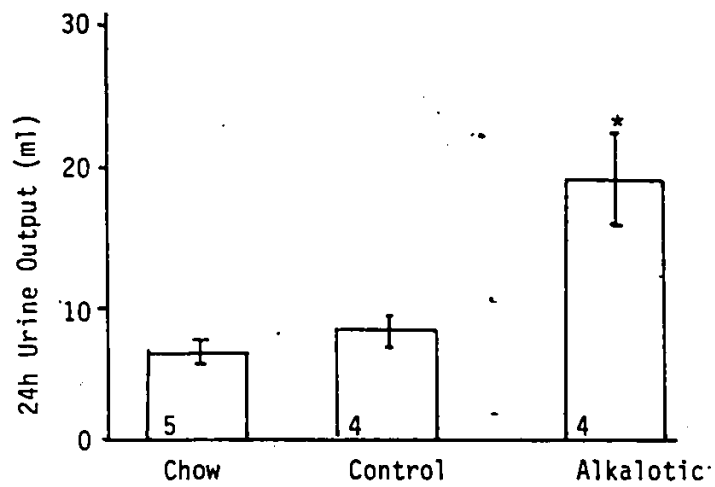
Table 4 shows the results obtained for A-B status on blood obtained by cardiac puncture at various times throughout the study period. Animals which underwent gastric drainage had significantly elevated pH ($p < 0.001$), pCO_2 ($p < 0.05$), and HCO_3 ($p < 0.001$) levels as compared to chow or control animals.

Figure 4



A histogram of 24h water intakes by chow, control and alkalotic rats in the preliminary study on the suitability of gastric drainage as a model for producing metabolic alkalosis (Study 1). Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histogram. $*=p<0.001$ compared to both chow and control groups using a one-way ANOVA and Scheffé test.

Figure 5



A histogram of 24h urine outputs in chow, control and alkalotic rats in Study 1. Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histogram. * $p < 0.001$ compared to both chow and control groups using a one-way ANOVA and Scheffé test.

Table 4

Group	pH	pCO ₂ (mmHg)	HCO ₃ (mEq/L)
Chow (5)	7.41 ± 0.01	41.16 ± 2.68	25.72 ± 0.82
Control (8) prior to drainage	7.40 ± 0.02	41.29 ± 2.04	26.48 ± 0.41
Control (4)	7.41 ± 0.01	41.45 ± 1.87	26.23 ± 0.52
Alkalotic (4)	7.49* ± 0.01	47.30** ± 2.23	31.30* ± 2.10

Acid-base status in chow, control and alkalotic rats in the study on urine concentrating ability (Study 2). Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.001 and ** = p<0.05 compared to chow and control both prior to drainage and post-drainage using a one-way ANOVA and a Scheffé test.

Table 5 shows results obtained from analyses of plasma electrolytes. Gastric drainage decreased plasma Na, K, and Cl levels similar to values obtained in Study 1. Plasma Cl was decreased to 83.50 ± 3.57 mEq/L from 101.50 ± 1.24 mEq/L ($p < 0.001$). Plasma Cl also decreased in control animals after two weeks on the Cl-free diet ($p < 0.05$) and 4 weeks later, ($p < 0.001$). The anion gap is a calculation which approximates the unmeasured anions in the plasma. Perturbations in plasma electrolyte concentrations may easily affect this calculation by either increasing or decreasing its value. The anion gap shown on Table 6 was significantly increased during alkalosis and during ingestion of the Cl-free diet ($p < 0.05$) compared to pre-drainage values.

Table 6 also lists the values obtained for hematocrit, plasma protein, and Max Uosm. Plasma protein was not affected by the gastric drainage or metabolic alkalosis. Hematocrit was increased in alkalosis ($p < 0.05$), possibly due to ECFV contraction. Maximum urinary osmolality was significantly decreased ($p < 0.001$) to 1376.20 ± 229.41 mOsm/kgH₂O from the pre-drainage level of 3117.50 ± 621.50 mOsm/kgH₂O.

As shown in Figure 6, alkalotic animals were polydipsic (71.42 ± 4.80 ml/24h) compared to chow (38.20 ± 4.71 ml/24h, $p < 0.001$) and control (34.60 ± 3.52 ml/24h, $p < 0.001$) rats.

Table 5

Group	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
Chow (5)	144.60 ± 0.60	--	106.20 ± 1.16
Control (8) prior to drainage	143.30 ± 0.80	3.68 ± 0.18	101.50** ± 1.24
Control (4)	139.40 ± 1.72	3.70 ± 0.50	90.00* ± 2.04
Alkalotic (4)	138.30 ± 1.70	2.90 ± 0.80	83.50* ± 3.57

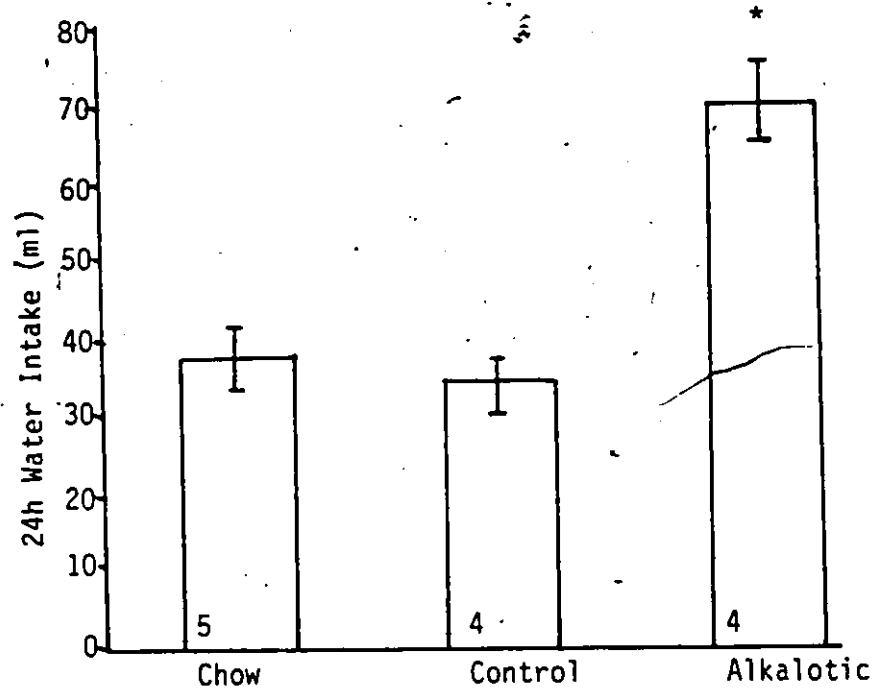
Plasma sodium (Na), potassium (K) and chloride (Cl) concentrations in chow, control and alkalotic rats in Study 2. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.001 versus chow and control prior to drainage and ** = p<0.05 versus chow rats using a one-way ANOVA and a Scheffé test.

Table 6

Group	Protein (mg/dl)	Hematocrit (%RBC)	Anion Gap (mEq/L)	Max Uosm (mOsm/kgH ₂ O)
Chow (5)	6.52 ± 0.27	38.00 ± 1.53	16.50 ± 1.50	2956.30 ± 37.10
Control (8) prior to drainage	6.74 ± 0.20	41.50 ± 2.25	19.50 ± 2.50	3117.50 ± 621.50
Control (4)	6.55 ± 0.50	43.00 ± 2.27	23.20* ± 2.80	3278.30 ± 131.80
Alkalotic (4)	6.73 ± 0.51	46.25** ± 2.53	23.50* ± 2.50	1376.30** ± 229.41

Plasma protein concentrations, hematocrits, anion gaps and maximum urine osmolality (Max Uosm) in chow, control and alkalotic rats in Study 2. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.001 compared to chow and control prior to drainage, ** = p<0.05 compared to chow and control prior to drainage and post-drainage using a one-way ANOVA and a Scheffé test.

Figure 6



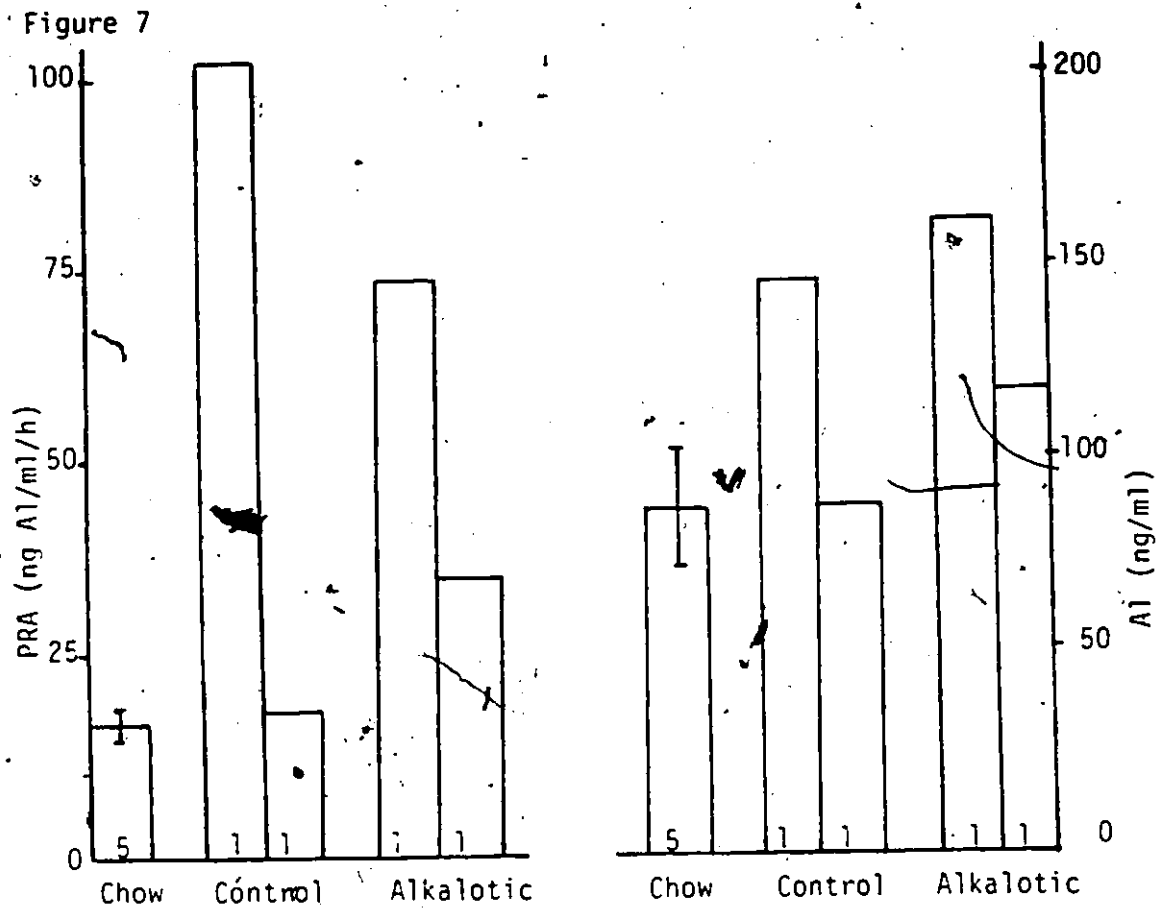
A histogram of 24h water intakes in chow, control and alkalotic rats in the study on urine concentrating ability (Max Uosm) (Study 2). Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histogram. * = $p < 0.001$ compared to both chow and control groups using a one-way ANOVA and a Scheffé test.

Figure 7 shows results obtained from RIA analyses of the terminal blood samples. Both PRA and Al are included in the table. There were no significant differences in PRA or Al levels in the control or alkalotic groups when compared to the chow group. This may be due to the large variation in values and the small number of animals (2) in the control and alkalotic groups. Although no significant changes occurred there is a slight trend in this preliminary data. The trend is for an increase in PRA and Al levels in both control and alkalotic groups with the larger increase occurring in the alkalotic group.

Table 7 shows the body weights during the baseline (prior to alkalosis) period, in chow, control, and alkalotic rats in Study 2. The control and chow rats show a significant weight gain ($p < 0.05$) versus baseline while the alkalotic animals did not gain a significant amount of weight. The alkalotic animals weigh significantly less ($p < 0.05$), than their chow and control counterparts.

(iii) Study 3 - The Second Study of Max Uosm.

Study 3 was undertaken to confirm some of the results obtained in Study 2 and also to investigate the effects of K-repletion on the urinary concentrating defect.



A histogram of plasma renin activity (PRA) and angiotensin 1 (AI) concentrations in chow, control and alkalotic rats in Study 2. The results for the chow animals are expressed as means \pm S.E.M. and as individual numbers for the control and alkalotic groups. The number of animals is shown in the lower left corner of the histograms.

Table 7

Group	Weight (g)
Baseline (15)	240.00 ± 1.30
Control and Chow (9)	265.63* ± 3.75
Alkalotic (4)	240.30** ± 2.00

Body weights in baseline (prior to gastric drainage), control and chow, and alkalotic rats in Study 2. Results are expressed as means \pm S.E.M. The number of animals is shown in parentheses. * = $p < 0.05$ versus baseline and ** = $p < 0.05$ versus control and chow using an un-paired t-test.

Table 8 compares A-B, electrolytes, and Max Uosm in chow and control animals. The only significant differences in these two groups due to the Cl-free diet alone were plasma Cl and Na levels. Both Na and Cl concentrations were decreased in plasma of rats on the Cl-free diet ($p < 0.05$). The chow results are not presented in this study except where noted, since there were no significant differences between this group and the control group except where already noted. The comparisons in this study are between the control and alkalotic groups of animals.

Table 9 compares blood A-B values in control and alkalotic animals. Alkalotic animals had significantly increased whole blood pH as well as plasma HCO_3 ($p < 0.001$) compared to controls. Plasma $p\text{CO}_2$ was also increased in the alkalotic group when compared to controls ($p < 0.01$).

Table 10 compares plasma electrolyte concentrations in control and alkalotic rats. Plasma Na was not significantly different in the two groups, but a significant decrease in both plasma K ($p < 0.05$) and plasma Cl ($p < 0.001$) was present. The alkalotic animals were, therefore, hypochloremic and hypokalemic.

Table 11 lists the results obtained for hematocrit, plasma protein, and anion gap in control and alkalotic animals. Neither the plasma protein levels nor the hematocrit were significantly altered in this study. The

Table 8

Group	pH	HCO ₃ (mEq/L)	Cl (mEq/L)	Na (mEq/L)	K (mEq/L)	Max Uosm (mOsm/kgH ₂ O)
Chow (3)	7.39 ± 0.02	26.65 ± 0.69	96.57 ± 1.49	142.60 ± 2.00	3.63 ± 0.09	2959.10 ± 167.62
Control (10)	7.39 ± 0.007	26.87 ± 0.54	91.22* ± 1.24	135.93* ± 1.06	3.96 ± 0.15	2843.20 ± 72.25

Acid-base status and plasma electrolyte concentrations and maximum urine osmolality (Max Uosm) in chow and control rats in the second study on urine concentrating ability (Study 3). Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.05 compared to chow rats using an un-paired t-test.

Table 9

Group	pH	HCO ₃ (mEq/L)	pCO ₂ (mmHg)
Control (10)	7.39 ± 0.007	26.87 ± 0.54	45.42 ± 0.88
Alkalotic (12)	7.47*** ± 0.01	35.82*** ± 1.30	50.76** ± 1.44

Acid-base status in control and alkalotic rats in Study 3. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. ** = p<0.01 and *** = p<0.001 compared to control rats using an un-paired t-test.

Table 10

Group	Cl (mEq/L)	Na (mEq/L)	K (mEq/L)
Control (10)	91.22 ± 1.24	135.93 ± 1.06	3.96 ± 0.15
Alkalotic (12)	78.60*** ± 2.94	134.92 ± 2.07	3.28* ± 0.20

Plasma chloride (Cl), sodium (Na) and potassium (K) concentrations in control and alkalotic rats in study 3. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.05 and *** = p<0.001 compared to control animals using an un-paired t-test.



Table 11

Group	Protein (mg/dl)	Hematocrit, (%RBC)	Anion Gap (mEq/L)
Control (10)	6.45 ± 0.11	46.85 ± 0.70	20.99 ± 1.13
Alkalotic (12)	6.39 ± 0.13	47.98 ± 0.98	24.04 ± 1.90

Plasma protein concentrations, hematocrits and anion gaps in control and alkalotic rats in Study 3. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses.

anion gap was not significantly changed.

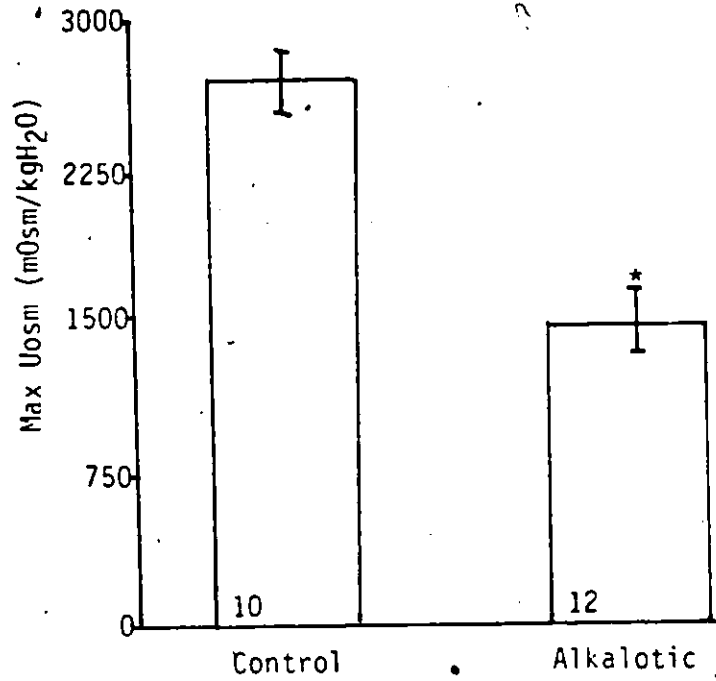
Figure 8 shows the results obtained for Max Uosm in both the control and alkalotic animals. The alkalotic animals had a significant urine concentrating defect as compared to control animals (1509.66 ± 82.30 mOsm/kgH₂O vs. 2843.20 ± 72.25 mOsm/kgH₂O, respectively, $p < 0.001$).

Table 12 shows body weights and 24h food intake in the baseline (prior to gastric drainage) period, for chow, control, and alkalotic rats. Chow and control rats gained significant amounts of weight when compared to the baseline values ($p < 0.05$) while the alkalotic rats did not gain a significant amount of weight. The alkalotic rats were significantly lighter than the controls of the same sex. 24h food intakes did not change significantly throughout the study.

Table 13 shows the results of the K-repletion study on the urinary concentrating defect. The results show that K-repletion did not repair the defect. The alkalotic animals concentrated to 1593.50 ± 143.67 mOsm/kgH₂O prior to K-repletion and 1713.00 ± 431.00 mOsm/kgH₂O post-repletion. These levels were significantly lower than those observed for the control animals.

Figure 9 shows the 24 h water intakes in chow, control, and alkalotic rats. These data show clearly that the alkalotic rats were polydipsic (57.92 ± 1.90 ml/24h) when

Figure 8.



A histogram of maximum urine osmolality (Max Uosm) in control and alkalotic rats in Study 3. Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histograms. * = $p < 0.001$ compared to control rats using an un-paired t-test.

Table 12

Group	Body Weight (g)		Food (g/24h)	
	♀	♂	♀	♂
Baseline	241.17 ± 3.00 (23)	382.50 ± 14.00 (4)	33.16 ± 1.38 (23)	34.00 ± 1.00 (4)
Chow (3)		433.33* ± 28.33		39.67 ± 3.00
Control (7)	264.00* ± 8.71		34.14 ± 3.29	
Alkalotic (11)	241.73** ± 4.18		30.90 ± 1.73	

Body weights and 24h food intakes in baseline (prior to gastric drainage), chow, control and alkalotic rats in Study 3. Results are expressed as means \pm S.E.M. The number of animals is shown in parentheses. The columns are paired off by the sex of the animals. * = $p < 0.05$ versus baseline and ** = $p < 0.05$ versus control using a one-way ANOVA and a Scheffé test. ♀ = female
♂ = male.

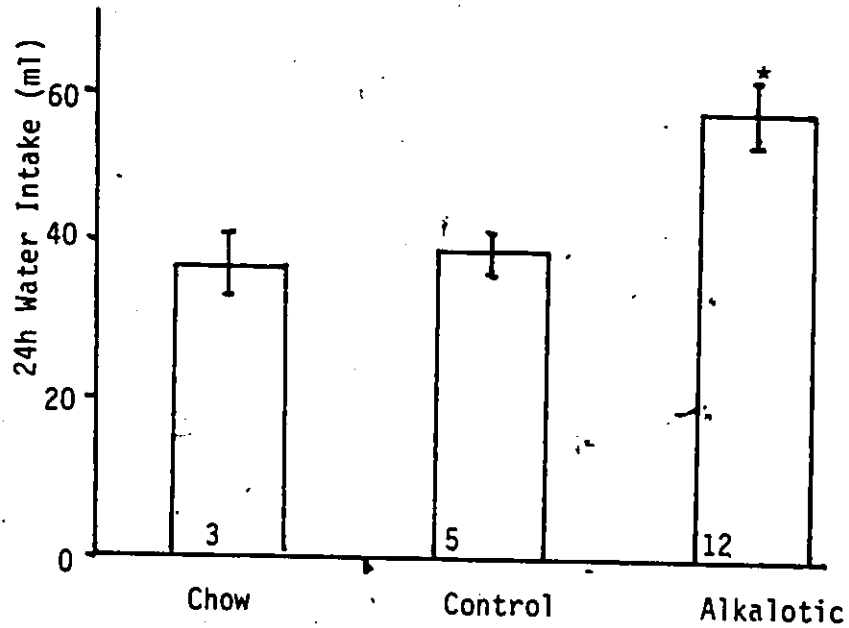
Table 13

Group	Max Uosm (mOsm/kgH ₂ O)		
	Pre-K	Post-K	Post-H ₂ O
Control	2790.38 ± 127.25 (8)	2440.00 ± 156.67 (3)	2602.40 ± 92.80 (5)
Alkalotic	1593.50* ± 143.67 (6)	1713.00** ± 341.00 (5)	1570.00 (1)

The effect of a K₂HPO₄ gavage on maximum urine osmolality (Max Uosm) in control and alkalotic animals in Study 3. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.001 versus control pre-K and ** = p<0.005 versus control post-K using an un-paired t-test (K=potassium).

5

Figure 9



A histogram of 24h water intakes in chow, control and alkalotic rats in the second study of Max Uosm (Study 3). Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histograms. * = $p < 0.01$ compared to both chow and control groups using a one-way ANOVA and a Scheffé test.

compared to either the chow (38.60 ± 4.71 ml/24h, $p < 0.01$) or control (39.33 ± 2.73 ml/24h, $p < 0.01$) groups of animals.

(iv) Study 4 - The Effect of Exogenous ADH Administration.

This set of experiments was undertaken to repeat Study 3, and to investigate the effects of exogenous ADH on the renal concentrating defect.

Table 14 compares A-B status, electrolyte concentrations, and Max Uosm in chow and control rats in this study. There were no significant differences between these two groups in any parameter studied. Therefore, only the control and alkalotic animals will be compared here.

Table 15 compares results for acid-base status in control and alkalotic animals. Alkalosis resulted in an increase in pH to 7.50 ± 0.01 from 7.41 ± 0.008 , pCO_2 to 56.64 ± 1.90 mmHg from 44.61 ± 1.48 mmHg, and HCO_3 to 42.81 ± 2.84 mEq/L from 27.15 ± 0.47 mEq/L ($p < 0.001$).

Table 16 compares plasma electrolyte concentrations in control and alkalotic rats. Plasma Cl was decreased to 76.50 ± 2.39 mEq/L from 98.40 ± 0.82 mEq/L for controls ($p < 0.001$). Plasma Na and K were also significantly decreased in the alkalotic rats ($p < 0.001$).

Table 17 compares body weight and 24 h food and water

Table 14

Group	pH	HCO ₃ (mEq/L)	Cl (mEq/L)	Na (mEq/L)	K (mEq/L)	Max Uosm (mOsm/kgH ₂ O)
Chow (7)	7.39 ± 0.02	26.65 ± 0.69	96.57 ± 1.49	142.60 ± 2.80	3.63 ± 0.09	2959.10 ± 167.62
Control (10)	7.41 ± 0.008	27.15 ± 0.47	98.40 ± 0.82	145.00 ± 0.72	3.48 ± 0.09	2843.20 ± 72.25

Acid-base status, plasma chloride (Cl), sodium (Na) and potassium (K) concentrations and maximum urine osmolality (Max Uosm) in chow and control rats in the study on the effect of exogenous ADH administration (Study 4). Results are expressed as means ± S.E.M. The number of animals is shown in parentheses.

Table 15

Group	pH	pCO ₂ (mmHg)	HCO ₃ (mEq/L)
Control (10)	7.41 ± 0.008	44.61 ± 1.48	27.15 ± 0.47
Alkalotic (12)	7.50* ± 0.01	56.64* ± 1.90	42.81* ± 2.84

Acid-base status in control and alkalotic rats in Study 4. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.001 compared to control values using an un-paired t-test.

Table 16

Group	Cl (mEq/L)	Na (mEq/L)	K (mEq/L)
Control (10)	98.40 ± 0.82	145.00 ± 0.72	3.48 ± 0.09
Alkalotic (12)	76.50* ± 2.39	136.70* ± 1.20	2.57* ± 0.12

Plasma chloride (Cl), sodium (Na) and potassium (K) concentrations in control and alkalotic rats in Study 4. Results are expressed as means \pm S.E.M. The number of animals is shown in parentheses. * = $p < 0.001$ versus control values using an un-paired t-test.

Table 17

Group	Body Weight (g)	Food (g/24h)	Water (ml/24h)
Baseline (25)	266.80 ± 2.89	26.28 ± 1.20	22.76 ± 1.16
Control (10)	287.00** ± 8.24	29.60 ± 1.51	25.90 ± 2.42
Alkalotic (10)	223.20* ± 3.95	26.93 ± 1.09	43.69* ± 4.60

Body weights and 24h food and water intakes in baseline (prior to gastric drainage), control and alkalotic rats in Study 4. The results are expressed as means \pm S.E.M. The number of animals is shown in parentheses. * = $p < 0.001$ compared to baseline and control values and ** = $p < 0.001$ compared to baseline using a one-way ANOVA and a Scheffé test.

intakes in control and alkalotic rats. Baseline values were obtained prior to surgery and gastric drainage. Control animals gained a significant amount of weight compared to the baseline value ($p < 0.001$), although 24 h food and water intake did not vary significantly from baseline values. These are total volumes and not normalized data. If the data were normalized the alkalotic polydipsia would be more severe and the animals would be hyperphagic. The alkalotic rats lost weight compared to the baseline value ($p < 0.001$). This may be due to ECFV contraction. The alkalotic rats also became polydipsic compared to baseline or control values ($p < 0.001$) although their food intake did not change significantly.

Table 18 compares plasma osmolality, protein, hematocrit, and anion gap in control and alkalotic rats. Plasma osmolality was significantly decreased in alkalosis ($p < 0.001$) and hematocrit was significantly increased ($p < 0.001$). This increase in hematocrit, coupled with the weight loss, are indicators of ECFV contraction. Plasma protein values were not significantly altered in alkalosis. The anion gap was not significantly altered in this study.

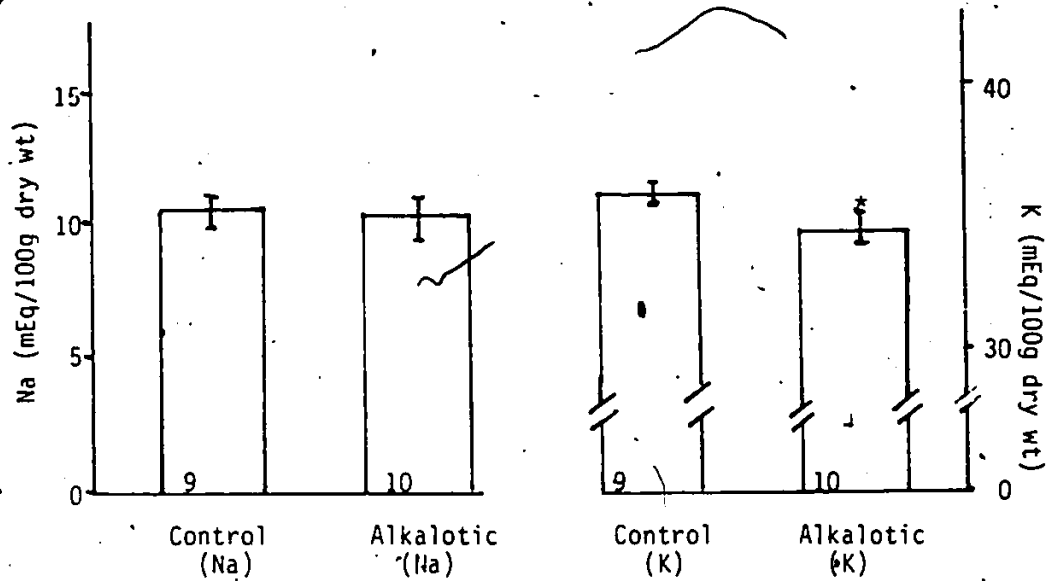
Figure 10 illustrates muscle Na and K levels in control and alkalotic animals. Muscle Na levels were unaffected in alkalosis (10.05 ± 0.53 mEq/100g dry wt versus 10.17 ± 0.44 mEq/100g dry wt for controls), but muscle K was decreased to

Table 18

Group	Plasma Osmolality (mOsm/kgH ₂ O)	Protein (mg/dl)	Hematocrit (%RBC)	Anion Gap (mEq/L)
Control (10)	308.40 ± 1.03	6.36 ± 0.08	44.35 ± 0.55	19.45 ± 0.55
Alkalotic (12)	288.73* ± 3.01	6.40 ± 0.08	49.55* ± 0.68	20.83 ± 2.41 (11)

Plasma osmolality, protein concentrations, hematocrits and anion gaps in control and alkalotic rats in Study 4. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * $p < 0.001$, compared to control values using an un-paired t-test.

Figure 10



A histogram of muscle sodium (Na) and potassium (K) in control and alkalotic rats in the study on the effect of exogenous ADH administration (Study 4). Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histograms. * = $p < 0.01$ compared to control rats using an un-paired t-test.

34.31 \pm 0.47 mEq/100g dry wt versus the control value of 36.21 \pm 0.16 mEq/100g dry wt ($p < 0.01$).

Table 19 compares Max Uosm in control and alkalotic rats under various experimental conditions. The first column compares values obtained prior to gastric drainage. There was no significant difference between the control and alkalotic groups prior to gastric drainage. Column two compares Max Uosm during alkalosis on ad libitum water intake. Alkalotic animals concentrated their urine to 1853 mOsm/kgH₂O compared to 2788 mOsm/kgH₂O for controls ($p < 0.001$) indicative of a urine concentrating defect. Column three compares Max Uosm values obtained during alkalosis when the alkalotic group was water restricted to control levels (ml/100g bwt/24h). From these results it can be seen that water restriction does not reverse the defect. The defect is therefore not totally dependent on polydipsia and the subsequent urinary washout. Column four compares Max Uosm during alkalosis and water restriction and during administration of exogenous ADH s.c. (a total of 1 I.U.). The results show that exogenous ADH partially repairs the concentrating defect. The rats concentrate their urine to 2342 mOsm/kgH₂O post-ADH versus 1967 mOsm/kgH₂O pre-ADH ($p < 0.05$), although the 2341 mOsm/kgH₂O level is still significantly lower than that for controls (2615 mOsm/kgH₂O) or for the pre-alkalosis period (2546 mOsm/kgH₂O) ($p < 0.05$).

Table 19

Group	Baseline	Ad. Lib.	Max Uosm (mOsm/kgH ₂ O)			
			Water Restr.	ADH	K ₂ HPO ₄	dH ₂ O
Control (9)	2753.70	2788.43	2850.90	2614.60	2517.80	2652.50
	± 145.10	± 157.60	± 129.99	± 75.56	± 105.75 (5)	± 277.71 (4)
Alkalotic (13)	2546.00	1853.00**	1966.73**	2341.80**#	1951.00**#	1933.25**#
	± 38.35	± 107.80	± 131.68 (10)	± 57.82 (10)	± 172.83 (6)	± 99.98 (4)

Maximum urine osmolality (Max Uosm) in control and alkalotic rats under baseline (prior to gastric drainage) conditions; on ad libitum water intake; with water intake of the alkalotic rats restricted to the level, in mls/100gwt/24h, drank by controls; with s.c. administration of ADH (2x 500 mU) and during acute K-repletion with a 450 mEq/L solution of K₂HPO₄ by gavage (or distilled water by gavage as a control for the stress of the procedure) in Study 4. Results are expressed as means ± S.E.M. The number of animals is shown in parentheses. * = p<0.05 versus control values in the same column and ** = p<0.001 versus control values in the same column. # = p<0.05 compared to the alkalotic baseline group. All statistical analyses utilized a one-way ANOVA and a Scheffé test.

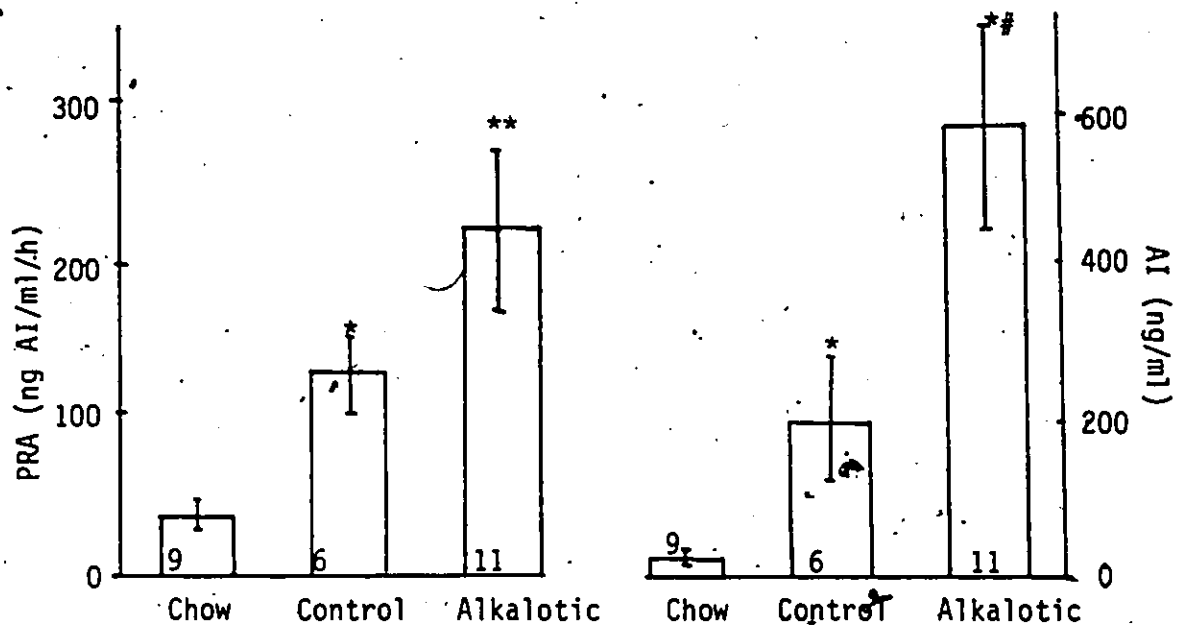
The final two columns illustrate data from the K-repletion studies. K-repletion did not correct the concentrating defect present during metabolic alkalosis of gastric origin in agreement with the results obtained in the previous study. It must be pointed out that the exact extent of the K-depletion was not known prior to the repletion and this makes interpretation of this experiment difficult.

Figure 11 illustrates both PRA and AI concentrations in chow, control, and alkalotic rats from study 4. PRA was significantly increased in both control (128.46 ± 28.01 ngAI/ml/h) and alkalotic (221.74 ± 58.94 ngAI/ml/h) groups ($p < 0.005$ and $p < 0.01$ respectively) compared to chow animals (37.42 ± 8.37 ngAI/ml/h). Plasma AI concentrations increased significantly in control (198.40 ± 64.01 ng/ml) and alkalotic rats (580.10 ± 133.27 ng/ml, $p < 0.005$ for both) as compared to chow rats (20.27 ± 4.51 ng/ml). AI was also significantly greater in the alkalotic rats when compared to control rats ($p < 0.05$).

Figure 12 illustrates plasma ADH values in chow, control, and alkalotic animals under the effect of anaesthesia. The alkalotic rats had a significantly lower plasma ADH (58.89 ± 1.04 pg/ml) compared to both chow (62.91 ± 1.35 pg/ml) and control (62.76 ± 1.23 pg/ml) rats ($p < 0.05$) under the same conditions of blood collection.

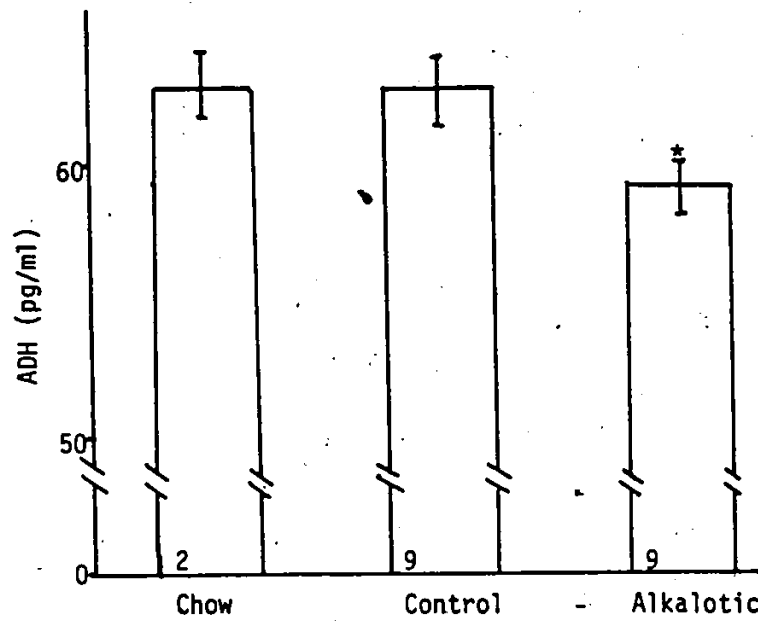
The kidneys of chow and control rats had no evidence of

Figure 11



A histogram of plasma renin activity (PRA) and angiotensin I (AI) concentrations in chow, control and alkalotic rats in Study 4. The results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histograms. * = $p < 0.005$ and ** = $p < 0.01$ versus chow and # = $p < 0.05$ versus controls using a one-way ANOVA and a Scheffé test.

Figure 12



A histogram of plasma antidiuretic hormone (ADH) concentrations in chow, control and alkalotic rats in Study 4. Results are expressed as means \pm S.E.M. The number of animals is shown in the lower left corner of the histograms. * = $p < 0.05$ compared to both chow and control groups using a one-way ANOVA and a Scheffé test.

renal pathology (Appendix 4). Three alkalotic rats showed evidence of mild nephrocalcinosis and, therefore, were not used in this study. Six of the other alkalotic animals seemed to be recovering from interstitial nephritis of a focal nature at the time of histological examination which would not have had a physiological effect upon their renal function (Dr. Sarkar, personal communication) (Appendix 4).

DISCUSSION

The results of this study indicate that gastric hydrochloric acid drainage in rats induces a chronic metabolic alkalosis which can be maintained by the ingestion of a Cl-free diet (Tables 1, 4, 7, 8, 12, and 13). This preparation is, therefore, a suitable model for investigating the renal and endocrine mechanisms underlying the urine concentrating defect seen in metabolic alkalosis.

Characteristics of the Model.

Metabolic alkalosis produced by gastric HCl drainage is characterized by hypochloremia, hyponatremia, hypokalemia, plasma hypo-osmolality, polyuria, polydipsia, and a renal concentrating defect. Decreased body weight and increased hematocrit were also observed in Studies 1, 2 and 4 (Tables 3, 6, and 16). These last two characteristics suggest ECFV contraction occurred.

This model of gastric drainage metabolic alkalosis used in these investigations is the first to be reported in the rat, and the first to be used in the study of how Cl-depletion may alter renal concentrating mechanisms. This model has several significant advantages over existing

models which use diuretics, steroids, and non-reabsorbable anions. Diuretics are known to alter renal function and increase acid excretion, salt loss leading to a contracted ECFV, and K loss possibly leading to hyperaldosteronism (Cogan et al., 1983), whereas steroids are known to cause K-depletion (Levine et al., 1973 and 1976). Steroids are also known to cause myocardial lesions (Levine and Sarkar, 1976). Use of non-reabsorbable anions lead to chloruresis, natriuresis, and kaliuresis (Gulyassy et al., 1962). In the present model, on the other hand, the major complicating factors are: 1) the degree of potassium depletion which, if severe, can lead to renal medullary pathology (Sarkar and Levine, 1978); and, 2) and the degree of ECFV contraction. These factors are discussed below.

Extra-cellular Fluid Volume Contraction

The alkalosis was induced in this study by gastric HCl drainage, but, it remained stable even after drainage was discontinued as long as the animals ingested the Cl-free diet. Kassirer and Schwartz (1966) indicated that alkalosis induced by vomiting or gastric drainage is maintained by ECFV contraction. It is well known that ECFV contraction is a potent stimulus for renin release and activation of the

renin-angiotensin-ADH system (Bonjour and Malvin, 1970; Dunn et al., 1973; Robertson, 1977; and Vander, 1967). Pitts et al. (1946) have stated that bicarbonate reabsorption is inversely related to ECFV and that a contracted ECFV leads to increases in plasma aldosterone levels and, therefore, to an increase in Na and bicarbonate reabsorption. They also showed that bicarbonate reabsorption was inversely proportional to plasma Cl levels. Since both ECFV contraction and hypochloremia were observed in this study, it is very likely that these changes contributed to the induction and maintenance of alkalosis, initially by increasing bicarbonate reabsorption and then by maintaining it at the increased level. It should be pointed out, however, that no direct measurements were made of ECFV in these studies.

Galla et al., (1983) have recently proposed that hypochloremic alkalosis can occur without the complication of ECFV contraction. These authors found that Cl-depletion is responsible for the maintenance of the alkalosis. In the present study a striking decrease in plasma Cl concentration to a level of 76.50 mEq/L (controls were at 98.40 mEq/L) was observed. It is possible that hypochloremia may have been responsible for the maintenance of the alkalosis since ECFV contraction was not always present. The possible influence of the minor degree of ECFV

contraction on intrarenal concentrating mechanisms was not, however, specifically studied in this research project.

Potassium-depletion.

The A-B and electrolyte results obtained in this study are in agreement with those obtained by Burnett et al. (1950), Luke et al. (1977), and Needle et al. (1964). These studies reported that selective removal of Cl or HCl results in metabolic alkalosis, hypochloremia, hyponatremia, and hypokalemia. Also, Atkins and Schwartz (1962), Bank and Aynedjian (1965), Cooke et al. (1952), Hutler et al. (1978), Helle Aires and Malnic (1972), and Seldin and Rector (1972) have all shown that metabolic alkalosis results in hypokalemia, which, if severe enough, can also lead to a renal concentrating defect.

Bennett (1970), Berl (1980), Berl et al. (1977), Carney et al. (1976), and Eknoyan et al. (1970) have shown that K-depletion alone, with or without concurrent alkalosis, results in a renal concentrating defect.

Potassium-repletion, induced by dietary means, intravenously or by dialysis, normally corrects this defect. In the present study, the defect observed in animals with chronic hypochloremic metabolic alkalosis was not corrected by

administration of K (Tables 11 and 17). The K-depletion in the present study was milder, however, than that seen in the studies on the concentrating defect in hypokalemia, and it is likely that these animals were not depleted enough to have developed a K-dependent concentrating defect. This would explain the lack of response to the gavage procedure.

Plasma levels of K were not measured immediately post-gavage and it is also not known to what extent, if any, plasma K was repleted. The extent of K-depletion immediately prior to the repletion was also not determined. The results of this experiment are, therefore, difficult to interpret. They suggest that the defect produced in this study was not wholly dependent upon plasma K levels. The plasma K concentrations and muscle K content in this study are evidence that the K-depletion in this model was much milder than that seen in classic diet-induced K-depletion (Levine et al., 1973).

One possible explanation for the concentrating defect in the present study is that the interaction between K and ADH at the distal tubule and collecting duct was hampered by the low K levels. This would render ADH less effective (depending on the amount of K present) as an antidiuretic agent (Cronin and Knoche, 1978; Nascimento and Calcagne, 1981; and Jamison and Robertson, 1979). Another explanation could be that hypochloremia inhibits solute reabsorption in

the TAL and thereby decreases the medullary hypertonicity and therefore urine tonicity. A third possible explanation is that plasma calcium or prostaglandin levels might have been altered, which may have affected the ADH responsiveness of renal tubules or the responsiveness of the central pathway responsible for ADH release. (Rutecki et al., 1982).

Renal Concentrating Ability.

The results of this study agree with recent findings (Abboud et al., 1979; Galla et al., 1981; and Luke et al., 1977) on the effects of hypochloremia on renal function. Burg and Green (1973) and Rocha and Kokko (1973) have implicated active Cl transport in the TAL as the driving force in the counter-current multiplication system of the kidney and, therefore, its ability to dilute or concentrate urine. Recently, Greger, (1981), Knepper et al., (1983), Hebert et al., (1981), and Velazquez et al., (1982) have provided evidence that the TAL actually actively transports Na. Chloride is co-transported in a secondary active transport process with Na. This active co-transport of Cl is also dependent upon co-transport of K with Na on the baso-lateral border (Na and K dependent Na-K-ATPase). The driving force for the Na and Cl co-transport is the

electrochemical gradient favouring Na entry into the cell from the lumen, as long as the Na-K-ATPase is removing Na from the cell at the basolateral border.

Wallin et al. (1973), Luke et al. (1977), and Kirchner et al. (1978) have shown that free water reabsorption is impaired in hypochloremia and this leads to production of a dilute urine. Urinary concentrating ability also depends on solute accumulation in the kidney, and an increase in the bicarbonate:Cl ratio leads to a decrease in concentrating ability. The alkalotic animals in this study were hypochloremic and had increased bicarbonate levels. These data therefore indicate that the plasma Cl level is responsible, at least in part, for the renal defect seen in hypochloremic alkalosis. The plasma Cl level may also be responsible for the increased PRA and AI concentrations found in this model.

The observation that exogenous ADH administration only partially corrects the defect supports the view that there is a nephrogenic component of the renal concentrating defect that is unresponsive to ADH. Several possible mechanisms could underlie such an intrarenal defect. One such possibility is that the increased pH has a direct effect on the renal tubular epithelium or on renal Na-K-ATPase pump activity.

Results of in vivo micropuncture studies suggest that

Cl-depletion decreases net reabsorption of NaCl by the TAL (Luke et al., 1978; and Galla et al., 1981). Gutsche et al., (1982) have recently shown that K-depletion impairs NaCl reabsorption by the TAL. The results suggest that the reduction in extra-cellular K is rate-limiting for peritubular Na-K-ATPase activity. Although the alkalotic animals in the present study show some degree of K-depletion evidenced by mild hypokalemia and a modest decrease in muscle cell K, the extent of the K-depletion does not appear to be of sufficient magnitude to cause an impairment in the NaCl reabsorption (Levine et al., 1973). Rather, the effect of metabolic alkalosis on TAL NaCl reabsorption may result from the accompanying decrease in Cl delivery to this loop segment. Gutsche et al., (1982), have recently obtained direct evidence in perfused nephrons that nitrate or bicarbonate substitution for Cl leads to impaired solute removal by the TAL in vivo. In vitro studies (Greger, 1983) have demonstrated that relatively high concentrations of Cl compared to Na or K are required to saturate the luminal transporter. For example, the affinity of the carrier for Na or K is so high that it is unlikely that either would be rate-limiting for the luminal uptake step. The possibility remains, however, that decreases in luminal Cl concentration within the physiological range could be rate-limiting at this transport step.

The persistent hyponatremia and hypo-osmolality in the present study suggest that there is an impairment in diluting ability. The results show that when water intake was matched to that of control animals, extra-cellular fluid hypo-osmolality persisted. However, since osmolality was significantly lower in alkalosis versus the control state before dehydration, osmolality may not have increased sufficiently to result in maximal secretion of ADH. It has been shown that increases in AII can stimulate ADH release (Bonjour and Malvin, 1970; Haack and Mohring, 1978; and Robertson, 1977) and, in the present study, AII levels (estimated from plasma AI concentrations, since at least 80% of the AI is converted to AII in one pass through the lungs) were significantly increased when compared to controls. Thus it appears that sufficient AII stimulus was present, but that the increase in ADH was only sufficient to contribute to impaired water excretion and not to achieve normal concentrating ability. While such an "inappropriate release of ADH" may have occurred in these animals, it seems more likely that insufficient Cl delivery to the TAL together with a defect in concentrating ability and diluting ability resulted from defective limb solute removal.

Another possibility is the involvement of $^{\circ}K$ with ADH and its effects at the renal tubular epithelium. However,

compared to dietary studies (Levine et al., 1973) the K-depletion present in this study was mild and may not be severe enough to have been involved in this way. The plasma hypo-osmolality may have been an over-riding stimulus to decrease ADH release in spite of high AII levels. While this may explain part of the urinary concentrating defect, it cannot explain the whole defect because exogenous administration of ADH did not totally correct the defect.

As noted earlier, polydipsia developed in alkalotic rats in the absence of an increase in solute intake and in the presence of a significant reduction in plasma osmolality. Since AII has been shown to be a potent dipsogen (Kucharczyk and Mogenson, 1975) and because AII is chronically elevated in the Cl-depleted alkalotic rats, the present results suggest that primary polydipsia may have been stimulated by AII.

The foregoing considerations strongly suggest that intrarenal water conservation may be impaired in this model of gastric alkalosis. Chloride-depletion could directly alter TAL function as a result of insufficient Cl delivery to transporting sites. The hypokalemia and modest whole body K-depletion could also alter TAL solute removal by interfering with baso-lateral NaCl transport (Gutsche et al., 1983). The results of histological examination using light microscopy rules out any complications due to renal

nephropathy. If even one focus of nephrocalcinosis was observed the data was not incorporated into the study. The animals which presented with mild focal interstitial nephritis were kept in the study, since the nephritis was of such a nature as to not have any physiological effect on normal renal function (Dr. Sarkar, personal communication). It is possible, however, that electron microscopy may show some pathological changes which were not apparent during the light microscopy study and this must be looked into in the future. Chloride-depletion, K-depletion, and structural changes could be important, in impairing intrarenal water handling.

Antidiuretic Hormone.

The renal concentrating defect in this study was not corrected by water-restriction and was only partially corrected by administration of exogenous ADH. The inability of ADH to reverse the polyuria observed in Luke's (1977) study may stem from hypochloremic changes in renal function. These could include: a decrease in receptor affinity for ADH; a change in receptor conformation or charge; a decrease in receptor number; a change in cell function; renal pathology; or, a change in the ADH molecule itself. No

studies on the subject have been published to date.

However, in the present study, administration of ADH partially corrected the defect. Decreased ADH release, due to plasma hypo-osmolality may partially explain the urine concentrating defect observed in alkalotic rats. Since exogenous ADH administration did not increase Max Uosm to control levels, it would appear that intrarenal factors, as well as reduced endogenous ADH production, may be involved in the urine concentrating defect. One possible explanation for the renal concentrating defect is medullary washout resulting from the large amounts of water that the alkalotic rats ingested. However, the results of the water-restriction experiments show that medullary washout, resulting from polydipsia, was not responsible for the renal concentrating defect. The results also suggest that changes in osmotic load were not responsible for the rats' inability to concentrate urine. Another explanation could be the K-depletion leading to increased prostaglandin levels and therefore decreased ADH effectiveness (Rutecki et al., 1982).

Renin-Angiotensin-ADH.

Numerous studies (Abboud et al., 1977; Kirchner et al.,

1978; Kotchen et al., 1976 and 1978; and Vander, 1967) have shown that Cl-depletion leads to renin release. It has been suggested that since Cl transport occurs as a secondary active process in co-transport with Na (Greger, 1981; and Knepper et al., 1983), with both occurring proximally in the thick ascending limb, and distally in the early segment of the distal tubule, the Cl effect on renin release may be mediated by a macula densa mechanism. Merrill et al. (1973) reported that hyponatremia leads to increased renin release, increased PRA, increased plasma AII and increased plasma aldosterone. Hyponatremia decreases renal conversion of AI to AII, approximately 4-fold, with no effect on plasma conversion. Thus, in the hypochloremic alkalotic preparation there are many stimuli such as hypochloremia, hyponatremia, ECFV contraction, and hypokalemia leading to hyperaldosteronism, which may activate the renin-angiotensin-ADH system. The plasma hypo-osmolality might be an over-riding stimulus to decrease ADH production directly in spite of the many stimuli for its release. The increase in plasma AII levels may contribute to the polydipsia observed in this alkalotic preparation. The polydipsia was not the mediator of the renal concentrating defect as was evidenced by the results of the water-restriction study.

There have been no studies to date which have attempted

to elucidate the role of the water and electrolyte homeostatic hormones, in metabolic alkalosis or its renal concentrating defect. Measurements of PRA and AI concentrations do imply that hypochloremia increases their values above normal levels, and that metabolic alkalosis increases them even further. The ADH levels were high, this may have been due to stress during the sampling procedure, due to the administration of pentobarbital anaesthesia (therefore the accuracy of the Wescor measurements need not be questioned) but alkalotic animals still produced significantly less ADH than control or chow rats. These high levels indicate that the alkalotic animals were capable of producing ADH, but under nonstressed conditions there may have been less ADH secreted due to the hypo-osmolality. If ADH is involved in the defect, as this study seems to indicate, it is involved in a complex manner. It may also be involved at the level of the medullary TAL as well as the collecting duct. One would expect high PRA and AI levels to increase ADH levels and low plasma osmolality to decrease it. It is possible that in hypochloremic alkalosis the osmotic stimulus is a more potent stimulus for ADH release than are plasma AII concentration or ECFV contraction.

Conclusion.

A new rat model of hypochloremic metabolic alkalosis produced rats which were chronically hyponatremic, hypokalemic, hypo-osmotic, polydipsic and showed evidence of a urinary concentrating defect. Measurements of Max Uosm, pair-drinking studies, and the response to exogenous ADH strongly suggest that: 1) an intrarenal concentrating defect is present which is only partially responsive to exogenous ADH; 2) polydipsia is primarily mediated by persistently high levels of AII; and, 3) hypo-osmolality and hyponatremia persist most likely as a result, of an intrarenal defect in TAL solute removal. The urinary concentrating defect thus appears to result from a disruption of both renal and extra-renal factors. The alkalotic animals presented with a chronic plasma hypo-osmolality and this may have been an important stimulus to prevent normal ADH release, despite stimuli to increase ADH release and may also explain, in part, the renal concentrating defect. The accompanying electrolyte disturbances may be responsible for the remainder of the concentrating defect seen in this model.

APPENDIX 1 - Calculations

1: The following are the calculations involved in determining plasma pCO₂ and HCO₃ concentrations.

pCO₂ = partial pressure of oxygen

tCO₂ = total CO₂ (measured)

H-H = Henderson-Hasselbach factor

dCO₂ = dissolved CO₂

0.0301 = solubility factor of CO₂ in the plasma

HCO₃ = plasma bicarbonate

pCO₂ = tCO₂/H-H factor

dCO₂ = pCO₂ x 0.0301

HCO₃ = tCO₂ - dCO₂

2: Calculations to determine plasma anion gap, which is a calculation of the unmeasured anions in plasma.

HCO₃ = bicarbonate

Cl = chloride

Na = sodium

Na - (HCO₃ + Cl) = anion gap

3: The following equation was used to determine where the significance in the one-way analysis of variance (ANOVA) occurred. It is called the Scheffé test.

M1 and M2 = means of the two groups to be compared

MSw = mean square within groups

n1 and n2 = group size of the two groups to be compared

k-1 = degrees of freedom

F = F-distribution factor for determining significance from the tables in any standard statistics text.

$$F = (M1 - M2)^2 / MSw(1/n1 + 1/n2)(k-1)$$

APPENDIX 2 - Antidiuretic Hormone Radioimmunoassay,

Extraction of Plasma

1: Wash the Bond Elut TM column with 3 ml 4% Acetic acid/ethanol, followed by 3 ml methanol and 3 ml distilled water.

Washing can be best accomplished by attaching the upper end of the column to the lower end of a disposable syringe and pushing the plunger slowly.

The syringe should be removed from the column each time when filling the column with the new solvent.

2: Apply 1 ml plasma or control sample to the top surface of the column material.

Attach the column with the syringe and push slowly over a period of one minute.

3: Wash the column with 3 ml distilled water. Dry the column by pushing the plunger in the syringe 2-3 times.

4: Elute the vasopressin with 1 ml 4% Acetic acid/ethanol, pushing slowly so that the solvent remains in contact with the column for a minimum of 2 minutes. Collect the eluate in 12 x 75 mm glass tube.

5: Evaporate the eluate to dryness in a 37°C water bath using compressed air or nitrogen. Specimens must be completely dry.

6: Reconstitute the sample with 0.5 ml of zero standard. Vortex to ensure complete reconstitution.

7: Assay 200 ul of the reconstituted sample, following assay procedure.

Assay Procedure

Reconstitute all reagents and allow to reach ambient temperature; mix thoroughly before using. Standards and unknowns should be assayed in duplicate.

1: Mark and arrange plastic test tubes in duplicate for all standards and unknowns.

2: Add 200 ul of the standard, control or extracted sample to the appropriate tubes.

3: Add 100 ul of Vasopressin Antiserum to all tubes except NSB and Total Count tubes. To NSB tubes, add 300 ul of 0

pg/ml standard.

4: Vortex all tubes.

5: Incubate at 2-8°C for 16-24 hours.

6: Add 100 ul of Vasopressin I¹²⁵ to all tubes.

7: Vortex all tubes.

8: Incubate at 2-8°C for 16-24 hours.

9: Add 1 ml of Precipitating Reagent to all tubes except Total Count tubes. This Reagent Should Be Mixed Thoroughly Before Use.

10: Vortex all tubes.

11: Incubate at room temperature for 15 minutes.

12: Centrifuge all tubes except Total Count tubes for 15 minutes at > 1500 g in a refrigerated centrifuge.

13: Decant the supernatant by inverting each tube or a rack of tubes. Keep the tubes inverted and allow them to drain

for 5-10 minutes on absorbent paper. After draining, blot the mouth of each tube to remove any droplets adhering to the rim before returning the tubes upright.

14: Count all tubes in a gamma counter for at least one minute.

Recovery is approximately 63-95% depending upon initial concentration. The highest recovery rates being at concentrations of about 12 - 13 pg/ml ADH. The cross-reactivity with lysine vasopressin is <0.01 and for other similar compounds it is <0.001. The sensitivity of this assay was 0.5 pg/ml ADH.

Taken from Vasopressin Arginine Radioimmunoassay Kit,
Diagnostic Systems Laboratories, Inc. Webster, Texas, 1983.

APPENDIX 3 - Angiotensin I Radioimmunoassay

Sample Collection

Blood must be drawn into a vacuum type collection tube or a syringe containing sufficient EDTA to yield a final concentration of 1 mg/ml. Maintain collected samples in an ice bath before separating the plasma in a refrigerated centrifuge at 4°C for 15 minutes at 1200 x g. The clear plasma should then be placed in an ice bath for immediate assay or stored frozen at -20°C.

Angiotensin I Generation - Specific Protocol

1. Known high and low control samples should be assayed with each run.
2. The frozen plasma samples are thawed in an ice bath or in a refrigerator, at 4°C.
3. Pipet 1 ml of each plasma sample into a clear polystyrene tube in an ice bath. Add 10 ul of the Dimercaprol solution and 10 ul of the 8-Hydroxyquinoline solution to each tube.

4. Add 2.0 ml of the pH 6.0 Maleate Buffer to each tube and mix thoroughly.

5. Transfer 1 ml of the contents fo each tube to a similarly labeled polystyrene tube. Incubate the 1 ml aliquots for 1 HOUR, AT 37°C, while maintaining the remainder of each solution in an ice bath for the same period of time.

6. At the end of the incubation period the samples incubated at 37°C are matched against their 4°C control in the ice bath and the angiotensin I measured by radioimmunoassay. (All samples may be stored frozen at this stage, if desired. Precautions must be taken to thaw later at temperatures that do not exceed 4°C.) Keep the incubated samples in an ice bath while pipetting all reagents.

Radioimmunoassay Protocol

Keep all reagents and tubes in an ice bath while pipetting.

1. Number a series of 16 tubes for the standard curve and 4 tubes for each sample to be run.

2. Pipet 500 ul of Assay Buffer into tubes 1 and 2 (Blank

tubes).

3: Pipet 100 ul of 5% BSA into tubes 1 and 2 (Blank tubes) and 3 and 4 ("0" Standard).

4: Pipet 100 ul of each Standard into the appropriate tubes, i.e., 0.1 ng/ml into tubes 5 and 6, 0.25 ng/ml into tubes 7 and 8, 0.5 ng/ml into tubes 9 and 10, 1.0 ng/ml into tubes 11 and 12, 2.5 ng/ml into tubes 13 and 14, and 5.0 ng/ml into tubes 15 and 16.

5: Pipet 100 ul of the sample as follows: 37°C generated sample into tubes 17 and 18 and the matched 4°C control into tubes 19 and 20. Continue in this manner for all other samples.

6: Pipet 100 ul of Tracer solution into all tubes.

7: Pipet 500 ul of Antiserum into all tubes except tubes 1 and 2.

8: Vortex all tubes for 2-5 seconds.

9: Incubate all tubes at 4°C for 18-24 hours.

10. Prior to the completion of the incubation, add a magnetic stir bar to the diluted Charcoal Suspension and initiate active stirring.

11. At the end of the 4°C incubation return all samples to an ice bath and pipet 1.0 ml of the Charcoal Suspension into each tube. Maintain active stirring of the Charcoal Suspension during the transfer.

12. Vortex all tubes and centrifuge at 1200 x g for 15-20 minutes. All tubes in the assay group should be processed as a unit to minimize variations in handling during the separation procedure.

13. Decant the supernatant fraction of each tube to appropriately numbered polystyrene tubes for gamma counting. Great care should be taken to transfer the supernatant free of charcoal particles. Maximize transfer by rimming the tubes. Antibody-bound angiotensin I is contained in the supernatant fraction and unbound angiotensin I is adsorbed by the charcoal.

14. Count the supernatant tubes in sequence using a well-type, solid-crystal scintillation counter. It is recommended that each tube be counted for at least 5

minutes.

15. All values were multiplied by the appropriate dilution factor.

The sensitivity of this procedure is approximately 2 pg of AI. The accuracy is within 0.02 + 0.16 to 0.08 + 0.13 ng of AI. The cross reactivity with angiotensinogen is 0.8% and is less than 0.008% with AII and less than 0.005% with AIII.

Taken from Instruction Manual, Rianen,
Angiotensin I (¹²⁵I) Radioimmunoassay Kit, New England
Nuclear, North Billerica, MA., 1983.

APPENDIX 4 - Renal Histology

(i) Preliminary Study of Model Viability

Figure 13a shows a sample of a normal kidney seen in all but 2 animals in this study. Figure 13b shows the kidney of the animal with medullary nephrocalcinosis and Figure 13c shows severe nephrocalcinosis extending into the cortex of the kidney. Figure 13d shows the kidney of the severely calcinotic rat prior to histological preparation. Figures 13A and B are at 400x magnification, Figure 13C is at 200x magnification and Figure 13D is at 4x magnification.

(ii) Effect of Metabolic Alkalosis on Max Uosm

Four animals died of a liver ailment post-surgically, but prior to the start of the study. Five animals died throughout the study as a result of cardiac puncture and anaesthetic overdose. The sixteen surviving animals were healthy. The results of 3 of the remaining animals were not presented due to the presence of varying degrees of nephrocalcinosis (Figure 14a, 13b). The 13 animals presented had a normal renal histology as represented by Figure 13a. Figures 14A and B are at 400x magnification.



Figure 13A: A photomicrograph of a normal rat kidney at the level of the medulla. The kidney was stained with H&E and paraffin mounted after slicing at 10 μ .

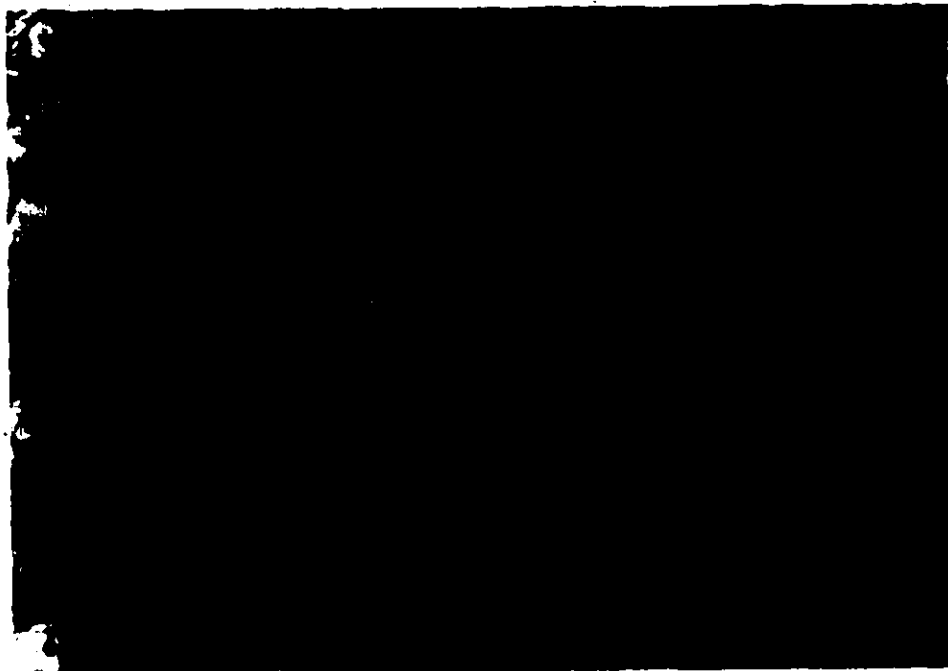


Figure 13B: A photomicrograph of a rat kidney with mild nephrocalcinosis. The arrow points out the calcium deposit.



Figure 13C: A photomicrograph of a rat kidney stained with H&E. This section shows severe nephrocalcinosis. The dark opaque spots are calcium deposits.



Figure 13D: A photograph of the same kidney prior to staining and sectioning. The white spots are calcium deposits. These deposits can be seen in both the medulla (arrow) and cortex (star).



Figure 14A: A photomicrograph of a rat kidney stained with H&E showing signs of nephrocalcinosis. A calcium deposit is marked by the arrow.

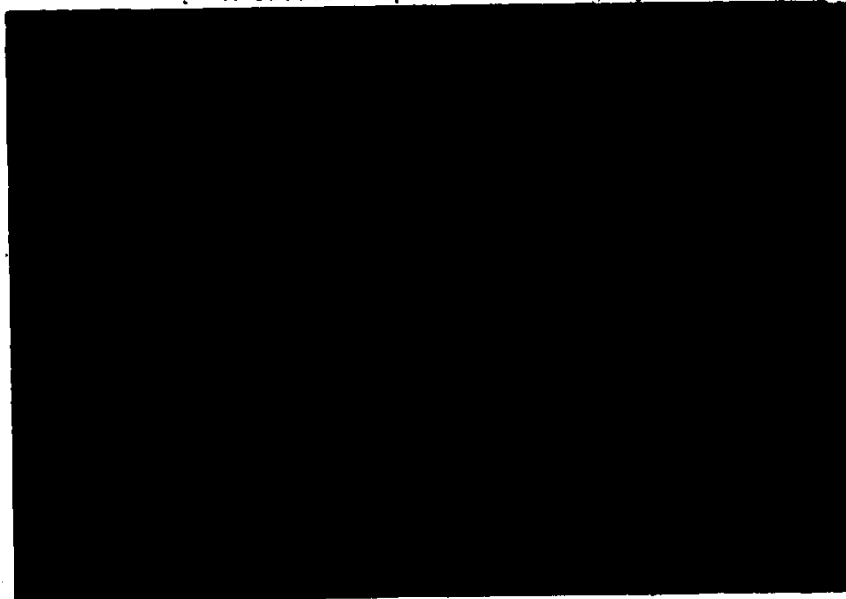


Figure 14B: A photomicrograph of a rat kidney stained with H&E with interstitial nephritis. Three such sites were observed in this kidney. The arrow points out one site of interstitial nephritis.

(iii) Effect of K-repletion on Max Uosm

In this study one animal died during cardiac puncture and another 2 were not presented due to the presence of nephrocalcinosis (Figure 13b). The resulting group of 25 animals had normal kidneys upon histological examination by light microscopy (Figure 13a).

(iv) Effect of exogenous ADH on Max Uosm

Figure 13a shows a representative kidney of the 20 animals without any observable renal pathology. Figure 13b is a representative photo of the 3 animals presenting with nephrocalcinosis. Figure 14b is a representation of the 6 animals showing evidence of interstitial nephritis and tubular regeneration. Four animals in this study died of complications following the cardiac puncture procedure.

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