1982

The extrarenal effects of diuretics on potassium homeostasis in the acutely nephrectomized rat

Lynn T. Tanoue
Yale University

THE EXTRARENAL EFFECTS OF DIURETICS ON POTASSIUM HOMEOSTASIS IN THE ACUTELY NEPHRECTOMIZED RAT

Lynn T. Tanoue

1982

The extrarenal effects of diuretics on potassium homeostasis in the acutely nephrectomized rat

Lynn T. Tanoue

B.A. Radcliffe College, 1978

A Thesis submitted to
the Yale University School of Medicine
in partial fulfillment of the requirement
for the degree of Doctor of Medicine
1982

to my parents

Acknowledgements

I would like to express my gratitude to Dr. Peggy Bia, who has been both a mentor and a friend. Her boundless energy and enthusiasm have been and will continue to be an inspiration to me.

I would also like to thank Dr. Peter Aronson, who kindly gave his time and effort toward co-advising this project.

Special thanks to Mrs. Karen Tyler who taught me the techniques used here and who saved me from countless lab difficulties, and also to the other members of the renal lab who made this project such a pleasure.

Table of Contents

Abstract

A potassium loading study was performed in acutely nephrectomized rats to determine the extrarenal effects of diuretics on potassium tolerance. Four diuretics were used - furosemide, hydrochlorothiazide, spironolactone, and bumetanide. In all animals studied, there was no significant difference between animals given diuretics and control animals in the observed increment in plasma potassium concentration. Keeping in mind the difficulties inherent in interpreting whole animal studies, these results suggest that:

1) If the recently postulated furosemide-inhibited cation cotransport mechanism does exist in the rat, then at the least this transport mechanism is not the dominant mechanism for determining potassium balance in the whole animal model.

2) There is no thiazide-induced extrarenal impairment or improvement of potassium tolerance.

3) Blockade of aldosterone with spironolactone does not cause impaired extrarenal potassium disposal as has been recently suggested.

Introduction

Diuretics are among the most commonly used pharmacologic agents in clinical medicine. They are classified according to chemical structure and by their major sites of action in the kidney. Yet, with the exception of the carbonic anhydrase inhibitors, relatively little is known about their mechanisms of action. That this should be so goes hand in hand with the fact that the intimate details of the structure and biochemical functions of the renal epithelium remain obscure. Elucidation of the mechanisms of action of the various classes of diuretics would doubtless provide invaluable insight into the mechanisms of ion transport in the tubular cell of the kidney, and in a broader sense to our understanding of renal physiology as well.

It appears that the actions of many diuretics are not limited to the renal epithelium. For example, furosemide has been shown to cause a prompt increase in venous capacitance which precedes its diuretic effect (1). This has been useful in the treatment of pulmonary congestion and pulmonary edema in the setting of elevated left ventricular filling pressure associated with acute myocardial infarction (1). Furosemide has also been used as an antihypertensive, as have diuretics of other chemical groups including thiazides and aldosterone inhibitors. Their effect of lowering blood pressure has been demonstrated to persist even after their diuretic effect has ceased (2-4).

Much of the work in this laboratory has been devoted to the study

of the regulation of potassium balance by the kidney and by extrarenal mechanisms. In view of the ubiquitous use of diuretics in clinical medicine, and in view of data suggesting that they have extrarenal effects not only on hemodynamic regulation as mentioned above but on regulation of ion transport as well, it seemed of interest to pursue study of the effects of these drugs on extrarenal potassium disposal. The background for this work is discussed in detail in later sections and briefly below.

1. Furosemide in large doses is often used to treat patients with acute renal failure in an effort to convert an oliguric state to a non-oliguric one. When successful, this effect is thought to involve changes in renal vasuclar resistance and renal blood flow (5-9). More recently, furosemide has been used in defining an ion cotransport mechanism which exists in several cell systems, including avian (10-13) and human red blood cells (14-18) and the loop of Henle (19 20). This system, which transports Na, K, and Cl intracellularly, is inhibited by furosemide. The ability of furosemide and other aminobenzoic acid derivatives such as bumetanide to act as inhibitors of the transport system is directly correlated with their diuretic potency (10), leading to speculation as to the significance of this mechanism within the renal epithelium. Moreover, if this cotransport system has major significance in the whole animal, then its effects on ionic, specifically potassium, balance must be considered. In the setting of acute renal failure, if use of furosemide could result in decreased potassium tolerance due to inhibition of cotransport, then there is a potential

for development of life-threatening hyperkalemia.

2. Thiazides are known to lower plasma potassium in certain disease states where hyperkalemia is found in the presence of normal renal function (21-24). Recently there has been a case report involving hyperkalemia associated with hypertension in which both abnormalities were corrected with thiazide administration (24). Interestingly, however, urinary K excretion did not significantly increase with the diuretic, implying that correction of the hyperkalemia was not due to increased potassium losses. In short, an extrarenal effect of thiazides on potassium disposal is suggested.

3. Aldosterone has recently been shown to have an extrarenal effect on potassium disposal (25). The effect of mineralocorticoids on extrarenal potassium homeostasis is discussed in detail in the following section. Briefly, mineralocorticoids appear to enhance K uptake by extrarenal tissues (26-28), and extrarenal K tolerance is diminished in their absence (29,30). By extrapolation, one would expect then that mineralocorticoid inhibition, for example with such agents as the aldosterone antagonist spironolactone, might have an opposite effect on extrarenal K balance..

There has been little work done on the role of diuretics on ion transport outside the kidney. We therefore undertook this study to examine the effects of diuretics of three chemical families on extrarenal potassium disposal. The diuretics used were furosemide,

bumetanide, hydrochlorothiazide, and spironolactone (Figure 1). As an understanding of extrarenal potassium regulation is essential to this project, a brief overview of factors currently believed to be involved in this regulation follows.

Background

A wealth of literature has been devoted to the discussion of the regulation of extracellular potassium concentration. Extracellular potassium represents only 2% of total body potassium, or approximately 65 mEq in a 70 kg man. Elucidation of the complex mechanisms by which this relatively small pool of potassium is controlled has proved to be a fascinating albeit extremely difficult task. While our understanding of the cellular mechanisms of potassium homeostasis has greatly expanded in recent years, it is still limited, and much of the regulation of cellular potassium transport as well as the broader area of cellular ion transport remains to be fully evaluated.

The kidney is a major regulator of potassium homeostasis; renal mechanisms are important determinants of extracellular potassium balance in chronic disease or health. However, it has become increasingly clear that extrarenal mechanisms play an important role in overall potassium homeostasis, particularly in settings of acute potassium imbalance (25). With the use of newer techniques and with increasing utilization of cellular probes in the form of drugs and hormones, our understanding of extrarenal potassium regulation has broadened. Moreover, a greater appreciation of the mechanisms of extrarenal cellular potassium transport and regulation can be applied to further our understanding of renal mechanisms of K transport, and to our understanding of the physiology of the kidney.

There are a number of extrarenal factors which interact to a greater or lesser degree with chronic as well as acute K homeostasis.

The details of these interactions have obviously not been fully worked out; however, it has become clear that renal potassium excretion may well not be the most important factor in the handling of an acute K load (25). It has been shown in humans (31-33), in dogs (31), and in rats (34) that less than 50% of an acute exogenous K load is excreted by the kidney in the first four to six hours following administration (25). The greater part of the remainder of the K load is transported into the intracellular compartment by extrarenal mechanisms, thus defending the extracellular fluid from life-threatening hyperkalemia. These mechanisms have been reviewed extensively in a recent review by Bia and DeFronzo (25), and will be discussed only briefly here.

Acid-Base

It has long been appreciated that acid-base balance can affect the distribution of potassium. Fenn and Cobb in 1934 showed that lowering the pH of a skeletal muscle preparation caused movement of K out of muscle cells into the surrounding medium (35) and suggested that there was a connection between intra- and extracellular hydrogen ion and potassium ion concentrations. Since then it has been the general conclusion that intra- and extracellular acid-base balance and potassium status are integrally and reciprocally related (36). Acidosis causes the plasma K concentration to increase due to displacement of K extracellularly in exchange for the buffering of excess H ions intracellularly, with the opposite exchange occuring during alkalosis. Burnell et al. quantitated the increments in K and pH in five patients with various types of acid-base disturbances and calculated that, on

the average, for every 0.1 unit change in extracellular pH there was an inverse change of 0.6 mEq/L in the serum K concentration (37). This numerical relationship is commonly used as a guideline to estimate a corrected K concentration in the presence of an acid-base disturbance. However, it has been pointed out in recent years that the relationship between hydrogen ion and potassium concentrations is far more complex than has been assumed (25,36), and that the variation of plasma K increments observed with an 0.1 unit pH change is considerable (25). As noted by Bia and DeFronzo (25) and by Adler and Fraley (38), a number of factors, including the nature of the disturbance (i.e. metabolic vs. respiratory), the type of anion accompanying an increase in hydrogen ion concentration, the duration of acidosis, and the extent of intracellular buffering, as well as the type of imbalance itself (i.e. acidosis vs. alkalosis) affect the relationship between the two ions. Moreover there is evidence that the plasma bicarbonate concentration also may alter potassium distribution, independent of the blood pH (38,39). Although the original observation of the reciprocal relationship between hydrogen ion and potassium concentration has been confirmed repeatedly, the complexity of the relationship has been underestimated, and the exact nature of the interactions between acid-base balance and potassium regulation remains to be defined.

Hormones

I. Insulin

Much effort has been concentrated recently on determining the effects of various hormones on extrarenal K regulation. Of the major

hormones known to affect potassium homeostasis, insulin is probably the best described. Harrop and Benedict (40) and Briggs et al. (41) in 1923 noted that insulin administered to the intact animal produced a decrease in serum potassium concentration. This observation has subsequently been demonstrated and further described by a number of investigators (32, 42-47) and it is now well established that insulin plays an important role in the disposal of an acute potassium load. It has been shown by several groups that infusion of KCl resulting in an elevation of plasma K to a significant degree of hyperkalemia (i.e. an increase of greater than 1.0-1.5 mEq/L (25)) causes an increase in peripheral insulin levels and a stimulation of pancreatic insulin secretion (45,46). The relative clinical importance of this relationship may be evident in the setting of acute hyperkalemia due to hemolysis, rhabdomyolysis, severe crush injuries, and burns, etc. Perhaps more importantly, however, it has recently been demonstrated that basal insulin levels play a role in potassium homeostasis (43). DeFronzo et al. (43) have shown that in dogs given a relatively small KCl infusion (0.375 mEq/kg/hr), serum K increased by only 0.5-0.7 mEq/L, remaining within the normal physiologic range, and under these conditions basal insulin levels remained unchanged. However, when somatostatin, a potent inhibitor of insulin and glucagon secretion, was infused with KCl, serum K levels doubled over control values. Moreover, suppression of basal insulin secretion in normal dogs, normal human subjects, and maturity-onset diabetics by somatostatin in the absence of KCl infusion was associated with a significant rise of baseline plasma K concentrations. This effect was independent of and preceded any changes in plasma glucose

and was also noted to be present in the absence of any change in renal K excretion, implying that the effect of basal insulin secretion on plasma K concentration was extra-renal in nature. That this was an effect of lack of insulin rather than glucagon was supported by the fact that in juvenile-onset diabetics lacking endogenous insulin secretion, no change in plasma K concentration was seen after infusion of somatostatin. A permissive role for basal insulin levels on K regulation is thus suggested. It should be noted, however, that peripheral measurements of insulin levels may not accurately reflect the secretion of insulin by the pancreas. Blackard and Nelson (48) have demonstrated that portal and peripheral vein insulin concentrations differ by a ratio of approximately 2-3:1. It has been shown that extremely small increments of plasma insulin (e.g. 25 μU/ml) can cause stimulation of K uptake by various tissues (49). Hence the postulated permissive role of basal insulin secretion on tissue K uptake must be modified to include the possibility that portal insulin levels may be sufficiently elevated to actively augment hepatic K uptake in the absence of any measurable increase in peripheral insulin levels (25). In this instance, use of a C-peptide assay as an analytic tool for measuring pancreatic insulin secretion might be a reasonable alternative.

In the absence of insulin the ability to tolerate an acute potassium load is markedly impaired. This has been demonstrated in pancreatectomized dogs (44,45,50) as well as in dogs, rats, and humans infused with somatostatin (34,43,46,47,51,52) and is independent of any change in renal K excretion. The ability to dispose of a potassium load intracellularly is restored when insulin is replaced. The clinical

importance of this mechanism is evident in such obvious ways as the use of insulin (along with glucose) in the treatment of life-threatening hyperkalemia. It is tempting to speculate that in the clinical setting of insulin lack, that is in the setting of diabetes, the not infrequent occurrence of hyperkalemia may be due at least in part to the insulinopenia itself as well as to the increase observed in plasma tonicity and, as recently postulated, in some cases also to concomitant hypoaldosteronism (26).

It has been shown by several groups that insulin-enhanced cellular K uptake involves muscle (53-57) and liver (58-60), which serve as the major body potassium storage pools. That the stimulation of cellular K uptake is distinct from the action of insulin on cellular glucose uptake is supported by the observations that a) the temporal courses of potassium and glucose movement differ (61), b) the potassium uptake can be shown to occur unchanged in glucose-free medium (55,56), and c) potassium uptake can be stimulated maximally by insulin concentrations too small to effect glucose transport (62).

The cellular mechanism by which K uptake is stimulated by insulin is thought to involve hyperpolarization of the cell membrane. Zierler et al. (55-57) in a system using rat skeletal muscle have shown that the movement of potassium is not sufficiently large enough nor does it occur rapidly enough to explain the insulin-induced increase in the transmembrane potential difference, and have surmised that the movement of potassium occurs as the result of the hyperpolarization rather than as its cause. They have suggested that perhaps K movement is enhanced due to some alteration of the permeability of the cell membrane secondary to the

hyperpolarization which allows the ion (and perhaps other substrates such as glucose) to move passively intracellularly (55,57).

II. Catecholamines

That catecholamines are involved in the regulation of potassium homeostasis was first noted by D'Silva, who in the 1930s demonstrated that injection of epinephrine into normal cats caused a transient increase in plasma potassium which was followed by a sustained decrease in plasma potassium (63,64). This initial hyperkalemic effect was presumed to be the result of increased hepatic potassium release, as supported by the observation that there was an increase in hepatic venous potassium concentration after epinephrine injection. The effect of sustained hypokalemia that followed was felt to be secondary to increased tissue uptake of potassium. Since then, catecholamines have been shown to play a significant role in the regulation of potassium in both the kidney and in extrarenal tissues (25,34,65-68).

DeFronzo et al. (68) recently demonstrated that infusion of KCl with epinephrine into normal humans leads to a marked inhibition of the increased renal K excretion that is observed with KCl infusion alone. Yet the rise in plasma K was less in subjects who had received both KCl and epinephrine as compared to subjects who received KCl alone, indicating that potassium tolerance is improved in the presence of catecholamines, presumably due to enhanced cellular uptake. Increased cellular uptake of K in response to catecholamine has been demonstrated clearly in skeletal muscle, the major potassium storage tissue (69-72),

and some investigators have also reported an increased uptake in liver (69-71). That this effect is distinct from the action of insulin has been demonstrated by Petit and Vick (73) in studies of pancreatectomized dogs. Response to epinephrine in experimental animals did not differ significantly from pancreatectomized controls, indicating that the enhanced cellular uptake of K did not depend on the release of insulin (73). Moreover, hyperglycemia per se was also found not to affect the response to epinephrine (74).

The mechanisms by which catecholamines bring about their biphasic effects on plasma K concentration have been worked out to some extent. Lockwood and Lum (69,75) and Todd and Vick (76) used adrenergic agonists and antagonists to show that the early hyperkalemic effect of catecholamines depends on both α- and β-adrenergic properties, while the secondary hypokalemic effect resulting from enhanced cellular uptake is apparently a purely β-adrenergic function. These authors have shown that β-agonist activity is protective against a KCl-induced hyperkalemia, and that this protective effect can be abolished by the β-blocking agent propranolol. Further work with more selective β_1 and β_2 agents have shown that the stimulation of cellular K uptake is most likely due to specific β_2-agonist activity (e.g. by the selective β_2-agonists salbutamol and soterenol) and can be prevented by selective β_2-antagonists (e.g. Butoxamine). Of clinical interest in this regard is the report by Wang and Clausen of the successful treatment of attacks in hyperkalemic familial periodic paralysis by salbutamol inhalation, presumably due to stimulation of cellular potassium uptake (77).

Recently, reports of impaired K tolerance following β-blockade in

man have prompted the study of the effects of β-blockade itself on potassium balance (67,68). As described above, infusion of KCl with epinephrine into normal subjects causes an inhibition of the increased renal K excretion observed with KCl infusion alone (68), yet plasma K rises less in those subjects given KCl and epinephrine than in those given KCl alone. β-blockade in the form of propranolol abolishes this effect of epinephrine. Interestingly, however, when propranolol is given with KCl infusion and no epinephrine is given, a greater increase in plasma K is noted than when KCl is given alone, suggesting that basal epinephrine levels may play a role in K regulation, as is thought to be the case with basal insulin levels (67,68).

Although the pharmacology of the catecholamine effect on cellular K uptake has become more clear in recent years, the mechanisms by which the epinephrine-stimulated K transport occurs have yet to be worked out. Moreover, the effect of potassium itself on regulation of catecholamine production is not known. It has been suggested that potassium may act as a stimulus to catecholamine release by the adrenals (78,79). Vogt demonstrated that injection of KCl into the celiac artery causes increased release of sympathomimetic amines into the adrenal vein (79), and Silberstein et al. have demonstrated that incubation of adrenal glands in media with elevated potassium concentrations results in increased activity of tyrosine hydroxylase (the first enzyme in the biosynthetic pathway of catecholamines) and in an increase in the amount of epinephrine released from the gland (78). However, Vogt failed to report the dose of KCl infusion used in her system, and the 50 mM K concentration of the medium used by Silberstein et al. seems hardly physiologic. The complex

interrelationships between potassium regulation and catecholamine synthesis, function, and effect are obviously still far from being fully understood.

III. Mineralocorticoids

The effect of mineralocorticoids in augmenting potassium secretion by the kidney has been described (80-83). This kaliuretic effect is observed in response to acute mineralocorticoid exposure as well as with prolonged treatment in contrast to the sodium "escape" phenomenon that occurs in the kidney with prolonged mineralocorticoid exposure (80). The action of these agents in the kidney has been localized to sites in the distal tubule and collecting system (84).

Hyperkalemia is known to act as a stimulus to aldosterone secretion (85,86). It has been suggested that in the absence of an intact renin-angiotensin system changes in potassium concentration may be the major regulator of aldosterone production (87). Laragh and Stoerk demonstrated that increased K intake resulted in aldosterone hypersecretion (88). Subsequently, Blair-West et al. demonstrated that KCl infusion leading to increased plasma K concentration within the normal physiologic range had an aldosterone-stimulating effect resulting in a significant elevation in adrenal vein aldosterone levels, and postulated that the effect observed earlier by Laragh and Stoerk had been due to a direct action of the increase in plasma K on the adrenal gland (89). Since then the biochemical synthetic pathway for aldosterone in the zona glomerulosa of the adrenal has been worked out, and the fact that potassium stimulates both an early and a late step in aldosterone synthesis (notably the

conversions of cholesterol to deoxycorticosterone and corticosterone to aldosterone (90)) is consistent with their earlier observations (26). That an increase in plasma K causes increased aldosterone secretion makes teleologic sense since a major physiologic effect of aldosterone is to increase K excretion via the kidney. However, aldosterone is thought to have significant effects on potassium regulation by extrarenal tissues as well.

Aldosterone is known to cause a decrease in the Na/K ratio in secretions of salivary glands, sweat glands, and colon (91-94). However, there have been conflicting views as to the effect of mineralocorticoids on K transport in other extrarenal tissues known to contain large amounts of exchangeable intracellular potassium, notably muscle and liver. Several groups have reported a decrease in potassium content of muscle after exposure to aldosterone (95,96); in contrast other groups have shown no change in muscle potassium content (27,28). Moreover it has been suggested that increased uptake of K occurs but that perhaps the potassium increment in any one tissue is too small to be measured (28,97,98). Bia and DeFronzo (25) have pointed out that these differences may have risen from a number of discrepancies among studies: species differences, failure to examine overall K balance, acid-base status, systemic hemodynamics, differences in aldosterone and potassium doses, and differences in the levels of other potassium-regulatory hormones. These authors, however, feel that mineralocorticoids do cause an enhancement of K uptake by extrarenal tissues (25). In vivo studies in normal rabbits infused with aldosterone (27) and in adrenalectomized rats treated with deoxycorticosterone acetate (28) have

shown that mineralocortioid administration decreases plasma K and that this decrease cannot be explained by increased renal or fecal K losses. In a similar vein, in adrenalectomized dogs an increase in plasma K was observed which could not be explained by decreased K losses in urine or stool (29). Moreover, in nephrectomized rats, hyperkalemia resulting from KCl loading was significantly greater in adrenalectomized animals versus controls (30). Furthermore, in the syndrome of hyporeninemic hypoaldosteronism in man in which hyperkalemia is often the presenting complaint, it has been noted that replacement of mineralocorticoid results in correction of the elevated plasma K without a concomitant increase in urinary K excretion (26).

Hence it appears that in the whole animal mineralocorticoid enhances extrarenal potassium disposal (25), and likely plays an important role in overall extrarenal potassium homeostasis. This is probably the result of mineralocorticoid-induced increased cellular uptake of K by extrarenal tissues; however, it has been difficult to document this in isolated tissue systems, and the mechanisms by which this uptake occurs remains obscure.

IV. Glucocorticoids

Glucocorticoids have been shown to produce a transient increase in renal potassium excretion which is distinct from the kaliuresis induced by mineralocorticoids (25). Some investigators have also reported a transient increase in plasma K with acute glucocorticoid administration (99,100), suggesting that there is an extrarenal effect of these hormones on K homeostasis. Studies done in hyperkalemic adrenalectomized

dogs in which chronic cortisone administration corrected the plasma K imbalance in the absence of a significant increase in renal K excretion support this postulate (101). Also, recent work done in human red blood cells has demonstrated that exposure to glucocorticoids causes an increase in ouabain sites (102). This respresents an increase in the number of Na-K ATPase pump sites, which may be the cellular mechanism responsible for the seemingly increased capacity to pump K intracellularly (102).

The effects of glucocorticoids on extrarenal tissues has been relatively unexplored with the exception of the gastrointestinal tract. Glucocorticoids are known to affect electrolyte transport across the intestinal epithelium, resulting in an increase in the transepithelial potential difference (103-105). In adrenalectomized rats, secretion of K and reabsorption of Na by colonic epithelial cells decreases, and there is a reduction in the transepithelial potential difference (105). Physiologic doses of dexamethasone replacement restore electrolyte transport and the potential difference to normal levels (105). Charney et al. (103) demonstrated that there is an association between intestinal electrolyte transport and the activity of mucosal Na-K ATPase activity. Administration of mineralocorticoid caused an increase in K secretion and a decrease in Na reabsorption in the intestinal epithelium, and this was felt to be related to the concomitantly increased ATPase activity. However, Binder more recently has shown a temporal distinction between the increase in membrane potential difference resulting from electrolyte transfer and the increase in the Na-K ATPase activity and has suggested that the increase in K transport seen with administration of dexamethasone

is not the result of increased enzyme activity as Charney et al. had postulated. It should be noted, however, that it is clear that the rate of K secretion is linked to the activity of the Na-K ATPase in the colonic epithelium, as has been recently confirmed by Hayslett et al. (106), albeit the glucocorticoid-induced increased K secretion may not operate via this mechanism.

V. Glucagon

In light of the major effects of insulin on extrarenal K balance, it would seem to follow that its endocrine pancreatic counterpart glucagon should also play a role in K homeostasis. A transient hyperkalemic effect of glucagon infusion distinct from its glycogenolytic action has indeed been identified (107,108). However, these studies were performed using pharmacologic doses of glucagon, and the effect of smaller, more physiologic amounts of the hormone on K balance remains to be established.

Cotransport

The cation cotransport system is a relative newcomer to the array of possible regulators of potassium homeostasis. A number of transport systems are known to operate within cell membranes to regulate intra- and extracellular concentrations of ions and organic compounds. In the particular case of potassium, two systems of K movement have been described: 1) the well-defined, ATPase-driven, ouabain-sensitive Na-K pump which causes Na efflux and K influx at the expense of energy in the form of ATP, and 2) passive K efflux which allows potassium to "leak" out of cells down its concentration gradient. Recently there has been

described a third form of ion cotransport which has been recognized in a variety of tissues including avian erythrocytes (10-13), mouse fibroblasts (114), human erythrocytes (14-18), Ehrlich ascites tumor cells (111,115), as well as the loop of Henle (19,20). While there has been a great deal of speculation about the exact nature of the system, it now appears that this cotransport mechanism is capable of producing net Na and K movement into cells and is dependent on the presence of extracellular chloride (15,110,111,113). Geck et al. in a model using Ehrlich ascites tumor cells have shown that the net flux of all three ions occurs inwardly, with an apparent stoichiometry of 1 K^+: 1 Na^+: 2 Cl^- (19, 114).

That this cotransport mechanism is distinct from the better known Na-K ATPase pump is made clear by the fact that it is ouabain-insensitive (10,11,14-17,114,115). In the presence of ouabain, the Na efflux and K influx of the Na-K ATPase pump ceases, but the cotransport flux continues. However, the cotransport mechanism is subject to hormonal and pharmacologic manipulations, and may also be sensitive to cellular volume (12,113,114,116,117), and is inhibited by propranolol (12) and by diuretic agents of the aminobenzoic acid family including furosemide (10,14,18,20,114,115) and bumetanide (10).

Energy for the cotransport process is probably derived from the Na (and perhaps also Cl) electrochemical potential difference (14,109). By coupling potassium to the process of Na movement, it can be effectively transported against its own concentration gradient. This mechanism would therefore fall under the category of secondary active transport. As recently reviewed by Aronson (119), secondary active transport mechanisms

cause the transport of one substnace against its concentration gradient by coupling its movement to the movement of another substance down a favorable gradient, and does not involve direct linkage to a metabolic process. Such mechanisms have been described in a variety of tissues in which the transport of amino acids, sugars, and ions are coupled to Na movement (109). This is as opposed to primary active transport in which transport is directly linked to a metabolic process. The Na-glucose cotransport that occurs in the proximal tubule is a well-described example of the former; the Na-K ATPase pump ubiquitous in cells is an example of the latter. Hence with secondary active transport electrochemically unfavorable events can by effected by coupling to electrochemically favorable ones without the direct expenditure of metabolic energy. In the cotransport mechanism described above, K transport would be coupled to the flow of Na down its electrochemical gradient. In this case Cl also moves down its concentration gradient, and probably contributes to the transport of potassium.

While the entity of this particular cotransport model seems well established, its physiologic function remains to be explained. Several authors have proposed that it operates in opposition to the Na-K ATPase as a regulator of cellular volume (11,12,113,114). It may also serve as a mechanism for transporting K into cells. Of particular interest to this study is the fact that drugs with diuretic properties are known to inhibit the cotransport system (10,14,18,20,114,115,119). Moreover, Palfrey et al. (10) have shown that within this class of agents, diuretic potency is directly related to inhibitory ability. That natriuretic effect should be directly correlated to the ability to specifically

inhibit the cotranport mechanism is a provocative finding, and it is tempting to speculate that this is the mechanism for "active" chloride reabsorption in the thick ascending loop of Henle as has been suggested (19,20). That this would have far-reaching implications in the understanding of the physiology of the renal epithelium is evident. However, this remains speculation, and the true physiologic importance of the cotransport system in vivo in the whole animal remains to be elucidated.

Materials and Methods

In order to evaluate the extrarenal effects of diuretics on potassium tolerance in acutely nephrectomized rats, a potassium loading study was performed in male Sprague Dawley rats (Charles River, Boston, MA). All rats weighed between 225-265 grams and were divided into the following four groups:

Group Ia - Furosemide group

Six rats received an acute intravenous dose of furosemide (20 mg/kg) 30 minutes prior to the infusion of KCl (Figure 2). In a separate study this dose of furosemide was shown to induce a diuresis in rats.

Ib - Furosemide control group

Six rats received an intravenous dose of D_5W equivalent in volume to an appropriate furosemide dose 30 minutes prior to the infusion of KCl.

Group IIa - Hydrochlorothiazide (HCTZ) group

Six rats receive an acute intravenous dose of HCTZ (5 mg/kg) 30 minutes prior to the infusion of KCl (Figure 2). The HCTZ was dissolved in 32% ethanol in D_5W. This dose of HCTZ was shown to induce a diuresis in rats in a separate study.

IIb - Hydrochlorothiazide control group

Six rats received an intravenous dose of 32% ethanol in D_5W equivalent in volume to an appropriate HCTZ dose 30 minutes prior to the infusion of KCl

Group IIIa - Spironolactone group

Six rats received spironolactone (10 mg/kg) subcutaneously 24-30 hours prior to study. This dose was repeated 210 minutes prior to KCl infusion (Figure 2). The spironolactone was dissolved in DMSO.

IIIb - Spironolactone control group

Six rats received a dose of DMSO equivalent in volume to an appropriate spironolactone dose 24-30 hours prior to study, and again 210 minutes prior to KCl infusion.

Group IVa - Bumetanide group

Three rats received bumetanide (0.25 mg/kg) intravenously 30 minutes prior to KCl infusion (Figure 2). The bumetanide was dissolved in 10% ethanol in D_5W.

IVb - Bumetanide control group

Three rats received an intravenous dose of 10% ethanol in D_5W equivalent in volume to an appropriate dose of bumetanide 30 minutes prior to KCl infusion.

During each K loading study, a diuretic treated rat was studied with a concomitant control in order to control for time-related changes in each parameter examined.

All animals were maintained on 15 grams of Standard Purina Rat Chow (Na and K content equal to 2.5 and 4.0 mEq/day, respectively) for 4-7 days before study. All rats consumed the entire 15 grams of chow each day. All rats were allowed free access to water.

Potassium loading study

Rats were fasted from the evening prior to study. In the case of animals in Group IIIa (spironolactone) and IIIb (DMSO), a dose of the appropriate drug was administered subcutaneously 24-30 hours prior to study and again at approximately 210 minutes before KCl infusion of the day of study. On the day of study, the animals were anesthetized with Inactin (Promonta, Hamburg, Germany), 12 mg/ 100 gm body weight intraperitoneally. Surgery was begun approximately 15 minutes after anesthesia. Body temperature was maintained between 37-38^{o} by means of a warming board and an overhead desk lamp; temperature was monitored by rectal thermometer.

A midline skin incision was made in the neck and a tracheostomy tube inserted to ensure adequate ventilation. The right external jugular vein and the left carotid artery were then cannulated with PE-50 tubing. All test substances were infused through the venous catheter and all blood samples were withdrawn through the arterial catheter. Blood pressure was measured via the carotid catheter.

Following neck surgery a midline abdominal incision was made and bilateral nephrectomy performed. The blood supply to the adrenal glands was left intact. All animals received a 1% body weight bolus of 0.15 M NaCl over 20 minutes following neck surgery, and a maintenance infusion of 0.15 M NaCl at 0.35 ml/ 100 gm body weight/ hour thereafter. A Harvard pump (Harvard Apparatus, Millis, MA) was used to deliver all infusions. The abdominal incision was closed with surgical staples.

Following an equilibration period of approximately 60 minutes (T = -60 to T = 0, see Figure 2), two carotid blood samples (E_1 and

E_2) were obtained 15 minutes apart (T = 0 and 15) for analysis of equilibration period plasma Na and K concentrations. Immediately following the second blood sample, an intravenous dose of the appropriate diuretic or its control substance was given in Groups Ia and Ib, IIa and IIb, and IVa and IVb, temporarily interrupting the maintenance NaCl infusion for approximately one minute. The volume of the dose of drugs and controls varied between 0.40 and 0.50 ml. No drug was given at this time in Group IIIa and IIIb. Two more carotid blood samples (B_1 and B_2) were obtained 15 minutes apart (T = 30 and 45) for analysis of baseline plasma Na and K concentrations. At T = 45 minutes, the saline infusion was replaced with an infusion of 0.5 M KCl administered at 0.35 ml/ 100 gm body weight/ hr to deliver 0.17 $\pm$ 0.02 mEq of potassium per 100 gm body weight per the hour period of KCl infusion. Four carotid blood samples (T_1, T_2, T_3, and T_4 at T = 75, 90, 105, and 120 minutes) were obtained.

All samples were collected in Red-Tip Heparinized Micro-Hematocrit capillary tubes (Fisher Scientific Co., Pittsburg, PA). The patency of the carotid catheter was maintained with heparinized saline (30 /ml). Approximately 0.20 ml of heparinized saline was given to each animal via the carotid catheter after each blood sampling to replace the volume of blood loss. Hematocrit was measured on every blood sample. Blood pressure was measured at T = 15, 45, 75, and 105 minutes. Blood pressure measurements were made with a U-tube mercury manometer connected by rigid tubing to the carotid artery catheter. The manometer was calibrated daily to correct for changes in barometric pressure.

In a separate study, the diuretic effectiveness of the doses used for Groups Ia and Ib and IIa and IIb were evaluated (Figure 3).

Experimental animals underwent neck surgery as outlined above, but nephrectomy was not performed. Instead, a lower midline abdominal incision was made and a catheter inserted into the bladder, taking care not to disturb the ureters. Following an equilibration of approximately 60 minutes, two carotid blood samples (P_1 and P_2) were obtained 30 minutes apart (T = 15 and 45 minutes) for analysis of baseline Na and K concentrations. Urine was collected over 30 minute intervals. Two baseline urine collections (U_1 and U_2) were obtained at T = 30 and 60 minutes (see Figure 3) and were analyzed for Na and K concentrations and volume. At T = 60 minutes, a chosen dose of diuretic or an equivalent volume of its respective control was given intravenously over approximately one minute. Four more carotid blood samples (PE_1, PE_2, PE_3, and PE_4 at T = 75, 105, 135, and 165 minutes) and four more urine collections (UE_1, UE_2, UE_3, and UE_4 at T = 90, 120, 150, and 180 minutes) were taken for analysis as outlined above. An effective diuretic dose was a dose that resulted in both an increase in urine volume and an increased natriuresis.

The dose of spironolactone used (10 mg/kg) was arbitrarily chosen as approximately five to ten times the average effective oral mg/kg dose in man. Administration of spironolactone to test animals was via a subcutaneous route. Each animal was given two doses of the diuretic, the first 24030 hours prior to study and the second approximately 210 minutes prior to KCl infusion.

Bumetanide, like furosemide, is a 3-aminobenzoic acid derivative, but bumetanide has a higher milligram potency. It is equipotent with furosemide at 1/40th the molar dose (119). However, bumetanide is ineffective as a diuretic in the rat (120). The dose of bumetanide used (0.25 mg/kg) was arbitrarily chosen as approximately 2.5 times the intravenous dose known to produce a diuresis in dogs (121).

Analytic methods

Hematocrits were measured by collecting blood samples in heparinized capillary tubes which were spun for three minutes on a Micro Hematocrit IEC MB Centrifuge (Demon/IEC Division, Needham Heights, MA), and subsequently read on a Micro Hematocrit Reader.

Plasma and urine Na and K concentrations were measured with a flame photometer (Instrumental Laboratories, Watertown, MA) using an internal lithium standard. Blood pH and plasma bicarbonate levels were not obtained.

Calculations

In Groups Ia, Ib, IIa, IIb, IVa, and IVb, mean baseline plasma Na and K concentrations were obtained by averaging two measurements obtained 15 minutes apart (B_1 and B_2) during the 30 minutes after animals had received their respective drugs or control substances, but before KCl infusion. In Groups IIIa and IIIb, mean baseline plasma Na and K concentrations were obtained in identical fashion in the 30 minutes prior to KCl infusion; in this case the drug and its control had been given at 24-30 hours prior to study and again at 210 minutes prior to

KCl infusion. In all groups, plasma Na and K increments above the baseline mean at T = 75, 90, 105, and 120 minutes were calculated for each animal. The rise in plasma K concentration at the end of each timed interval was used as an index of K tolerance. Changes in plasma Na and K concentrations at these times as well as changes in hematocrit and blood pressure were calculated for all animals. Results of each group were then compared to its respective control group and analyzed by the Student t-test. Results are listed in Tables 1 and 2. All values are given as the mean $\pm$ the standard error of the mean.

Results

Weights

Mean body weight in each group a was not significantly different from its respective control group b. Mean weights at the time of study were:

Group Ia	241.3 ± 4.1 grams	Ib	242.2 ± 4.3 grams
IIa	234.3 ± 7.6 grams	IIb	233.7 ± 6.7 grams
IIIa	247.0 ± 4.1 grams	IIIb	246.8 ± 3.7 grams
IVa	235.0 ± 4.7 grams	IVb	236.3 ± 5.2 grams

Blood pressure

Mean blood pressures during the equilibration period prior to administration of a diuretic, during the baseline period immediately following equilibration, and at the end of the study were compared to respective controls. Results are listed in Table 1. As can be seen, mean blood pressures in each group a did not differ significantly from its respective control group b.

However, it should be noted that in group IIa (HCTZ) and IIb (HCTZ control) there was a significant fall in blood pressure in both groups (-14.3 ± 3.4 and -18.3 ± 3.3 mm Hg, respectively) immediately following administration of either the drug or its control. This was in contrast to all other groups where no such change in blood pressure was observed, and was probably due to the vasodilatory effects of the fairly large ethanol percentage required to dissolve the HCTZ. However, blood pressures in group IIa did not differ significantly from its control group IIb, and by the end of the study blood pressures in both

groups had risen to levels comparable to the other groups.

Hematocrit

The mean hematocrit in each group a at the beginning of the study was not significantly different from its respective control group b. Mean hematocrits at the end of the study and the increment between initial and final hematocrits were also not significantly different between experimental and control groups.

KCl dose

The amount of KCl infused was similar in all groups, and ranged between 0.17 $\pm$ 0.1 mEq/100 gm body weight/hour.

Potassium loading study

Mean plasma K and Na concentrations were similar in all groups during the baseline period (see Table 2). The results of the potassium loading studies are shown in Table 2 and Figures 4-7.

The maximal increment in plasma K concentration after one hour of KCl infusion (ΔT_4) is listed for each group in Table 2, and was similar in each group. As can be seen graphically in Figures 4-7, the rise in plasma K concentration during KCl infusion in the groups of rats given diuretics closely approximated the rise in plasma K in their respective controls. Differences in increments between plasma K at given time intervals and plasma K during baseline periods ($\Delta T_1 - \Delta T_4$) were not statistically significant.

Plasma Na concentration at baseline varied from 137.2 $\pm$ 2.7 to 143.2 $\pm$ 1.30 mEq/L. There were no significant changes in plasma Na concentrations during study.

Discussion

It has been a long standing assumption that diuretics have little effect on electrolyte transport outside the kidney (122). However, in view of the fact that more needs to be known regarding their mechanisms of action within the kidney and their effects of electrolyte homeostasis, further study of their actions both within the kidney and in extrarenal tissues is warranted.

The purpose of this study was to examine the effects of diuretics of three different chemical families on extrarenal potassium disposal. The results indicate that furosemide, bumetanide, hydrochlorothiazide, and spironolactone at the doses employed do not significantly alter the ability of the acutely nephrectomized rat to tolerate a potassium load. The increments in plasma K following intravenous KCl administration were not significantly different in animals given diuretics as compared to controls given no diuretics.

While the present study shows no significant changes in potassium disposal with the diuretics used, there is information in the medical literature that made us suspect that these agents could have an extrarenal effect on potassium homeostasis. Hypokalemia is a common complication of diuretic therapy in man (120) and is generally attributed to potassium losses in the urine. However, there have been suggestions made in recent years that the etiology of the observed hyperkalemia may not be as simple as an increase in potassium excretion, and that changes in internal potassium balance may be among the extrarenal effects which diuretics could possess. Since the reasons

for pursuing an extrarenal effect on potassium disposal differ with each diuretic, the three families of diuretics used in this study will be discussed separately here.

I. Furosemide and Bumetanide

Furosemide, like the other "high ceiling" diuretics including bumetanide (Figure 1), inhibits sodium and chloride reabsorption in the thick ascending loop of Henle (122,123). Although the mechanism of its action is not fully understood, it appears to involve primarily inhibition of the active transport of Cl at the luminal membrane of tubular cells in the ascending limb (122,123), resulting in increased flow to the distal tubule and collecting system. This causes an impairment of urinary concentrating and diluting ability and increased urinary Na, Cl, K, and water losses. Like the rest of the high ceiling diuretics, the diuretic potency of furosemide far exceeds diuretics of other classes (124,125). Bumetanide appears to have an action similar to that of furosemide, and has a higher milligram potency in dogs and in man (119,120).

A number of extrarenal effects of furosemide on systemic hemodynamics have been described, including its action on pre-load reduction (1) and its action as an antihypertensive agent (126-128). As mentioned earlier, furosemide has been shown to induce acutely an increase in venous capacitance which precedes its natriuretic effect (1,129,130). The observed decrease in left atrial and left ventricular filling pressures is probably due to increased renal blood flow as well as to increased peripheral venous capacitance (1,131). As noted by Dikshit

et al. (1), this furosemide-induced hemodynamic effect is more rapid than the inotropic effect of digitalis, and may be more beneficial in the setting of acute myocardial infarction not only due to its effects on relieving pulmonary edema and congestion but also to its relative lack of demand on myocardial oxygen requirements.

Furosemide has been used widely as an antihypertensive agent. Immediate decreases in blood pressure are probably mediated by volume changes. However, the mechanism of its hypotensive effect in chronic use is felt to be distinct from its diuretic activity. This is supported by the observation that furosemide in large doses has been noted to be an effective antihypertensive in the presence of renal insufficiency (132,133). Although the effect of decreasing blood pressure is not fully understood, it probably involves a number of factors, including increased peripheral venous capacitance (1,134), decreased peripheral arteriolar resistance (126,135), and increased peripheral blood flow (126,135).

There has recently been described an electrolyte cotransport system capable of producing net sodium, potassium, and chloride movement intracellularly (10,14,15,19,108-113). The in vivo physiologic significance of this mechanism is not known. However, it has been postulated that it acts in opposition to the Na-K ATPase which extrudes Na from dells, and that it may have a significant role in the regulation of cellular volume as well as in electrolyte balance (11,12,112,113). Moreover, it is of interest that the mechanism has been demonstrated to exist in the loop of Henle (19) and is inhibited by furosemide. The cotransport is probably the mechanism

of "active" chloride transport in the ascending limb. This postulate does not necessarily contradict evidence that chloride transport in the thick ascending loop is dependent in some way on Na-K ATPase activity (123). As outlined by Aronson (118) and Crane (136), secondary active transport is by definition not directly dependent on active metabolic expenditure. However, there must be an indirect linkage to some such primary active process in order for it to continue. For example, many known secondary active transport systems involve coupling of the transport of substances against their electrochemical potential differences to the movement of sodium down its electrochemical gradient. In order to maintain the sodium gradient oriented to allow sodium flux into cells, an active transport mechanism to extrude sodium from cells must exist. Otherwise the gradient would dissipate and any transport process coupled to sodium movement would cease. For example, Crane has proposed this form of couple transport as the mechanism of active intestinal glucose absorption (136). In the intestinal epithelium, a primary active mechanism in the basolateral membrane actively pumps Na out of cells at the expense of ATP, hence maintaining the Na electrochemical gradient directed toward allowing Na movement into cells. In this manner, secondary active transport of such compounds as glucose can continue across the luminal membrane. Frizzell et al. (137) have proposed a similar mechanism for active chloride transport. A similar type of transport may be effective in the ascending loop of Henle. That is, the postulated Na-K-Cl cotransport mechanism may be linked secondarily to a primary active Na-K ATPase pump. Hence, inhibition of the pump, for example by ouabain, would indirectly

but surely block Cl uptake in the ascending limb as has been observed (123).

As mentioned, the physiologic significance of this cotransport system is not known. However, if the mechanism is an important regulator of electrolyte balance not only in the loop of Henle as has been suggested, but in the whole animal as well, then one would expect that inhibition of the transport could have significant *in vivo* consequences. Of particular interest to this study are the possible effects of cotransport inhibition on extrarenal potassium homeostasis. The nephrectomized rat model used for the present study excludes renal effects on electrolyte balance. If blocking the cotransport mechanism with furosemide or bumetanide causes inhibition of potassium movement into cells, then one would expect to see a decrease in K tolerance in response to an acute K load. There is precedent to look for these effects in the whole animal in that the cotransport system is known to exist extrarenally in human red blood cells (14-18). However, as is evident in Figures 4 and 5, this was not the case with either furosemide or bumetanide. The timed increments in serum potassium observed in both furosemide-treated and bumetanide-treated animals were not significantly different from the increments observed in their controls. These results argue against the postulate that the cotransport mechanism is a major regulator of *in vivo* extrarenal K homeostasis.

It is not surprising that bumetanide failed to cause any significant change in K tolerance. As pointed out by Palfrey et al. (10), the ability of the aminobenzoic acid derivatives to inhibit the cotransport

mechanism is directly related to diuretic potency. Bumetanide is not effective as a diuretic in the rat (120), and the results obtained here support their conclusion. However, furosemide is known to be an effective and potent diuretic in the rat, and it was expected that it might cause a change in K tolerance.

It should be noted that the cotransport mechanism has never been studied in the whole animal. It is certainly possible that it does function as an important regulator of extrarenal electrolyte homeostasis, but that its effects on electrolyte balance are not detectable by the techniques used here. As outlined previously, the mechanism has been studied in isolated cell systems in which other factors known to influence K homeostasis in vivo can be controlled. The physiologic constraints of using a whole animal model can be numerous.

Keeping this in mind, the fact that no effect on K tolerance was seen does not necessarily imply that the cotransport system is not operative. It may be that other regulators of K homeostasis are either more important in vivo or dominate K regulation in this particular setting. For example, the stress of anesthesia and surgery in these animals has been documented to result in persistently elevated epinephrine levels (30). β-adrenergic agents are known to stimulate cotransport in isolated cell systems; their effects oppose those of such agents as furosemide. Therefore, in the experimental set-up used here, one would expect the cotransport mechanism to be maximally stimulated by epinephrine prior to administration of furosemide. The results obtained showing no change in K tolerance in furosemide-treated

animals when compared with controls may be explained by this maximal β-adrenergic stimulation. This may indicate either that β-adrenergic stimulation is the dominant regulator of the cotransport mechanism or that β-adrenergic stimulation is not reversible at the doses of furosemide used. This raises another issue. The transport mechanism is known to be inhibited by concentrations of furosemide in the range of 10^{-5} - 10^{-3} M (10). Furosemide, like other organic acids, is actively secreted into the proximal tubule (138) and concentrated in the tubular lumen, and is known to inhibit chloride reabsorption at concentrations of 10^{-6} - 10^{-5} M in the lumen of the ascending limb (122). The volume of distribution of furosemide is 10% body weight, and in plasma, the drug is 95% protein bound (139). At the dose used in these experiments (20 mg/kg), one would estimate that the concentration of free circulating drug would be approximately 3×10^{-5} M. This is near the lower limit of concentrations at which an effect is seen on cotransport in isolated cell systems, and it is possible that the free fraction of drug in this experimental setting may not have been sufficient to effect cotransport inhibition. It would be of interest to accurately determine if concentrations obtained with the dose used are within the range known to produce inhibition in vitro; however, this was beyond the scope of the present experiments.

The role of cotransport on electrolyte homeostasis merits further work. It would be interesting to repeat these experiments in a setting in which factors which might interact or interfere with its effects could be better controlled. In particular, its significance in tissues known to participate in K storage and regulation, such as muscle or liver,

should be pursued. There are also a number of ways in which the design of these experiments could be modified and possibly improved. For example, pH, bicarbonate levels, and hormone levels were not measured in the present study. Had there been significant changes in K tolerance noted, these would have been explored more thoroughly. Also, it is possible that following another electrolyte besides K would be informative. The postulated stoichiometry of the system is 1K : 1Na : 2Cl. It may be that the changes brought about in K balance were too small to be quantitated in the face of the large K load administered. Under these circumstances, measuring chloride concentrations might have yielded more significant results, since twice as much Cl is transported as K. If K infusion could be used in the same manner to stress the cotransport system, but with an anion other than Cl, then monitoring response to the inhibition of the cotransport mechanism by following chloride concentration might be more fruitful.

II. Hydrochlorothiazide

Thiazides are among the most commonly used diuretic agents. The mechanism of the action of thiazides within the kidney is not fully understood, but appears to involve inhibition of reabsorption of sodium and chloride in the distal tubule via an interaction with specific renal receptors (120,140).

Numerous extrarenal effects of thiazides have been described. Their use in the treatment of hypertension is perhaps the most obvious. Initially thiazides cause a decrease in blood volume and a drop in

cardiac output due to their diuretic activity. However, during chronic therapy, cardiac output returns to normal and peripheral resistance falls (141,142), thereby maintaining a relatively hypotensive state (3,4). This effect is not related temporally or in magnitude to the diuretic effect per se, and persists after normalization of the cardiac output.

Other extrarenal effects of thiazides include an association of their use with impaired glucose tolerance (144-147). The mechanism of this effect is not known but is felt perhaps to involve production of hypokalemia which then limits insulin secretion (147,148). Thiazides also have major effects on metabolic balance. Koppel et al. (149) have demonstrated an extrarenal effect of thiazides on calcium balance. In patients with end stage renal disease and hypertension treated with thiazides, a distinct hypercalcemic effect was noted. These authors postulate that this may have resulted from potentiation of the action of parathyroid hormone in these patients who often have underlying parathyroid gland hyperplasia secondary to their renal disease. A hypermagnesemic effect was also noted in these patients, which was felt to be attributable to decreased renal Mg excretion and perhaps to an increased release of Mg from bone or soft tissues.

Of most interest to the present study is the suggestion by several investigators that thiazides have an extrarenal effect on potassium homeostasis. There is significant controversy over what effect thiazides have on serum and total body potassium. Some investigators report significant reductions in total body potassium with thiazide use (150-152) while others report no reduction in total

body K (151,153-155). Oh and Carroll (156) have pointed out that a mathematically significant fall in total body K may not be physiologically significant. Nevertheless, the hazard of hypokalemia in patients on thiazides is evident, particularly in the setting of myocardial disease, hypertension, digitalis use, and liver cirrhosis in which they are often used.

It is a common clinical observation that a significant number of patients using thiazide diuretics have a fall in serum potassium (157). Chronic hypokalemia is thought to result from increased aldosterone secretion due to elevated renin levels secondary to chronic diuretic-induced oligemia (158). This is supported by the work of Sweet and Gaul (157) who noted that thiazide-induced hypokalemia could be corrected by adding a β-adrenergic blocking agent, theoretically by decreasing plasma renin activity. However, these authors point out that the diuretic produced a lowering of serum potassium even when plasma renin was suppressed to pre-treatment levels by β-blockade. It is striking that thiazide-induced hypokalemia is so commonly observed in view of multiple demonstrations that total body potassium is unchanged even with prolonged thiazide use. Recently a number of groups have postulated that the hypokalemia associated with thiazide administration may reflect redistribution of potassium rather than an absolute reduction of total body potassium (23,24,159).

In a study on the use of thiazides in hypertensive patients, Talso and Carballo (159) noted that 85% of these patients demonstrated a decrease in serum potassium concentration, yet simultaneous determinations of total body potassium revealed no significant changes

in the body content of the cation. They postulated that alterations in acid-base balance, notably the metabolic alkalosis often observed with thiazide use, might have a significant effect on the production of hypokalemia by causing redistribution of potassium. However, while alterations in extracellular pH and replacement of hydrogen ion in the form of ammonium chloride did favorably alter serum potassium status during thiazide administration, these authors noted that supplementation of the extracellular hydrogen ion concentration was not the sole mediator of the extracellular potassium concentration, and suggested that other factors, including perhaps an effect of thiazides on the permeability of the cell membrane to potassium, were involved in determining the level of serum potassium.

There have been a number of cases described in the pediatric population of hyperkalemia occurring in the setting of normal renal and adrenal function and associated with acidosis and hypertension (21, 22). Recently, the phenomenon of hyperkalemia following renal transplantation but unrelated to graft rejection has been noted in a significant percentage of post-transplant patients (23). DeFronzo et al. (23) noted that this was present in 23 of 75 consecutive transplant patients within three months of transplantation, and was present in the absence of acidosis, renal insufficiency, and rejection. It was also noted that the observed hyperkalemia was corrected by administration of hydrochlorothiazide. The authors have postulated that the transient defect in tubular K secretion observed post-transplantation was due either to decreased tubular basolateral membrane K uptake or to decreased luminal membrane permeability to K, and that the effectiveness of

thiazide in reversing the hyperkalemia may be due to alteration of either or both of these cellular defects. The authors also point out the possibility that thiazides may have actions on extrarenal potassium distribution which could account for or at least contribute to the observed decrease in serum K.

In another recent study, Farfel et al. (24) have described two cases in which hypertension and hyperkalemia associated with normal renal function, low plasma renin activity, and normal plasma aldosterone levels were corrected with thiazide administration. The authors have postulated that the etiology of the observed syndrome lies in a defect in cell membrane potassium transport. Of particular interest to the present study was the fact that thiazide administration caused serum K to fall to normal values in the absence of a significant increase in urinary K excretion in either patient. In general it has been assumed that the hypokalemic effect of diuretics is due to their action of increasing renal K losses. However, these results support the theory that an extrarenal effect of thiazides causing cellular K redistribution plays a significant role in their hypokalemic effect.

There is, then, precedent to believe that thiazides may have extrarenal effects on potassium homeostasis. The model used for the present study was chosen for its exclusion of renal effects. If the hypokalemic effect of thiazides is caused by cellular redistribution of potassium, one would expect that tolerance to a potassium load would be improved in their presence. However, as is evident graphically in Figure 6, this was not the case. The timed increments in serum potassium observed in thiazide-treated animals were not different from

the increments observed in their controls. These data argue against the postulated extrarenal hypokalemic effect of thiazides. It is unlikely that the dose of hydrochlorothiazide was insufficient since this is a maximally natriuretic dose (143), and since it is several times greater than the usual daily oral dose in human subjects. However, it is possible that an effect does exist, but that its magnitude is not great enough to maintain a significant lowering of plasma potassium in the face of the large KCl infusion used in these experiments.

III. Spironolactone

The introduction of the aldosterone-antagonist spironolactone in 1957 (160) represented one of the first cases in which knowledge of the biochemical structure of a compound with known biologic effect was used to search deliberately for and synthesize a specific pharmacologic agent. In view of the fact that aldosterone itself had not been isolated until 1952 (161), this was a remarkable accomplishment.

Spironolactone is a competitive inhibitor of aldosterone. It does not inhibit secretion of aldosterone or of any of the other adrenal steroids, but antagonizes the effect of aldosterone by combining with specific target tissue receptor sites (162,163). In the kidney it antagonizes the aldosterone-stimulated K secretion and Na reabsorption that occurs in the distal tubule (120). In the absence of aldosterone, spironolactone has no effect on renal electrolyte excretion (163), but if aldosterone is present spironolactone promotes a natriuresis regardless of the level of mineralocorticoid (164). Potassium retention

occurs only if there is a concomitant low sodium intake, and this action can be overcome by sufficiently increased levels of aldosterone (165,166). Use of spironolactone may induce hyperkalemia; however, with chronic use serum K levels return toward pre-treatment levels (167,168).

Spironolactone induces a modest hypotensive effect in most hypertensive patients, and has a synergistic hypotensive effect with other diuretics (169). That this action is distinct from a purely volume depleting effect due to its diuretic action is shown in that expansion of the extracellular fluid volume with dextran, plasma, or saline does not abolish its effect (170).

Evidence exists that mineralocorticoids increase extrarenal tissue K uptake in the whole animal (25,27-29). As this has been discussed in the introduction, studies supporting this hypothesis will not be reiterated here. The availability of spironolactone as a selective inhibitor of aldosterone activity can be used as a tool to further investigate this theory. Moreover, in view of the fact that there is precedent in the example of insulin to believe that basal levels of hormones may play a role in K regulation, it is of interest to examine the effects of selective aldosterone blockade on extrarenal potassium disposal.

If, as recently postulated, extrarenal potassium tolerance is impaired in the setting of aldosterone deficiency (25,30), then one would expect to see a decrease in the ability of animals given spironolactone to tolerate a potassium load. The results of the present study indicate, however, that administration of spironolactone

did not significantly alter the hyperkalemic response in nephrectomized rats given an acute potassium load. As can be seen in Figure 7, the timed increments in plasma potassium observed in spironolactone-treated animals were not different from the increments observed in control animals. The conclusion to be drawn from these results is that blockade of peripheral aldosterone activity does not impair the ability of extrarenal tissues to dispose of potassium as has been suggested. This argues against the hypothesis that aldosterone plays an important role in extrarenal potassium regulation.

The possiblity exists, of course, that the results obtained here do not accurately reflect the effects of aldosterone inhibition. It has been noted clinically as well as experimentally that several days of spironolactone administration are required to observe the effects of aldosterone inhibition (169,171). Although the doses used in the present study should have been sufficient to induce aldosterone inhibition, it is possible that a longer duration of administration is necessary. This was avoided in this study to prevent the effects of spironolactone-induced increases in body potassium stores which are observed with long term use of the drug.

Another possible explanation for failure to see decreased K tolerance is the method used to administer the drug. French and Manery (172) have pointed out that intermittent administration of depot preparations of aldosterone are not reliable in producing its mineralocorticoid effect. It would not be unreasonable to consider that this might also be true of spironolactone, since the compounds are chemically very similar. One means of checking this would be to

obtain plasma and tissue levels of the drug, but this was not feasible here.

Conclusion

The effects of furosemide, bumetanide, hydrochlorothiazide, and spironolactone on extrarenal potassium homeostasis were studied in acutely nephrectomized rats. None of these agents altered the ability of experimental animals to tolerate a potassium load. We conclude that, within the limits of the experimental design of this study, diuretics do not modify extrarenal potassium disposal. This study provides no evidence that the changes in serum potassium concentration observed with diuretic use are caused by any changes other than alterations in renal potassium excretion.

Table 1

Group	Blood Pressure (mm Hg) Equilibrium mean (E)	Baseline mean (B)	T_4	Increment in Blood Pressure (mm Hg) (E) - (B)	T_4 - (E)
Ia	100.0 ± 5.3	104.0 ± 3.9	107.0 ± 3.1	4.0 ± 4.2	3.0 ± 3.0
Ib	102.3 ± 5.6	103.0 ± 5.8	106.7 ± 7.0	0.7 ± 2.6	3.7 ± 4.2
IIa	106.0 ± 7.2	87.0 ± 8.8	94.7 ± 5.7	-14.3 ± 3.4	6.0 ± 6.2
IIb	110.3 ± 10.9	94.3 ± 11.7	103.3 ± 8.4	-18.3 ± 3.3	9.0 ± 3.8
IIIa	111.7 ± 7.6	113.7 ± 6.1	115.0 ± 5.6	3.0 ± 5.8	1.3 ± 3.0
IIIb	111.3 ± 5.8	111.3 ± 4.1	115.0 ± 2.5	-0.3 ± 2.2	2.0 ± 3.5
IVa	112.0 ± 6.1	111.3 ± 9.4	108.7 ± 10.7	-0.6 ± 6.4	-2.7 ± 2.7
IVb	110.7 ± 11.6	100.7 ± 11.0	103.3 ± 8.8	-6.7 ± 2.4	2.7 ± 8.2

Table 1. Blood pressure during equilibrium period (E), during baseline period (B), and at the end of study (T_4), and increments in blood pressure between periods (E) and (B) and between periods T_4 and (E). All values are given as the mean ± SEM.

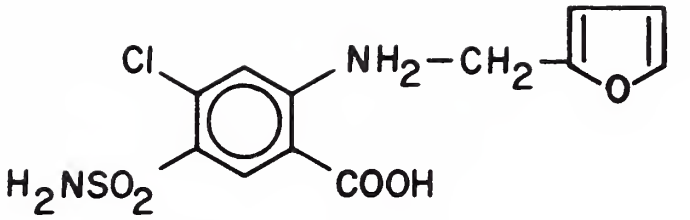
Table 2

Group	Baseline mean plasma K (mEq/L)	Increment in plasma K above baseline (mEq/L) ΔT_1	ΔT_2	ΔT_3	ΔT_4
Ia	5.21 ± .13	.71 ± .14	1.89 ± .19	2.60 ± .16	3.11 ± .17
Ib	4.95 ± .11	.96 ± .18	1.89 ± .05	2.58 ± .10	3.21 ± .14
IIa	4.57 ± .15	.78 ± .12	1.50 ± .12	2.20 ± .13	2.74 ± .18
IIb	4.91 ± .14	.67 ± .17	1.50 ± .17	2.10 ± .17	2.69 ± .26
IIIa	5.12 ± .11	.88 ± .07	2.15 ± .06	2.85 ± .07	3.32 ± .12
IIIb	4.83 ± .19	.97 ± .13	1.92 ± .20	2.68 ± .21	3.08 ± .22
IVa	4.87 ± .51	.61 ± .16	1.59 ± .18	2.55 ± .16	3.31 ± .25
IVb	4.66 ± .44	1.02 ± .03	1.81 ± .08	2.94 ± .15	3.67 ± .20

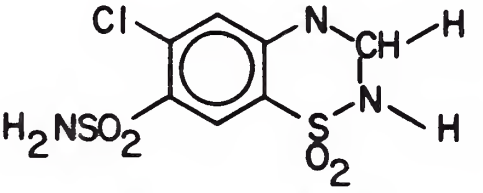
Table 2. Baseline mean plasma K concentrations.

Increments in plasma K concentrations above baseline at timed intervals during KCl infusion.

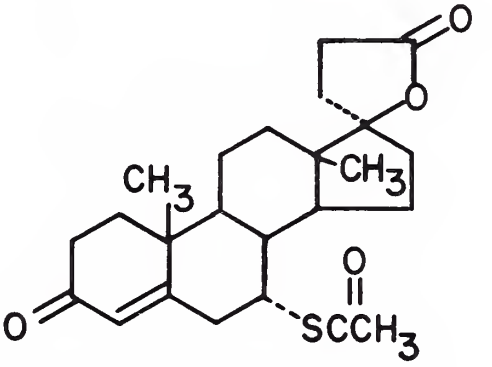
All values are given as the mean ± SEM



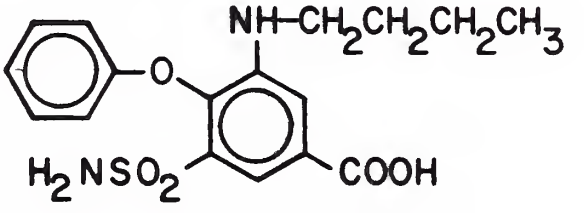
Furosemide



Hydrochlorothiazide



Spironolactone



Bumetanide

Figure 1. Chemical structures of furosemide, hydrochlorothiazide, spironolactone, and bumetanide.

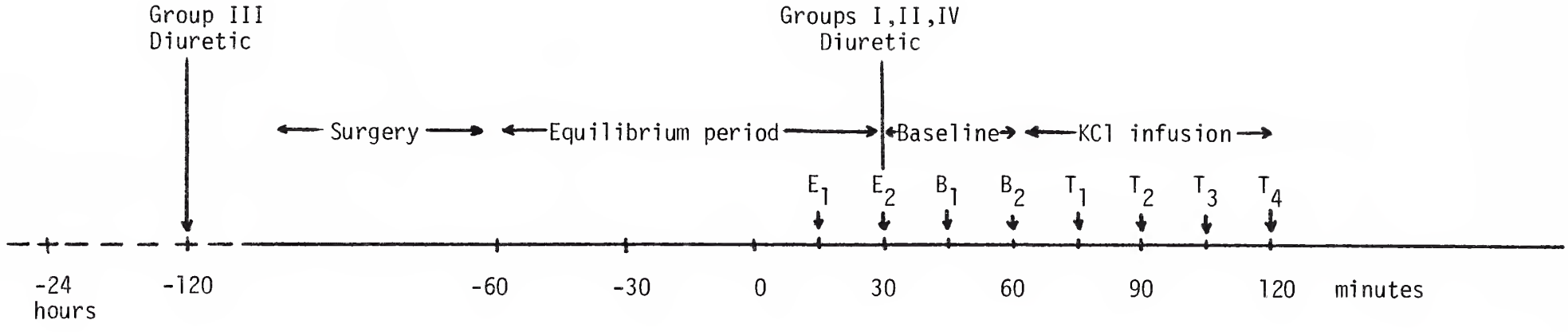


Figure 2. Protocol for potassium loading study

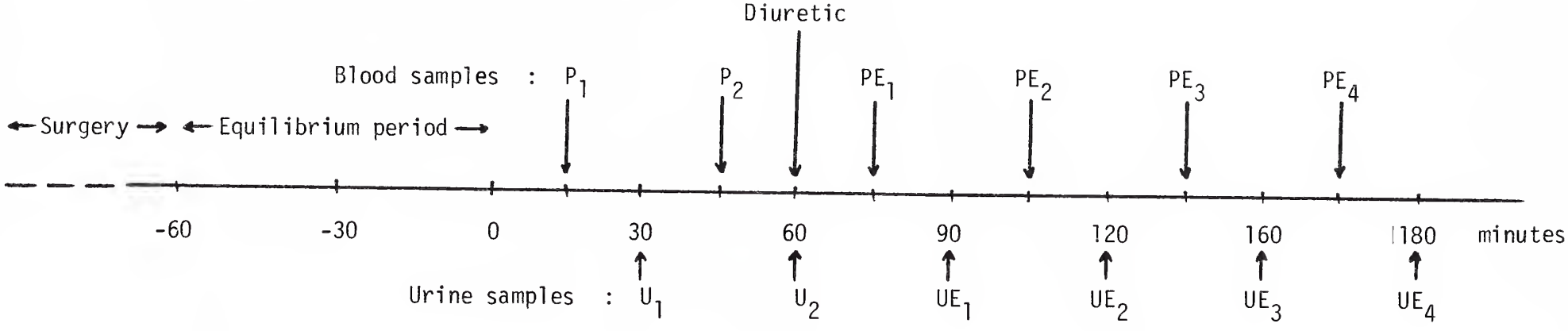


Figure 3. Protocol for study to test diuretic effectiveness.

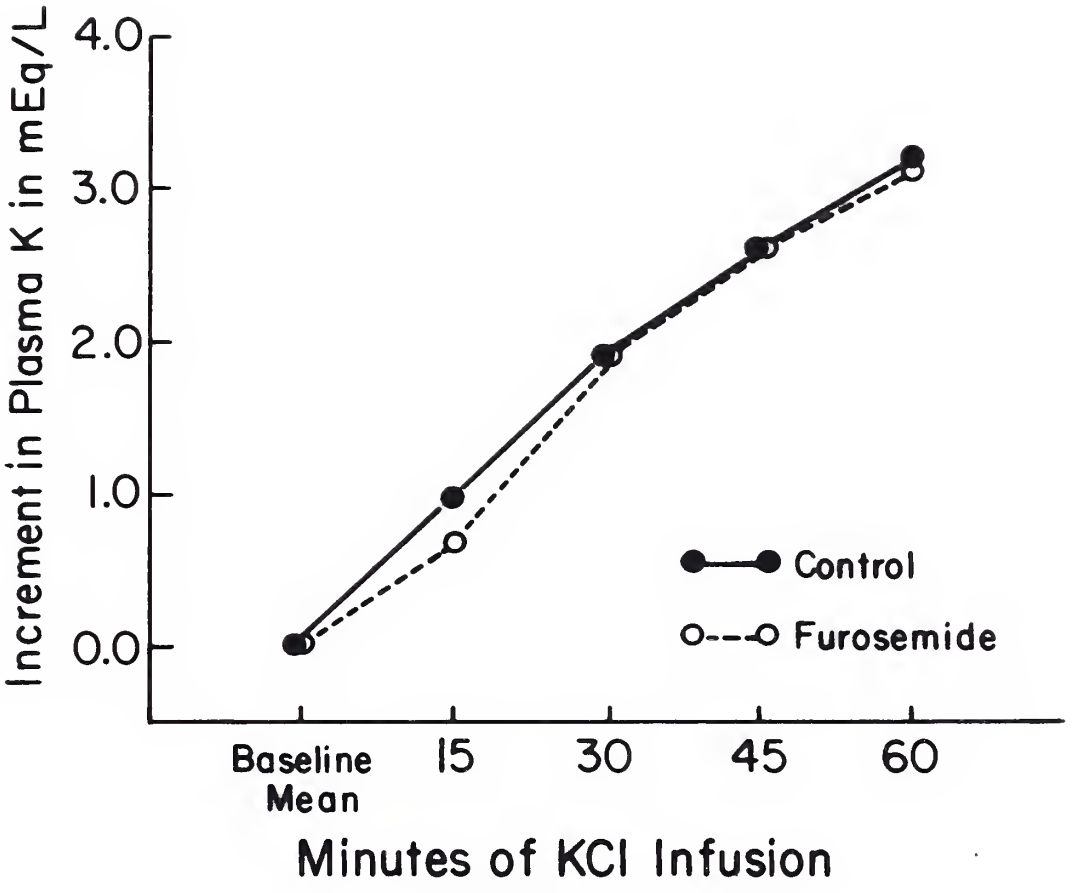


Figure 4

Figure 4. Increment in plasma K concentration above baseline in control (●——●) and furosemide-treated (o- -o) animals. KCl was infused from T = 0 to T = 60 minutes.

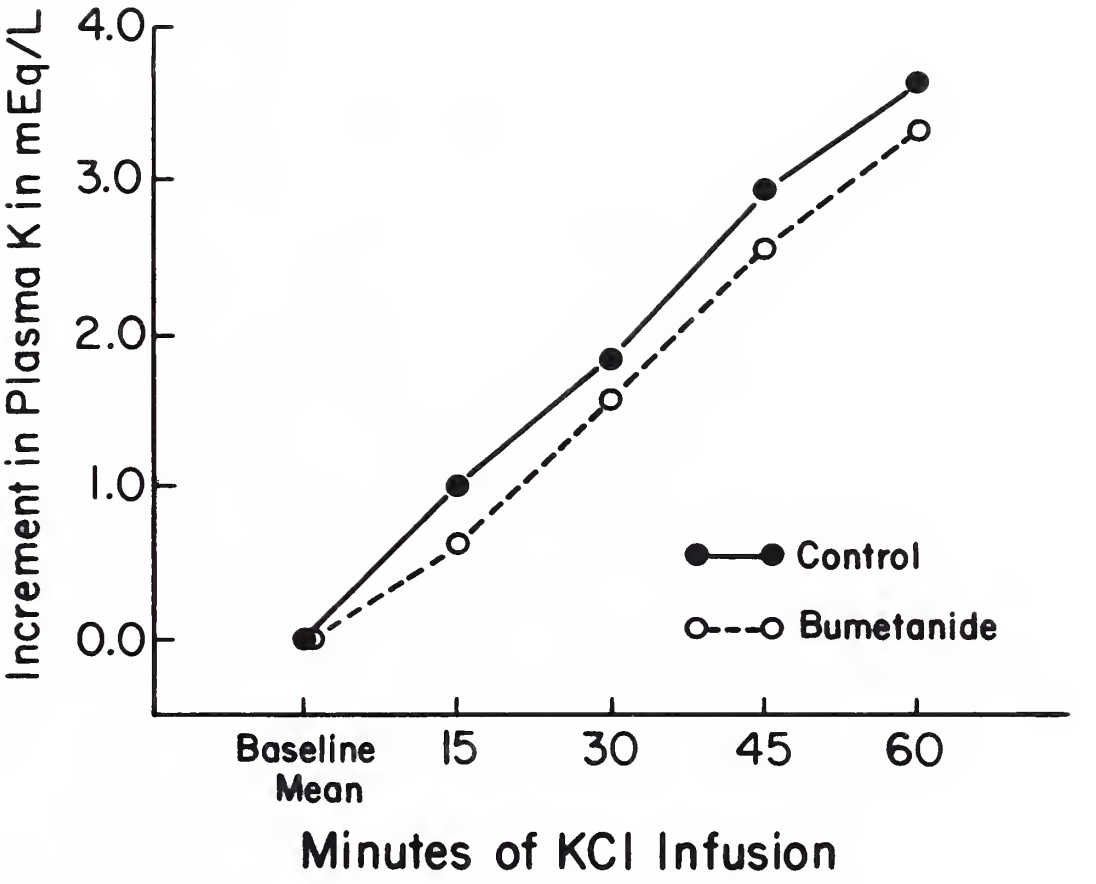


Figure 5

Figure 5. Increment in plasma K concentration above baseline in control (●—●) and bumetanide-treated (o--o) animals. KCl was infused from T = 0 to T = 60 minutes.

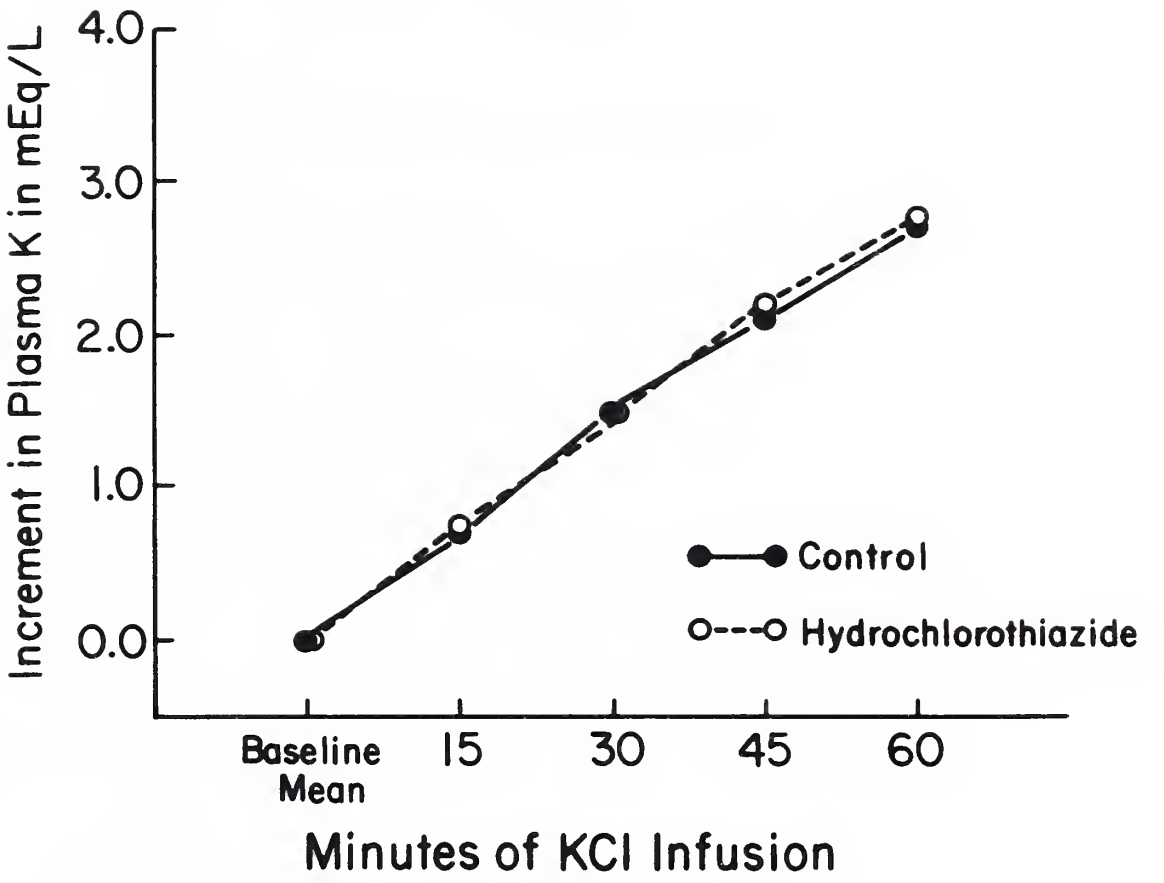


Figure 6

Figure 6. Increment in plasma K concentration above baseline in control (●—●) and hydrochlorothiazide-treated (o--o) animals. KCl was infused from T = 0 to T = 60 minutes.

Bibliography

1. Dikshit, K., J.K. Vyden, J.S. Forrester, K. Chatterjee, R. Prakesh, and H.J.C. Swan. Renal and extrarenal effects of furosemide in congestive heart failure after acute myocardial infarction. New Eng J Med 288:1087-1090, 1973.

2. Freis, E.D. Salt, volume, and the prevention of hypertension. Circ 53:589-595, 1976.

3. Wilkins, R.W., W. Hollander, and A.V. Chobanian. Chlorothiazide in hypertension: Studies on its mode of action. Ann NY Acad Sci 71: 465-474, 1958.

4. Conway, J. and H. Palmero. The vascular effect of the thiazide diuretics. Arch Int Med 111:203-207, 1963.

5. Cantarovich, F., C. Galli, L. Benedetti, C. Chena, L. Castro, C. Correa, J. Perec Loredo, J.C. Fernandez, A. Locatelli, and J. Tizado. High dose furosemide in established renal failure. Br Med J 4:449-450, 1973.

6. Montoreano, R., J. Cunarro, M.T. Mouzet, A. Ruiz-Guinazu. Prevention of the initial oliguria of acute renal failure by the administratio of furosemide. Postgrad Med J (Suppl) :7-10, 1971.

7. Schroder, K. and U. Gessler. The influence of mannitol, ethacrynic acid, and furosemide on glomerular filtration in experimental acute renal failure. Postgrad Med J (Suppl) :11-12, 1971.

8. Cantarovich, F., A. Locatelli, J.C. Fernandez, and J. Perez Loredo. Furosemide in high doses in the treatment of acute renal failure. Postgrad Med J (Suppl) :13-17, 1971.

9. Fries, D., N. Pozet, N. Dubois, and J. Traeger. The use of large doses of furosemide in acute renal failure. Postgrad Med J (Suppl) :18-20, 1971.

10. Palfrey, H., P.W. Feit, and P. Greengard. cAMP-stimulated cation cotransport in avian erythrocytes: inhibition by "loop" diuretics. Am J Physiol 238:C139-C148, 1980.

11. Kregenow, F.M. and T. Caryk. Co-transport of cations and Cl during the volume regulatory response of duck erythrocytes. Physiologist 22:73a, 1979.

12. Kregenow, F.M., D.E. Robbie, and J. Orloff. Effect of norepinephrine and hypertonicity on K influx and cyclic AMP in duck erythrocytes. Am J Physiol 231:306-311, 1976.

13. Gardner, J.D., G.D. Aurbach, A.M. Spiegel, and E.M. Brown. Receptor function and ion transport in turkey erythrocytes. Recent Prog Horm Res 32:567-595, 1976.

14. Wiley, J.S. and R.A. Cooper. A furosemide-sensitive cotransport of sodium plus potassium in the human red cell. J Clin Invest 53:745-755, 1974.

15. Dunham, P.B., G.W. Steward, and J.C. Ellory. Chloride-activated passive potassium transport in human erythrocytes. Proc Nat Acad Sci 77:1711-1715, 1980.

16. Sachs, J.R. Ouabain-insensitive sodium movements in the human red blood cell. J Gen Physiol 57:259-282, 1971.

17. Beauge, L.A. and N. Adragna. The kinetics of ouabain inhibition and the partition of rubidium influx in human red blood cell.s J Gen Physiol 57:576-595, 1971.

18. Brazy, P.C. and R.B. Gunn. Furosemide inhibition of chloride transport in human red blood cells. J Gen Physiol 68:583-599, 1976.

19. Greger, R.F. and E. Schlatter. Coupled Transport of 2 Cl^-, 1 Na^+, and 1 K^+ at the luminal membrane of the rabbit cortical thick ascending limb of Henle's loop (cTAL). Kidney Int 21:274a, 1982.

20. Burg, M.B. Renal handling of sodium, chloride, water, amino acids, and glucose, in The Kidney, edited by B.M. Brenner and F.C. Rector, Jr. Philadelphia, W.B. Saunders Co., 1981, pp. 328-370.

21. Spitzer, A., C.M. Eledmann, Jr., L.D. Goldberg, and P.H. Henneman. Short stature, hyperkalemia and acidosis: A defect in renal transport of potassium. Kidney Int 3:251-257, 1973.

22. Weinstein, S.F., D.M.E. Allan, and S.A. Mendoza. Hyperkalemia, acidosis, and short stature associated with a defect in renal potassium excretion. J Ped 85:355-358, 1974.

23. DeFronzo, R.A., M. Goldberg, C.R. Cooke, C. Barker, R.A. Grossman, and Z.S. Agus. Investigations into the mechanisms of hyperkalemia following renal transplantation. Kidney Int 11:357-365, 1977.

24. Farfel, Z., A. Iaina, T. Rosenthal, U. Waks, S. Shibolet, and J. Gafni. Familial Hyperpotassemia and hypertension accompanied by normal plasma aldosterone levels, possible hereditary cell membrane defect. Arch Int Med 138:1828-1832, 1978.

25. Bia, M.J. and R.A. DeFronzo. Extrarenal potassium homeostasis. Am J Physiol 240:F257-F268, 1981.

26. DeFronzo, R.A. Hyperkalemia and hyporeninemic hypoaldosteronism. Kidney Int 17:118-134, 1980.

27. Dawborn, J.K. and L. Watson. Effect of prolonged administration of aldosterone on potassium and magnesium metabolism in rabbits. Med J Austr 2:304-307, 1968.

28. Alexander, E.A. and N.G. Levinsky. An extrarenal mechanism of potassium adaptation. J Clin Invest 47:740-748, 1968.

29. Loeb, R.F., D.W. Atchley, E.M. Benedict, and J. Leland. Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. J Exp Med 57:775-792, 1933.

30. Bia, M.J., K.A. Tyler, and R.A. DeFronzo. Regulation of extrarenal potassium homeostasis by adrenal hormones in rats. Am J Physiol (in press).

31. DeFronzo, R.A., R.S. Sherwin, M. Dillingham, R Hendler, W.V. Tamborlane, and P. Felig. Influence of basal insulin and glucagon secretion on potassium and sodium metabolism. J Clin Invest 61: 472-479, 1978.

32. DeFronzo, R.A., P.A. Taufield, H. Black, P. McPhedran, and C.R. Cooke. Impaired renal tubular potassium secretion in sickle cell disease. Ann Intern Med 90:310-316, 1979.

33. Gonick, N.D., C.R. Kleeman, M.E. Rubini, and M.H. Maxwell. Functional impairment in chronic renal disease. Studies of potassium excretion, Am J Med Sci 261:281-290, 1971.

34. DeFronzo, R.A., R. Lee, A. Jones, and M. Bia. Effect of insulinopenia and adrenal hormone deficiency on acute potassium tolerance. Kidney Int 7:586-595, 1980.

35. Fenn, W.O. and D.M. Cobb. The potassium equilibrium in muscle. J Gen Physiol 17:629-645, 1934.

36. Adler, S. and D.S. Fraley. Potassium and intracellular pH. Kidney Int 11:433-442, 1977.

37. Burnell, J.M., M.F. Villamil, B.T. Uyeno, and B.H. Scribner. Effects in humans of extracellular pH change in relationship between serum potassium concentration and intracellular potassium. J Clin Invest 35:935-939, 1956.

38. Fraley, D.S. and S. Adler. Correction of hyperkalemia by bicarbonate despite constant blood pH. Kidney Int 12:354-360, 1977.

39. Kim, W.G. and E.B. Brown. Potassium transfer with constant extracellular pH. J Lab Clin Med 71:678-685, 1968.

40. Harrop, G.A., Jr. and E.M. Benedict. The role of phosphate and potassium in carbohydrate metabolism following insulin administration. Proc Soc Exp Biol Med 20:430-431, 1923.

41. Briggs, A.P., I. Koechig, E.A. Poisy, and C.J. Weber. Some changes in the composition of blood due to the injection of insulin. J Biol Chem 58:721-730, 1923.

42. Davidson, M.B. and N. Hiatt. Effects of KCl administration on insulin secretion in dogs. Isr J Med Sci 8:752-754.

43. Hiatt, M., L. Morgenstern, M.B. Davidson, G. Bonorris, and A. Miller. Role of insulin in the transfer on infused K to tissue. Horm Metab Res 5:84-88, 1973.

44. Hiatt, M., M.B. Davidson, and G. Bonorris. The effect of KCl on insulin secretion in vivo. Horm Metab Res 4:64-68, 1972.

45. Petit, G., R.L. Vick, and A.M. Swandler. Plasma K and insulin changes during KCl infusion in normal and nephrectomized dogs. Am J Physiol 228:107-109, 1975.

46. Santeusano, F., G. Faloona, J.P. Knochel, and R.H. Unger. Evidence for a role of endogenous insulin and glucagon in the regulation of K homeostasis. J Lab Clin Med 81:809-817, 1973.

47. Blackard, W.G. and N.C. Nelson. Portal and peripheral vein immunoreactive insulin concentration before and after glucose infusion. Diabetes 19:302-305, 1970.

48. DeFronzo, R.A., P. Felig, E. Ferannini, and J. Wahren. Effect of graded doses of insulin on splanchnic and peripheral potassium metabolism in man. Am J Physiol 238:E421-E427, 1980.

49. Hiatt, M., T. Yamakawa, and M.B. Davidson. Necessity for insulin in transfer of excess infused K to intracellular fluid. Metabolism 23:43-49, 1974.

50. Petit, G.W. and R.L. Vick. Contribution of pancreatic insulin to extrarenal K homeostasis: a two-compartment model. Am J Physiol 226:319-324, 1974.

51. DeFronzo, R.A., C.R. Cooke, R. Andres, G.R. Faloona, and P.J.Davis. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest 55:845-855, 1975.

52. Clausen, T. and O. Hansen. Active Na-K transport and the rate of ouabain binding. The effect of insulin and other stimuli on skeletal muscle and adipose tissue. J Physiol 270:415-430, 1977.

53. Gourley, D.R.H. Effect of insulin on potassium exchange in normal and ouabain-treated skeletal muscle. J Pharmacol Exp Ther 148: 339-347, 1965.

54. Zierler, K.L. Hyperpolarization of muscle by insulin in a glucose-free environment. Am J Physiol 197:524-526, 1959.

55. Zierler, K.L. Effect of insulin on potassium efflux from rat muscle in the presence and absence of glucose. Am J Physiol 198:1166-1070, 1960.

56. Zierler, K.L. Effect of insulin on membrane potential and K content of rat muscle. Am J Physiol 197:515-523, 1959.

57. Burton, S.D. and T. Ishida. Effect of insulin on potassium and glucose movement in perfused rat liver. Am J Physiol 209: 1145-1151, 1965.

58. Kestens, P.J., J.J. Haxhe, L. Lambotte, and C. Lambotte. The effect of insulin on the uptake of potassium and phosphate by the isolated perfused canine liver. Metabolism 12:941-950, 1963.

59. Burton, S.D., C.E. Mondon, and T. Ishida. Dissociation of potassium and glucose efflux in isolated perfused rat liver. Am J Physiol 212:261-266, 1967.

60. Andres, R.M., A. Baltzan, G. Cader, and K.L. Zierler. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J Clin Invest 42:108-114, 1962.

61. Zierler, K.L. and D. Rabinowitz. Effect of very small concentrations of insulin on forearm metabolism. Persistence of its actions on potassium and free fatty acids without its effect on glucose J Clin Invest 43:950-962, 1953.

62. D'Silva, J.L. The action of adrenaline on serum potassium. J Physiol 82:393-398, 1934.

63. D'Silva, J.L. The action of adrenaline on serum potassium.JJ Physiol 86:219-228, 1936.

64. Todd, E.P., R.L. Vick, and J.T. Turlington. Influence of epinephrine upon plasma potassium concentration: Changes with time during constant infusion. Proc Soc Exp Biol Med 128:188-191, 1968.

65. Todd, E.P., R.L. Vick, F.M. Bonner, and D.W. Luedke. The influence of the rate of infusion on the kalemotropic effect of epinephrine. Arch Int Physiol Biochem 77:33-45, 1969.

66. Rosa, R.M., P. Silva, J.B. Young, L. Landsberg, R.S. Brown, J.W. Rowe, and F.H. Epstein. Adrenergic modulation of extrarenal potassium disposal. New Eng J Med 302:431-434, 1980.

67. DeFronzo, R.A., M. Bia, and G. Birkhead. Epinephrine and potassium homeostasis. Kidney Int 20:83-91, 1981.

68. Lockwood, R.H. and B.K.B. Lum. Effects of adrenergic agonists and antagonists on potassium metabolism. J Pharm Exp Ther 189: 119-129, 1974.

69. Hiatt, N., L.W. Chapman, and M.B. Davidson. Influence of epinephrine and propranolol on transmembrane K transfer in anuric dogs with hyperkalemia. J Pharm Exp Ther 209:282-286, 1979.

70. Vick, R., E. Todd, and D. Luedke. Epinephrine-induced hypokalemia: Relation to liver and skeletal muscle. J Pharm Exp Ther 181: 139-146, 1972.

71. Clausen, T. and J.A. Flatman. The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. J Physiol 270:383-414, 1977.

72. Petit, G.W. and R.L. Vick. An analysis of the contribution of the endocrine pancreas to the kalemotropic actions of catecholamines. J Pharm Exp Ther 190:234-242, 1974.

73. Todd, E.P. and R.L. Vick. A re-evaluation of the effects of single rapid injections of epinephrine on plasma K. Proc West Pharmacol Soc 12:114-118, 1969.

74. Lum, B.K.B. and R. Lockwood. Effects of nicotine and sympathomimetic amines on potassium intoxication. J Pharmacol Exp Ther 181:147-154, 1972.

75. Todd, E.P. and R.L. Vick. Kalemotropic effect of epinephrine: analysis with adrenergic agonists and antagonists. Am J Physiol 220:1963-1969, 1971.

76. Wang, P. and T. Clausen. Treatment of attacks of hyperkalemic familial periodic paralysis by inhalation of salbutamol. Lancet I:221-223, 1976.

77. Silberstein, S.D., L. Lemberger, D.C. Klein, J. Axelrod, and I.J. Kopin. Induction of adrenal tyrosin hydroxylase in organ culture. Neuropharmacol 11:721-726, 1972.

78. Vogt, M. The secretion of the denervated adrenal medulla of the cat. Br J Pharmacol 7:325-330, 1952.

79. Giebisch, G., G. Malnic, and R.W. Berliner. Renal transport and control of potassium excretion. in The Kidney, edited by B.M. Brenner and F.C. Rector, Jr. Philadelphia. W.B. Saunders Co., 1981, pp. 408-439.

80. Duarte, C.G., F. Chomety, and G. Giebisch. Effect of amiloride, ouabain, and furosemide on distal tubular function in the rat. Am J Physiol 221:632-640, 1971.

81. Hierholzer, K. and M. Wiederholt. Some aspects of distal tubular solute and water transport. Kidney Int 9:198-213, 1976.

82. Mulrow, P.J. and B.H. Forman. The tissue effects of mineralocorticoids. Am J Med 53:561-572, 1972.

83. Knochel, J.P. and M.G. White. The role of aldosterone in renal physiology. Arch Int Med 131:876-884, 1973.

84. Boyd, J.E. and P.J. Mulrow. Further studies of the influence of potassium upon aldosterone production in the rat. Endocrinol 90L299-301, 1972.

85. Boyd, J.E., W.P. Palmore, and P.J. Mulrow. Role of potassium in the control of aldosterone secretion in the rat. Endocrinol 88:556-565, 1971.

86. Linas, S.L. and R.W. Schrier. The renin-angiotensin-aldosterone system and the etiology of hypertension in renal disease. in The Kidney, edited by B.M. Brenner and F.C. Rector, Jr. Philadelphia. W.B. Saunders Co., 1981, pp.2344-2379.

87. Laragh, J.H. and H.C. Stoerk. A study of the mechanism of secretion of the sodium-retaining hormone (aldosterone). J Clin Invest 36:383-392, 1957.

88. Blair-West, J.R., J.P. Coghlan, D.A. Denton, J.R. Goding, M. Wintour, and R.D. Wright. The control of aldosterone secretion. Recent Prog Horm Res 19:311-383, 1963.

89. Bayard, F., Cooke, C.R., Tiller, D.J., Beitins, I.Z., Kowarski, A., Walker, W.G., and C.J. Migeon. The regulation of aldosterone secretion in anephric man. J Clin Invest 50:1585-1595, 1971.

90. Charron, R.C., L.E. Lemme, D.R. Wilson, T.S. Ing, and O.M. Wrong. The effect of adrenal steroids on stool composition, as revealed by in vivo dialysis of feces. Clin Sci 37:151-167, 1969.

91. Conn, J.W. Aldosteronism in man. Some clinical and climatological aspects. J Am Med Assoc 183:775-781, 1963.

92. Davidson, M.B. and N. Hiatt. Effect of KCl administration on insulin secretion in dogs. Isr J Med Sci 8:753-754, 1973.

93. Simpson, S.A.S. and J.F. Tait. Recent progress on methods of isolation, chemistry, and physiology of aldosterone. Recent Prog Horm Res 11:183-219, 1955.

94. Adler, S. An extrarenal action of aldosterone in mammalian skeletal muscle. Am J Physiol 218:616-621, 1970.

95. Flückiger, E. and F. Verzar. Die wirkung von aldosteron (electrocortin) auf den natrium, kalium, and glykogen stoffwechsel des isolierten muskels. Experientia 10:259-261, 1954.

96. Woodbury, D.M. and A. Koch. Effects of aldosterone and deoxycorticosterone on tissue electrolytes. Proc Soc Exp Biol Med 94:720-723, 1957.

97. Santos, R.F. Extrarenal action of adrenal glands on potassium metabolism. Am J Physiol 197:643-647, 1959.

98. Bartter, F. and P. Fourman. The different effects of aldosterone-like steroids on urinary excretion of potassium and acid. Metab Clin Exp 11:6-20, 1962.

99. Knight, R.D., D.S. Kornfeld, G. Glaser, and P. Bondy. Effects of intravenous hydrocortisone on electrolytes of serum and urine in man. J Clin Endocrinol 15:176-181, 1955.

100. Roberts, K.E. and R.F. Pitts. The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog. Endocrinol 50:51-60, 1952.

101. Thakkar, U., D.M. Kaji, K. Torelli, and T. Kahn. Glucocorticoid-induced increase in (H_3) ouabain binding sites on human erythrocytes. Clin Res 28:269a, 1980.

102. Charney, A.N., M.D. Kinsey, L. Myers, R.A. Gianella, and R.E. Gots. Na-K activated adenosine triphosphatase and intestinal eletrolyte transport. J Clin Invest 56:653-660, 1975.

103. Binder, H.J. Effect of dexamethasone on electrolyte transport in the large intestine of the rat. Gastroenterol 75:212-217, 1978.

104. Basti, C.P., H.J. Binder, and J.P. Hayslett. Role of glucocorticoids and aldosterone in maintenance of colonic cation transport. Am J Physiol 238:F181-F186, 1980.

105. Hayslett, J.P., N. Myketey, H.J. Binder, and P.S. Aronson. Mechanism of increased potassium secretion in potassium loading and sodium deprivation. Am J Physiol 239:F378-F382, 1980.

106. Petit, G.W., R.L. Vick, and M.D. Kastello. The contribution of renal and extrarenal mechanisms to hypokalemia reduced by glucagon. Eur J Pharmacol 41:437-441, 1977.

107. Burton, S.D., C.E. Mondon, and T. Ishida. Dissociation of potassium and glucose efflux in isolated perfused rat liver. Am J Physiol 212:261-266, 1967.

108. Schultz, S.G. and P.F. Curran. Coupled transport of sodium and organic solutes. Physiol Rev 50:637-718, 1970.

109. Frizzell, R.A., M. Field, and S.G. Schultz. Sodium-coupled chloride transport by epithelial tissues. Am J Physiol 236: F1-F8, 1979.

110. Geck, P., C. Pietrzyk, B.C. Burckhardt, B. Pfeiffer, and E. Heinz. Electrically silent cotransport of Na, K, and Cl in Ehrlich cells. Biochim Biophys Acta 600:432-447, 1980.

111. Casteels, R. The distribution of chloride ions in the smooth muscle cells of the guinea pig's taenia coli. J Physiol 214: 225-243, 1971.

112. Ellor, J.C. and P.B. Dunham. Volume-dependent passive potassium transport in LK sheep red cells. in Membrane Transport in Erythrocytes, edited by U.V. Lassen, H.S. Ussing, J.O. Wieth. Copenhagen, 1980, pp. 408-423.

113. Gargus, J.J. and C.W. Slayman. Mechanism and role of furosemide-sensitive K transport in L cells: A genetic approach. J Membrane Biol 52:245-256, 1980.

114. Heinz, E., P. Geck, C. Pietrzyk, and B. Pfeiffer. Electrogenic ion pump as an energy source for active amino acid transport in Ehrlich cells. in Biochemistry of Membrane Transport, edited by G. Semenza and E. Carafoli. Springer Verlag, New York, 1977, pp. 236-249.

115. Kregenow, F.M. The response of duck erythrocytes to norepinephrine and an elevated extracellular potassium. Volume regulation in isotonic media. J Gen Physiol 61:509, 1973.

116. Kregenow, F.M. The response of duck erythrocytes to hypertonic media. Further evidence for a volume controlling mechanism J Gen Physiol 58:396, 197T.

117. Kregenow, F.M. Transport in avian red cells. in Membrane Transport in Red Cells, edited by J.C. Ellory and V.L. Lew, Academic Press, London, 1977, pp. 383-426.

118. Aronson, P. Identifying secondary active solute transport in epithelia. Am J Physiol 240:F1-F11, 1981.

119. Murchison, L.E. and P.D. Bewsher. Lack of effect of bumetanide on body potassium contents in hypertension. Br J Pharmacol 2: 87-96, 1975.

120. Mudge, G. Agents affecting volume and composition of body fluids. in The Pharmacologic Basis of Therapeutics, 6th edition, edited by A.G. Goodman, L.S. Goodman, and A. Gilman. MacMillan Publishing, New York, 1980, pp. 848-884.

121. Duchin, K.L. and D.E. Hutcheon. Comparison of bumetanide and hydrochlorothiazide on renal potassium and hydrogen ion excretion. J Clin Pharm 17:453-460, 1977.

122. Burg, M., L. Stoner, J. Cardinal, and N. Green. Furosemide effect on isolated perfused tubules. Am J Physiol 225:119-124, 1973.

123. Burg, M.B. Tubular chloride transport and the mode of action of some diuretics. Kidney Int 9:189-197, 1976.

124. Frazier, H.S. and H. Yager. The clinical use of diuretics, part I. New Eng J Med 288:246-249, 1973.

125. Frazier, H.S. and H. Yager. The clinical use of diuretics, part II. New Eng J Med 288:455-457.

126. Mukherjee, S.K., M.A. Katz, U.FL Michael, and D.A. Ogden. Mechanisms of hemodynamic actions of furosemide: Differentiation of vascular and renal effects on blood pressure in functionally anephric patients. Am Heart J 101:313-318, 1981.

127. Davidov, M., N. Kakaviatos, and F.A. Finnerty, Jr. Antihypertensive properties of furosemide. Circ 36:125-135, 1967.

128. Mroczek, W.J., M. Davidov, F.A. Finnerty, Jr. Large dose furosemide therapy for hypertension. Am J Cardiol 33:546-549, 1974.

129. Sjögren, A. The early hemodynamic effects of ouabain and furosemide in patients with acute myocardial infarction and raised pulmonary artery diastolic pressure. Acta Med Scand (Suppl) 510:53-68, 1970.

130. Biagi, R.W. and B.N. Bapat. Furosemide in acute pulmonary edema. Lancet I:849, 1967.

131. Hesse, B., I. Nielsen, H. Lund-Jacobsen. The early effects of intravenous furosemide on central hemodynamics, venous tone, and plasma renin activity. Clin Sci Mol Med 49:551-555, 1975.

132. Muth, R.G. Diuretic properties of furosemide in renal disease. Ann Int Med 69:249-261, 1968.

133. Berman, L.B. and A. Ebrahimi. Experience with furosemide in renal disease. Proc Soc Exp Biol Med 118:333-336, 1965.

134. Mierzwiak, D.S. Acute effects of furosemide on left ventricular contractility in dogs. Arch Int Pharmacodyn Ther 213:180-185, 1975.

135. Cantarovich, F., L. Benedetti, and J.C. Fernandez. High doses of furosemide and sodium in hypertension. Nephron 12:133-139, 1974.

136. Crane, R.K. The gradient hypothesis and other models of carrier-mediated active transport. Rev Physiol Biochem Pharmacol 78: 99-159, 1977.

137. Frizzell, R.A., M. Field, and S.G. Schultz. Sodium-coupled chloride transport by epithelial tissues. Am J Physiol 236: F1-F8, 1979.

138. Ferguson, D.R. and B.R. Twite. Furosemide - Pharmacology and cellular mode of action. Naunyn-Schmiedeberg's Arch Pharmacol 281:295-300, 1974.

139. Anderson, R.J., J.G. Gambertoglio, and R.W. Schrier. Furosemide. in Clinical Use of Drugs in Renal Failure. Thomas Books, Springfield, Ill., 1976, pp. 150-153.

140. Ross, C.R. and E.J. Cafruny. Blockade of the diuretic action of benzothiadiazines. J Pharmacol Exp Ther 140:125-132, 1963.

141. Dustan, H.P., E.L. Bravo, and R.C. Tarazi. Volume-dependent essential and steroidal hypertension. Am J Cardiol 31:606-615, 1973.

142. Lund-Johansen, P. Hemodynamic changes in long-term diuretic therapy of essential hypertension. Acta Med Scand 187:509-518, 1970.

143. Ludens, J.H. and H.E. Williamson. Effect of chlorothiazide on renal blood flow in the conscious dog. Proc Soc Exp Biol Med 135:197-199, 1970.

144. Bengtsson, C. Impairment of glucose metabolism during treatment with antihypertensive drugs. Acta Med Scand (Suppl) 628:63-67, 1979.

145. Goldner, M.G., H. Zarowitz, and S. Akgun. Hypoglycemia and glucosuria due to thiazide derivatives administered in diabetes mellitus. New Eng J Med 262:403-405, 1960.

146. Zatuchni, J. and F. Kordasz. The diagetogenic effect of thiazide diuretics. Am J Cardiol 7:565-567, 1961.

147. Arnery, A., P. Berthau, T.C. Bulpit, M. Deruytere, A. de Schaepdryver, C. Dolery, R. Fagard, F. Forette, J. Hellemans, P. Lund-Johansen, A. Mutses, and J. Tuomilehto. Glucose intolerance during diuretic therapy. Lancet 1:681-683, 1978.

148. Hastings, A.B. and J.M. Buchan. Role of intracellular cation on liver glycogen formation in vitro. Proc Nat Acad Sci 28:478-482, 1942.

149. Koppel, M.H., S.G. Massry, J.H. Shinaberger, D.L. Hartenbower, and J.W. Coburn. Thiazide-induced rise in serum calcium and magnesium in patients on maintenance hemodialysis. Ann Intern Med 72:895-901, 1970.

150. Healy, J.J., T.J. McKenna, B. St J. Canning, T.G. Brien, G.J. Duffy, and F.P. Muldowney. Body composition changes in hypertensive subjects on long-term oral diuretic therapy. Br Med J 1:716-719, 1970.

151. Lanzoni, B., G. Smith, and A.V. Chobanian. Influence of metolazone, hydrochlorothiazide and spironolactone on potassium balance in hypertensive patients. in Systemic Effects of Antihypertensive Agents, edited by M.P. Sambhi. Stratton Intercontinental Medical Book Corporation, New York, 1976, pp. 171-181.

152. Edmonds, C.J. and B. Jasani. Total-body potassium in hypertensive patients during prolonged diuretic therapy. Lancet 2:8-12, 1972.

153. Dargie, H.J., K. Boddy, A.C. Kennedy, P.C. King, P.R. Read, and D.M. Ward. Total body potassium in long-term frusemide therapy: is potassium supplementation necessary? Br Med J 4:316-319, 1974.

154. Wilkinson, P.R., H. Issler, R. Hesp, and E.B. Raftery. Total body and serum potassium during prolonged thiazide therapy for essential hypertension. Lancet L:759-762, 1975.

155. Anderson, J., B.E Godfrey, D.M. Hill, A.D. Munro-Faure, and J. Sheldon. A comparison of the side effects of hydrochlorothiazide and furosemide in the treatment of hypertensive patients. Quart J med 40:541-560, 1971.

156. Oh, M.S. and H.J. Carroll. The renin-aldosterone system and thiazide-induced depletion of total body potassium in essential hypertension. Nephron 21:269-276, 1980.

157. Sweet, C.S. and S.L. Gaul. Attenuation of hydrochlorothiazide induced hypokalemia in dogs by a -adrenergic blocking drug, timolol. Eur J Pharmacol 32:370-374, 1975.

158. Dustan, H.R., R.C Tarazi, and E.L. Bravo. Diuretic and diet treatment of hypertension. Arch Int Med 133:1007-1013, 1974.

159. Talso, P.J. and A.J. Carballo. Effects of benzothiadiazines on serum and total body electrolytes. Ann NY Acad Sci 88:822-840, 1960.

160. Kagawa, C.M., J.A. Cella, and C.G. van Arman. Action of new steroids in blocking effects of aldosterone and deoxycorticosterone on salt. Science 126:1015-1016, 1957.

161. Tait, J.F., S.A. Simpson, and H.M. Grundy. The effect of adrenal extract on mineral metabolism. Lancet 1:122-124, 1952.

162. Goldberg, M. The renal physiology of diuretics. in Handbook of Physiology, edited by J. Orloff and R.W. Berliner, American Physiological Society, Washington, D.C., 1973, pp.

163. Coppage, W.S. and G.W. Liddle. Mode of action and chemical usefulness of aldosterone antagonists. Ann NY Acad Sci 88: 815-821, 1960.

164. Conn, J.W., L.H. Louis, S.S. Fajans, D.H.P. Streeten, R.D. Johnson, J.R. Moorhouse, M.G. Crane, A.F. Berker, and E. Ramirez. Metabolic effects in normal men and in primary aldosteronism of a synthetic "aldosterone antagonist". J Lab Clin Med 52:805-806, 1958.

165. Kagawa, C.M., F.M. Sturtevant, and C.G. van Arman. Pharmacology of a new steroid that blocks salt activity of aldosterone and deoxycorticosterone. J Pharmacol Exp Ther 126:123-130, 1959.

166. Liddle, G.W. Aldosterone antagonists. Arch Int Med 102:998-1004, 1958.

167. Gantt, C.L. Diretic Therapy. Disease-A-Month. Yearbook Publishers, Chicago, Ill., 1967.

168. Nash, D.T. Antihypertensive effect and serum potassium homeostasis: Comparison of hydrochlorothiazide and spironolactone alone and in combination. J Med 8:367-377, 1977.

169. Ross, E.J. Aldosterone and its antagonists. Clin Pharmacol Ther 6:65-106, 1965.

170. Hollander, W. and A.V. Chobanian. Anti-hypertensive action of spironolactone (SC-9420) steroidal antagonist. Circ 20:713, 1959.

171. Crane, M.J. and J.J. Harris. Effect of spironolactone in hypertensive patients. Am J Med Sci 260:311-330, 1970.

172. French, I.W. and J.F. Manery. The effect of aldosterone on electrolytes in muscle, kidney cortex, and serum. Can J Biochem 42:1459-1476, 1964.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE