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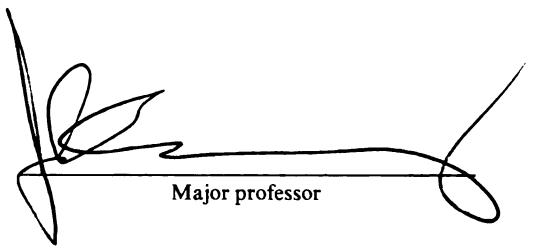
ATRIAL NATRIURETIC PEPTIDE
IN
REDUCED RENAL MASS

presented by

MARILYN AUDREY BRANDT

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology



Major professor

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IN
REDUCED RENAL MASS

by

Marilyn Audrey Brandt

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

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The maintenance of sodium balance in animals with experimentally induced reduced renal mass (RRM) is accomplished by a progressive increase in the absolute rate of sodium excretion per nephron so that the serum concentration of this solute remains normal. The increased fractional sodium excretion per nephron is a hallmark of chronic renal disease, the mechanism of which is unknown. The recently discovered atrial natriuretic peptide have potent natriuretic and diuretic activity and increased plasma ANP concentration occurs in volume expansion. I therefore hypothesized that plasma concentration of ANP increases with RRM thereby aiding in the control of sodium excretion. The present study was undertaken in conscious chronically instrumented rats with 5/6 nephrectomy and in sham operated control rats so that repeated measurements

ABSTRACT

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The maintenance of sodium balance in animals with chronic impairment, induced reduced renal mass (RRM) is accomplished by a progressive increase in the absolute sodium excretion per nephron so that the concentration of this solute remains normal. The increased fractional sodium excretion per nephron [illegible] chronic renal disease, the mechanism of which is unclear. The recently discovered atrial natriuretic peptide (ANP) exerts natriuretic and diuretic activity and its plasma ANP concentration occurs in volume expansion. The present study was undertaken to test the hypothesized that plasma concentration of ANP increases with RRM thereby aiding in the control of sodium excretion. The present study was undertaken to test the hypothesized that plasma concentration of ANP increases with RRM thereby aiding in the control of sodium excretion. Sprague-Dawley rats with 5/6 nephrectomy and sham operated control rats so that operated measure [illegible] chronically instrumented rate with 5/6 nephrectomy and sham operated control rats so that operated measure

could be obtained in the same animal over time. All rats were housed in metabolism cages and received 1 mEq of sodium intravenously per day for 1 week; after which some animal were switched to 6 mEq of sodium per day for 1 week, while the remainder of the rats continued to receive 1 mEq of sodium per day. Plasma ANP was elevated from an average control value of 196 fmoles/ml to 417 fmoles/ml and 444 fmoles/ml on experimental day 10 and 14 (E10 and E14) in the rats which received 6 mEq of sodium per day. Mean arterial pressure was also significantly elevated in this group to 135 mmHg, 137 mmHg, 152 mmHg and 149 mmHg on days E8, E10, E12 and E14 respectively, as compared to its own control of 108 mmHg. The elevation of plasma ANP was not caused by reduced renal mass per se because ANP only increased when animals with RRM were maintained on a high salt intake. The elevation in ANP may be due to the elevation in arterial pressure in the rats maintained on 6 mEq of sodium per day because neither ANP nor MAP increased in animals with RRM on 1 mEq of sodium per day. Both RRM groups remained in sodium balance. If the elevation of ANP was caused by extracellular fluid volume expansion, it was not detected by the balance studies.

[illegible] the difference in the same animal over time. All rats were switched to a mEq of sodium per day for 1 week, then maintained on metabolism cages and received 1 mEq of sodium per day for 1 week after which some [illegible] per day for 1 week after which [illegible] sodium per day. Plasma ANP was elevated from an [illegible] pCi/ml to 1?6 [illegible] pCi/ml on experimental day 10 and 14 (Elv and elev in the [illegible] ml/min. Data are presented as means ± SEM. Plasma ANP was also significantly elevated in rats given 2 mEq of sodium per day. Mean [illegible] 132 mmHg. 137 mmHg, 152 mmHg and 149 mmHg on days 2, 7, 10, and 14 respectively, as compared to its own control value of 120 mmHg. The elevation of plasma ANP was [illegible] regional mass per se because ANP only [illegible] the [illegible] with. BRM were maintained on a high salt [illegible] elevation in ANP may be due to the elevation in [illegible] in the rats maintained on a mEq of sodium per day because neither ANP nor MAP increased in animals with [illegible] mEq of sodium per day. Both BRM groups remained in [illegible] volume expansion. It was not detected by the [illegible] dose. It the elevation of ANP was caused by extracellular

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INTRODUCTION

Reduction of renal mass, either experimentally or during the course of chronic renal disease, places a substantial demand on the remaining nephrons to maintain the bodily fluids compatible with life. Each remaining nephron must assume an increasing share of the total excretory function provided there are not concomitant decreases in the amount of substances which require excretion by the kidneys. In order to preserve life, solute specific adaptations occur in the remaining nephrons. Some substances undergo "no regulation", others undergo "partial regulation" while still others undergo "complete regulation". Sodium is an example of a substance which is completely regulated by the kidneys. That is, as nephron population decreases, and hence GFR, the remaining nephrons are faced with the task of excreting an even greater load of the filtered sodium so that the animal maintains external sodium balance. This increase in the fractional excretion of sodium occurs immediately and before any compensatory change in GFR occur. The maintenance of sodium balance is an important feat because the extracellular fluid volume is regulated by the renal excretion of sodium.

INTRODUCTION

Over the years, many different hypotheses have been postulated to account for the increased fractional sodium excretion noted upon a reduction of renal mass. Some of these include an increase in renal blood flow, an increase in cardiac output, a change in plasma mineralocorticoid levels, an increased solute diuresis per nephron and natriuretic hormone.

The recent discovery of atrial natriuretic peptide (ANP), a hormone synthesized and released from the atrial myocytes, has been showm to posses marked natriuretic, diuretic and vasorelaxant properties. ANP has recently been suggested to account for the associated natriuresis noted in patients after a paroxysmal tachycardia episode and the escape from the sodium retaining effects of mineralocorticoids. Acute instances of volume expansion, sudden increases in arterial pressure and atrial distension have additionally been shown to stimulated its release. Thus, it appears that this peptide is released in instances of atrial distension or when the extracellular fluid volume is expanded. An increase in plasma volume and an increase in arterial pressure produced in chronic renal failure could increase atrial volume and atrial stretch thereby stimulating ANP release. This led us to hypothesize that this peptide may play an important role in the regulation of sodium excretion when renal function is compromised. We therefore hypothesized ANP might be markedly elevated in animals exhibiting reduced renal mass.

LITERATURE REVIEW

Regulation of the Effective Circulating Volume

The body regulates both the volume and the ionic composition of the extracellular fluid. Sodium is both the major osmotically active solute of the extracellular fluid and the major determinant of the extracellular fluid volume (Smith 1957). Changes in the extracellular fluid volume reflect changes in total body sodium as long as the osmolality of both the intra and extracellular fluid are similar (Strauss et al. 1958). On the other hand, if the total body sodium content remains constant, changes in osmolality will primarily reflect changes in body water balance (Leaf et al. 1952). Thus, extracellular fluid volume control is considered in terms of the regulation of body sodium while control of osmotic pressure of the body fluids may be considered in terms of the regulation of total body water balance.

The major means by which the extracellular fluid volume is regulated is by the control of renal sodium excretion. Strauss et al. (1958) stated that the kidneys are the primary regulators of sodium balance. These organs

Regulation of the Effective Circulating Volume

respond, by varying sodium excretion, to changes in the extracellular fluid volume actively perfusing the tissues, more appropriately referred to as the effective circulating volume. In the study, subjects were maintained on a sodium diet of 5 mEq per day. Upon reaching sodium balance, the subjects were challenged with 150 ml of intravenously administered hypotonic saline, the sodium content of which constituted 1% of the extracellular fluid sodium content. Sodium excretion increased within 30 minutes following the saline infusion without any detectable increase in GFR. Since the infusion was hypotonic, plasma sodium concentration could not have risen. This study showed that a change in the effective circulating volume was the only stimulus for the kidneys to respond to the very small change in total sodium content. This study concluded that conditions which lead to an increase in the effective circulating volume lead to an increase in renal sodium excretion whereas conditions which lead to a decrease in the effective volume lead to a decrease in renal sodium excretion (Strauss et al. 1958). The noted changes in sodium excretion will occur irrespective of the plasma sodium concentration (Leaf et al. 1953). However, subjects with a body sodium deficit will not respond to a sodium load with a natriuresis until the body sodium deficit has been replaced. After which and only at this time will a further increment in administered sodium be excreted as surplus sodium to the body requirement (McCance 1936). This

experiment indicates that the usual lag period required before urinary sodium excretion readjusts to a change in dietary intake is dependent upon the occurrence of a sufficient change in the effective circulating volume. Thus a volume receptor must be part of the control loop regulating sodium excretion (Smith 1957).

The major means by which osmotic pressure is regulated is by the renal excretion of water under the influence of ADH (Robertson et al. 1976). The regulation of water excretion serves to maintain a constant level of osmotic pressure in the body fluids in addition to causing adjustments in the extracellular fluid volume to correspond to alterations in sodium balance. An increase in the plasma osmotic pressure enhances ADH secretion resulting in water retention while a reduction in the plasma osmotic pressure decreases ADH secretion resulting in water excretion.

The site responsible for the detection of a change in osmotic pressure is located in the brain. Verney et al. (1946) compared the effects of infusing a hypertonic saline solution into either the carotid artery or femoral artery. Infusion of hypertonic saline into the carotid artery resulted in an increase in plasma ADH and subsequent antidiuresis even though systemic plasma osmolality was not changed. The increase in osmolality is sensed as an osmotic gradient between the plasma and the supraoptic cells of the hypothalamus. Dunn et al. (1973) raised plasma osmolality by infusing hypertonic saline or glucose. These impermeant

substances decreased cell volume and subsequently released ADH. When plasma osmolality was raised with a permeant substance such as urea, cell size was not affected and ADH secretion did not occur. Thus, the actual stimulus for ADH release was not a increase in osmolality per se, but rather a reduction in cell volume of the supraoptic cells of the hypothalamus. Dunn et al. (1973) also found that these osmoreceptors were very sensitive, responding to changes in plasma osmolality of as little as 1%.

Afferent Mechanisms in the Regulation of the Effective Circulating Volume

Several sites have been suggested as the location of extrarenal volume receptors and will be discussed in the following sections. These include arterial baroreceptors located in the aortic arch and carotid sinus, intrathoracic low pressure receptors in the cardiac atria and great veins, liver or portal vein receptors and cerebrospinal fluid sodium receptors. Several intrarenal detectors of extracellular volume have also been suggested.

Arterial Volume Receptors

Volume receptors reside in the high pressure arterial system, capable of sensing the fullness of this system and thus modulating renal sodium excretion. Experimental evidence favoring an arterial receptor system was obtained

from a study of an experimentally produced AV fistula. Upon closure of an AV fistula, a decreased rate of emptying of the arterial volume into the venous side occurred. As a consequence, diastolic pressure was elevated while pressures in the great veins, right atrium and pulmonary vessels were decreased. A fall in venous return, cardiac output, stroke volume and heart rate additionally occurred. The kidneys appeared to respond to the increased filling of the arterial tree, rather than to a decreased filling of the the venous vascular beds, by increasing sodium excretion, which suggested that the effective arterial blood volume was critical in controlling overall extracellular volume (Davis et al. 1964).

Arterial baroreceptors, located in the aortic arch and the carotid sinus, can sense changes in pressure which in turn can influence ADH release. ADH release is inversely proportional to acute changes in systemic arterial pressure. Davidov et al. (1969) found that an increase in arterial pressure decreased ADH secretion while Schrier et al. (1971) found that a decrease in arterial pressure increased ADH secretion. These responses were blocked by carotid sinus denervation, suggesting that they were mediated by alterations in tone in the parasympathetic afferents in the carotid sinus nerves (Anderson et al. 1974).

The arterial pressure receptors may act as volume receptors. Volume depletion could decrease cardiac output

which in turn would decrease mean arterial pressure even in the face of a reflex increase in vascular resistance. For example, Anderson et al. (1974) found that acute thoracic inferior vena cava constriction caused a pooling of blood in the venous system and thus a reduction of venous return to the heart, which in turn lowered cardiac output and subsequently arterial pressure. Urine osmolality increased and free water clearance decreased. The urinary response was blocked by either prior denervation of the arterial baroreceptors or prior hypophysectomy. Thus the increase in ADH release, which in turn increased water reabsorption, was mediated via baroreceptor afferents.

However, the arterial system may not be the best place to detect small changes in blood volume because only 15% of the blood volume resides there. Arterial distensibility and compliance are low. Arterial baroreceptors may be involved in the renal excretion of water, but there is little evidence that these baroreceptors are directly involved in the regulation of sodium excretion (Schrier et al. 1977).

Low Pressure Volume Receptors

The venous bed is a highly distensibile structure, the capacitance being 20 times that of the arterial capacitance (Gauer et al. 1970). Approximately 85% of the circulating blood volume is contained within the venous circulation (Gauer et al. 1970). The capacitance of the extrathoracic and intrathoracic compartments are approximately equal

(Gauer et al. 1970). However, since the intrathoracic compartment has a smaller volume than the peripheral capacitance vessels, its wall tension must be greater for an equal change in blood volume. The low pressure system effectively registers changes in blood volume as changes in vascular wall tension. That is, the distensibility of the low pressure system is constant as long as the changes in blood volume are less than 10 to 15% of the total blood volume (Echt et al. 1974).

Several types of receptors strategically located in the intrathoracic low pressure regions serve as volume receptors. B receptors, located at the entrance of the great veins into the atria (Paintal et al. 1973); A receptors, located in the atrial wall (Arndt et al. 1971); and J receptors located near the pulmonary capillaries (Paintal et al. 1973) play an important role in the modulation of plasma volume (Gauer et al. 1970). That is, any stimulus which increases filling of the thoracic vascular compartments is associated with a diuresis and natriuresis. This was best demonstrated by the work of Epstein et al. (1978) who immersed subjects in a water bath at different temperatures in order to control the amount of redistribution of blood volume from the periphery into the thorax. This procedure provided a stimulus for increased salt and water diuresis that correlated with the increment in central blood volume. The study showed that intrathoracic venous or atrial receptors were sensitive to

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small changes in volume expansion.

ADH secretion varies inversely with left atrial pressure. Claybaugh et al. (1973) found that volume depletion resulted in a decrease in left atrial pressure, increasing ADH secretion, while volume expansion resulted in an increase in left atrial pressure, decreasing ADH secretion. These effects were abolished by vagal denervation.

Hepatic Volume Receptors

The potential for the liver to play a role in the afferent limb of volume control is attractive, because its location is ideal for the detection of sudden changes in dietary salt and water intake. Hypotonic (Haberich et al. 1968), isotonic (Perlmutt et al. 1975) and hypertonic (Daley et al. 1967) saline solutions all evoke a greater natriuresis when infused into the portal vein than when the same solutions are infused into a systemic vein. The natriuretic response to hypotonic and hypertonic saline infusion could only be abolished by cervical vagotomy or by sectioning the hepatic branch of the vagus respectively. The release of a humoral substance from the liver was speculated to account for the response to isotonic saline infusion because cervical vagotomy or section of the hepatic branch of the vagus did not abolish the response to isotonic saline. Thus, these studies suggest the presence of a hepatic receptor for the regulation of sodium as well

as water excretion.

Cerebrospinal Fluid Sodium Receptors

Evidence has accumulated regarding the potential role of changes in the sodium concentration of the cerebrospinal fluid in renal salt handling. It is possible to evoke a natriuresis by experimentally inducing elevations in the sodium concentration of the cerebrospinal fluid (Andersson et al. 1974). Conversely, a reduction in the sodium concentration of the cerebrospinal fluid evokes an antinatriuretic effect (Olsson 1973). These changes occur without changing the osmolar concentration of the cerebrospinal fluid. Addition of angiotensin II to hypertonic saline infusions into the third ventricle potentiates the natriuretic response elicited by hypertonic saline infusions alone (Eriksson 1976).

Intrarenal Volume Receptors

Volume receptors reside in the kidneys, capable of sensing changes in the effective circulating volume. Juxtaglomerular cells, residing in the wall of the afferent arteriole, serve as baroreceptors (stretch receptors) capable of detecting changes in pressure. A decrease in stretch corresponds to a decrease in pressure and hence volume, while an increase in stretch corresponds to an increase in pressure and hence volume (Tobian et al. 1959a). The macula densa cells, located in the distal

tubule, are also sensitive to volume changes. That is, changes in sodium chloride delivery to these cells brought about by changes in the effective volume are detected by these cells. Both the juxtaglomerular cells and the macula densa cells are collectively called the juxtaglomerular apparatus and are ultimately involved in renin release, which is discussed below under the sections of compensatory and corrective measures to changes in the effective circulating volume. Finally, Niijima (1975) described mechanoreceptors in the kidney which could detect changes in volume as measured by afferent nerve traffic from renal nerves.

Efferent Mechanisms in the Regulation of the Effective Circulating Volume

Once the body has detected a perturbation in the effective circulating volume, it responds by first evoking compensatory measures followed by corrective measures. The sympathetic nervous system plays an important role in the initiation of compensatory measures by appropriately changing cardiac output and vascular resistance. Final corrective measures are brought about by the kidney to control renal salt and water excretion in order to restore normal volume balance. Thus, an increase in volume can only be corrected by the renal loss of sodium and excess water. However, when the effective circulating volume depletion is

due to heart failure or cirrhosis with ascites, the associated renal sodium retention is only compensatory as complete correction cannot occur without reversal of the underlying process.

Compensatory Action of the Sympathetic Nervous System

Sympathetic nervous tone is altered during changes in the effective circulating volume. For example, volume expansion reduces both sympathetic neural tone and adrenal medullary secretion of catecholamines (Frye et al. 1960), where as volume depletion augments sympathetic neural tone and adrenal medullary secretion of catecholamines (Freis et al. 1951).

As previously mentioned, when volume depletion occurs, a series of afferent neural impulses traveling to the brain stem initiate a series of events, via the sympathetic nervous system, to restore tissue perfusion. Freis et al. (1951) reported that venoconstriction occurs which in turn would decrease the volume of blood in this reservoir, thereby increasing blood flow to the heart. Whether venoconstriction actually occurs is a controversial point. It is known that cutaneous veins do not participate in this response (Epstein et al. 1968). However the splanchnic capacitance vessels in animals have been reported to participate in the cardiovascular regulation exerted by the arterial baroreceptors (Brooksby et al. 1971; Brender et

al. 1969; Hadjimians et al. 1968; Shoukas et al. 1973). The possible increase in blood delivery to the heart could increase contractility and heart rate. Both an increase in venous return and an increase in heart rate would serve to increase cardiac output. Arteriolar vasoconstriction via direct sympathetic innervation does occur. This results in an increased peripheral resistance which inturn would raise arterial pressure toward normal. Renin secretion is enhanced by hypotension, primarily due to the increase in sympathetic tone. Renin in circulating blood cleaves angiotensinogen to form angiotensin I which is converted to angiotensin II by kininase II in the pulmonary endothelium. Angiotensin II can also serve to increase arteriolar constriction. Angiotensin II acts on the adrenal cortex to release aldosterone. Both angiotensin II and aldosterone exert a direct action on the kidney to control salt and water excretion to control the effective volume. Both of these topics will be discussed in the following section, "Corrective Action by the Kidney to Control Salt and Water Excretion".

Corrective Actions by the Kidney to Control Salt and Water Excretion

Corrective measures by the kidney in response to a change in the effective circulating volume include changes in the renal excretion of salt and water. There are many factors which contribute to the renal handling of sodium or

sodium excretion. In contrast, the renal handling of water is controlled primarily by ADH.

Control of Sodium Excretion

Sodium is freely filterable at the glomerulus and actively reabsorbed by the proximal tubule, loop of Henle, distal tubule and collecting duct. It is not secreted by the tubule. Thus the final amount of sodium excreted in the urine depends on: 1) the plasma sodium concentration, 2) the rate of glomerular filtration of plasma and 3) tubular reabsorption of sodium from the filtrate as it passes through the nephron. It is therefore possible to alter sodium excretion by changing any one of the three variables mentioned above.

Plasma Sodium Concentration

Effects of alterations of plasma sodium concentration on net sodium excretion may be particularly pronounced in experimental situations where the plasma sodium concentration has been artificially manipulated by hypertonic or hypotonic saline administration to hypernatremia or hyponatremia respectively (Schrier et al. 1969, 1971b). Outside of these experimental conditions, day to day plasma sodium concentrations are carefully maintained within a narrow range by ADH and thirst mechanisms and do not play an important role in the regulation sodium excretion (Robertson et al. 1976). Even

in instances of hyponatremia or hypernatremia, variations in the effective circulating volume can override the effects of plasma sodium concentration on sodium excretion (Leaf et al. 1953).

Control of GFR

An increase in GFR may contribute to the natriuretic response after a sodium load only if the extracellular volume is expanded (Lindheimer et al. 1974). However, animals with a reduction in renal mass, and hence GFR, are able to adjust sodium excretion to match intake (Peters 1963). The primary reason why changes in the filtered load of sodium do not appreciably affect net sodium excretion is a phenomenon known as glomerular tubular balance. That is, the absolute rate of sodium reabsorbed in the proximal tubule varies directly with the glomerular filtration rate. If, for example, GFR is experimentally reduced, then the absolute amount of sodium reabsorbed by the proximal tubule and to a lesser extent the loop of Henle, the distal tubule and the collecting duct will decrease leading to increased sodium excretion (Glabman et al. 1965).

Control of Sodium Reabsorption

The control of sodium reabsorption is more important for long term sodium regulation than acute and temporary changes in GFR. Many factors are known to alter sodium reabsorption. Some of the ones which will be discussed

include the renin-angiotensin-aldosterone system, the sympathetic nervous system, renal physical factors, and natriuretic substances.

Renin-Angiotensin-Aldosterone System

A major controller of sodium reabsorption is the release of aldosterone from the zona glomerulosa cells of the adrenal cortex. Aldosterone stimulates the distal tubule and collecting ducts to reabsorb sodium thereby restoring the effective volume (Landon et al. 1966). Aldosterone can be released by several stimuli. The first is a rise in the plasma sodium concentration (Dufau et al. 1969). As previously mentioned, plasma sodium does not change appreciably in healthy states, but may increase or decrease in pathological conditions. Even in these cases, it is still a weak stimulus for aldosterone release. Another stimulus for the release of aldosterone is an increase in the plasma potassium concentration (Boyd et al. 1973). Adrenocorticotrophic hormone or ACTH is also an important stimulator of aldosterone release (Tucci et al. 1967). Finally, angiotensin II can directly cause aldosterone secretion (Laugh et al. 1960). Angiotensin II is initially generated by renin release.

Renin has been shown to be synthesized and stored in the granules of the juxtaglomerular apparatus with most of the renin containing cells located in the afferent arteriole (Davis et al. 1976). Stimuli which affect release

of renin include stretch of the intrarenal baroreceptor, tubular sodium chloride delivery to the macula densa, the activation of renal sympathetic nerves and angiotensin itself. Tobian et al. (1959b) first recognized that mean arterial perfusion pressure appeared to be a primary factor controlling renin release and the concept was subsequently termed the baroreceptor or stretch receptor hypothesis. Blaine et al. (1970) developed a model in which ureteral ligation and renal artery ischemia decreased the delivery of fluid past the distal tubule. Alterations in renal perfusion pressure were produced and it was found that hemorrhage or aortic constriction were potent stimuli for renin release.

Many studies have supported the hypothesis that the macula densa acts as a sensor to detect a signal provided by the renal tubular fluid which in turn alters the rate of renin release (Davis et al. 1976). A functional relationship between the macula densa and the juxtaglomerular cells of the renal afferent arteriole was first derived from anatomical and histochemical studies by Goormagtigh in 1939. There is still considerable controversy as to the exact signal received by the macula densa which alters renin release. Vander et al. (1964) have proposed that an increase in sodium chloride to the macula densa of the distal tubule might decrease renin release whereas a decrease in distal tubule sodium delivery would stimulate renin release. Conversely, Thurau (1964) has

suggested that the macula densa senses an increase in sodium chloride concentration which subsequently leads to an increase in renin release. Kirchner et al. (1978) suggested that the maucla densa senses chloride rather than sodium and plays an integral role in tubuloglomerular feedback in which alteration in fluid delivery to the distal tubule affects glomerular filtration rate, renin release and the subsequent generation of angiotensin II, thereby making up the efferent limb of the feedback process.

The sympathetic nervous system has been implicated in the release of renin. Direct stimulation of the renal nerves leads to an acute increase in renin release from the kidney (Vander 1965). The renal nerves have been shown to be involved in the response to upright posture (Cuche et al. 1972), tilting (Zanchetti et al. 1975), exercise, and the cold pressor test (Perytremann et al. 1972). A direct effect of the renal sympathetic nerves on the juxtaglomerular cells in controlling the rate of renin release was demonstrated by Johnson et al. (1971) in the non filtering kidney. Reid et al. (1972) have postulated that a beta-adrenergic receptor mediates the response to circulating catecholamines and norepinephrine liberation at the renal nerve endings based on the finding that isoproterenol caused a marked increase in renin release when administered directly into the kidney.

There are additional inputs which are known to affect

renin release. Intravenous administration of angiotensin II decreases plasma renin activity and the rate of renin release and appears to occur without measurable alterations in renal perfusion pressure, suggesting a direct inhibitory effect on juxtaglomerular cells (Bunag et al. 1967). ADH inhibits renin release at physiological concentrations independent of any hemodynamic effects of the hormone (Bunag et al. 1967). Other stimuli are known to inhibit or stimulate renin release and for a complete review on the subject, please see Keeton et al. (1981).

Sympathetic Nervous System

The sympathetic nervous system was first implicated in the control of the circulating volume by Bernard in 1859 when the phenomenon of denervation natriuresis was reported. In denervation natriuresis, an increase in urine volume and electrolyte excretion result from denervation of the kidneys. Bonjour et al. (1969) showed that following acute renal denervation, sodium excretion increased despite a reduction in GFR and in the absence of an increase in renal perfusion pressure. A more complete study by Bello-Reuss et al. (1975) found that several days following renal denervation, a five fold increase in sodium excretion occurred with out any change in GFR, single nephron GFR or renal plasma flow. Proximal tubular fractional reabsorption of sodium decreased and late distal tubular fluid sodium concentration increased. Hence, the large reduction in

proximal fractional reabsorption of sodium was partially compensated for by increased reabsorption in the loop of Henle and distal and collecting tubules.

Functional anatomical evidence was reported by Barajas et al. (1973) of direct innervation of the proximal tubule, and the juxtaglomerular apparatus and surrounding tubules. These anatomical studies provide a morphological basis for the functional effects of changes in sympathetic nerve traffic.

Among the first evidence that sympathetic nerves might control the extracellular fluid volume via cardiopulmonary reflexes was the finding that left atrial distension caused an increase in urinary sodium excretion (Ledsome et al. 1961). Prosnitz et al. (1978) extended these findings by demonstrating that increased left atrial pressure decreased renal nerve activity 40% and increased urinary sodium excretion by approximately 80%. These changes occurred with out a change in glomerular filtration rate and under conditions in which renal perfusion pressure was held constant with an aortic clamp proximal to the renal arteries. Bilateral cervical vagotomy was able to abolish both renal nerve activity chnages and sodium excretion. Interestingly, constant infusion of vasopressin abolished the diuretic effect of left atrial distension, but not the natriuretic effect.

Thames et al. (1982) also suggested that cardiopulmonary baroreceptors participate in the reflex

control of renal sympathetic nervous activity. After volume expanding dogs with a dextran-saline solution these investigators found an inverse correlation between pulmonary wedge pressure and renal nerve activity.

Direct renal nerve stimulation which did not alter intrarenal blood flow in saline loaded dogs caused a significant decrease in urinary sodium excretion with out a significant change in GFR or renal blood flow. Later it was found that these aforementioned results could be blocked by both phenoxybenxamine and guanethidine (DiBona et al. 1978)

Infusion of norepinephrine or epinephrine into an isolated perfused kidney resulted in increased sodium and water reabsorption that could be inhibited by propanolol but not phenoxybenxamine. However, infusions of both isoproterenol and phenylephrine could mimic the results noted upon norepinephrine infusion. It was concluded that the anitinatriuretic effects of sympathetic stimulation do not fit simple alpha and beta adrenergic receptor mechanisms (Besbarb et al. 1977)

The primary nephron site influenced by catecholamines is the proximal tubule. Utilizing micropuncture in denervated kidneys, renal nerve stimulation decreased sodium reabsorption in the proximal tubule, but not the distal tubule (Bello-Reuss et al. 1980). In vitro evidence for a direct effect of adrenergic nerves on tubule function again comes from the work of Bello-Reuss et al. (1980) who microperfused isolated tubule segments. In microperfused

pars convoluta of proximal tubules, a significant and reversible increase in fluid transport was noted upon the addition of norepinephrine to the bath. This effect was not noted in the pars recta of the proximal tubule or the straight portion of the proximal tubule.

A possible role for dopaminergic neural transmitter release in the regulation of sodium excretion has also been suggested. Dinerstein et al. (1979) reported that dopaminergic receptors have been found in the kidney and Alexander et al. (1974) reported a direct relationship between sodium excretion and urinary dopamine excretion. Bello-Reuss et al. (1982) demonstrated that dopamine exerts an inhibitory effect on isolated perfused proximal tubule sodium reabsorption rates.

Physical Factors

Physical factors, which refer to hydrostatic (Martino et al. 1968) and oncotic pressures (Davidman et al. 1967) in the peritubular interstitium and circulation of the kidney, have been implicated in the control of sodium excretion. These effects will be viewed separately in the various segments of the nephron beginning with the proximal tubule and finishing with the collecting tubule.

Changes in hydrostatic and oncotic pressure in the peritubular microcirculation may have some effects on proximal tubule reabsorption under some but not all circumstances. Proximal reabsorption involves two steps,

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namely tubule electrolyte transport across the tubular epithelium into the interstitial space and the uptake of reabsorbate by the peritubular capillaries. The first evidence suggesting peritubular capillary control of proximal reabsorption was put forth by Lewy et al. (1968). In their study, an increase in venous pressure, caused by venous occlusion, served to increase hydrostatic pressure. Using free-flow micropuncture and the split drop method in the proximal tubule, a decreased fractional reabsorption of sodium was noted. Dresser et al. (1971) noted that acute hypertension was associated with an increased peritubular capillary hydrostatic pressure and a decrease in proximal reabsorption. Brenner et al. (1969) noted that reabsorption in the proximal tubule varied directly with the protein concentration in the peritubular capillaries in response to intravenous infusion of saline or protein. Finally, Brenner et al. (1971) perfused peritubular capillaries with colloid free Ringers solution and various albumin containing solutions to test the relationship between peritubular capillary oncotic pressure and fluid reabsorption by the proximal tubule. They found that colloid-free perfusion increased the rates of reabsorption. However, there is evidence not in support of peritubular capillary control of proximal sodium reabsorption. Rumrich et al. (1968) found that perfusion of peritubular capillaries with colloid free solutions or with the addition of albumin to the perfusate did not affect reabsorption by the proximal tubule. The

difference in this finding and that of Brenner et al. (1971) might be that Brenner et al. had bicarbonate concentrations which were higher than those used by Rumrich et al., thereby altering the pH of the cells surrounding the perfusate. Finally, Conger et al. (1976) using peritubular capillary microperfusion examined the effects of protein free and hyperoncotic plasma on proximal fluid reabsorption and found no detectable effect on the rate of proximal tubule reabsorption.

Microperfusion rate has been shown to be of greater importance than the peritubular capillary oncotic pressure in determining proximal tubular reabsorption. Banks et al. (1972) found that a high perfusion rate was associated with a decreased reabsorption by the proximal tubule possibly due to an increased hydrostatic pressure in the peritubular capillaries.

A change in the hydrostatic pressure of the peritubular capillaries has been shown to alter net reabsorption. Strandhoy et al. (1974) examined the effect of renal vasodilators, which have the effect of increasing the hydrostatic pressure. They found that marked increases in peritubular capillary and interstitial hydrostatic pressure did not decrease proximal sodium reabsorption. Thus, changes in Starling forces in the peritubular capillaries do not change proximal reabsorption.

Sodium reabsorption in the proximal tubule and urinary sodium excretion may be dissociated. Knight et al. (1977)

increased the delivery of sodium out of the proximal tubule without changing urinary sodium excretion. In addition, systemic infusions of hyperoncotic albumin solution decreased the rate of proximal fluid transport similar in magnitude to that seen with acute saline infusion, yet urinary sodium excretion is much greater following saline infusion than hyperoncotic albumin (Howards et al. 1968).

The loop of Henle is sensitive to transepithelial hydrostatic pressure changes, and a relationship between the renal medullary blood flow and the reabsorption capacity of the loop of Henle. According to this hypothesis, increased medullary blood flow decreases net sodium reabsorption by the loop of Henle by a decrease in medullary interstitial hypertonicity. Fadem et al. (1982) compared the effect of three vasodilators of total renal blood flow, two of which were natriuretic, the other non natriuretic. The two vasodilators which were natriuretic increased papillary plasma flow whereas the non natriuretic vasodilator did not. They concluded that the increased papillary plasma flow washed the solute gradient out of the medullary interstitium, and caused the natriuresis.

In contrast to the the evidence favoring a role of physical forces on sodium reabsorption in loop of Henle, there is little evidence that physical forces affect sodium reabsorption in the distal tubule. Diezi et al. (1980) found that distal tubule sodium reabsorption was not altered by volume expansion. Sodium delivery to the

superficial early distal tubule was maintained constant during volume expansion by clamping the aorta proximal to the kidney. Under these conditions sodium reabsorption in the distal tubule was similar to that without volume expansion. Morgan et al. (1969), using microperfused superficial distal tubules found sodium reabsorption was not altered by volume expansion with saline.

The effects of hydrostatic and oncotic pressures on sodium reabsorption in the collecting ducts were evaluated by Gross et al. (1976) using in vitro microperfusion methods. An inverse relationship between hydrostatic perfusion pressure and transtubular potential difference was demonstrated in the collecting tubule. The decrease in perfusion pressure was thought to reflect an inhibition of active sodium transport. However, Helman et al. (1971) found that if they increased the bath hydrostatic pressure, an increased in the transtubular potential difference of the collecting tubule occured, presumably reflecting active sodium transport in this segment of the nephron. They concluded that the effects of changes in the hydrostatic pressure may also relate to the degree of stretch of the tubule wall.

The effect of oncotic pressures on sodium reabsorption in the collecting duct was studied by Schwartz et al. (1978). They observed that upon the addition of albumin to the bath, their was no effect on transepithelial voltage, lumen to bath sodium transport or on potassium excretion.

Natriuretic Hormone

There are instances where the above mentioned factors can not account for the increased sodium excretion seen in some experimental conditions. DeWardener et al. (1961) initiated a series of cross-circulation experiments which suggested that natriuresis noted upon saline administration was due to the action of a humoral substance, other than aldosterone, on the tubules. Briefly, blood from a volume-expanded dog was cross-circulated to a recipient dog, where it was noted to produce a natriuretic response in the recipient dog. Livensky (1966) ruled out that an increase in GFR, and hence the filtered load of sodium, or a decrease in the concentration of aldosterone cannot account for the increased sodium excretion noted after a saline load. In the experiment, animals received large doses of both ADH and aldosterone in order to prevent fluctuations in plasma concentration of these hormones which could alter sodium excretion. Control GFR and plasma sodium concentrations were measured, which enabled the filtered load of sodium to be calculated, in addition to how much sodium was excreted. After a control period, .9% sodium chloride, which has the same osmolality as the extracellular fluid, was infused into the animals. Both the filtered load of sodium and the excreted load of sodium increased. While the infusion of saline continued, a clamp was placed around the aorta just proximal to the renal arteries, a maneuver which served to decrease the filtered

load of sodium. However, even though the filtered load of sodium decreased, the amount of sodium that was excreted increased in proportion to the decreased filtered load. Thus, the increase in sodium excretion cannot be accounted for by an increase in GFR or a decrease in aldosterone, and hence some other "factor" must be responsible for the increased sodium excretion.

Further evidence of a natriuretic factor was suggested by Bourgoinie et al. (1971). In this study, serum fractions were obtained from both normal individuals and patients with chronic renal failure - a stiuation where a natriuretic factor has been suggested to exist. It was found that serum fractions from uremic patients evoked a greater natriuretic response when injected intravenously into an assay rat than did the same serum fraction obtained from normal individuals.

Other Substances Known to Influence Sodium Excretion

Other substances are known to alter sodium excretion. Two of more common substances include renal prostaglandins and the kinin-kallikrein system. Both of these substances appear to increase sodium excretion by increasing renal blood flow. Cortisol, estrogen, growth hormone and insulin decrease sodium excretion, while glucagon, progesterone and parathyroid hormone increase sodium excretion. For a complete review on the subjects, see the text by Brenner and Rector (Brenner et al. 1981).

Atrial Natriuretic Peptide

Atrial Granules

The muscle cells of the atrial myocardium contain, in addition to the contractile elements and other organelles, numerous electron-dense granules. The existence of these granules was first described by Kisch in 1956 in the atria of guinea pigs (Kisch, 1956). Bompiani et al. (1959), who were studying the conductive tissue in rat hearts, also noticed the presence of osmophilic dense bodies close to the Golgi apparatus. Jamieson et al. (1964) were the first to describe these granules as specific granules that were different from lysosomes and similar to pancreatic protein secretory granules. These granules have been found exclusively in the atria, and not in ventricular tissue, of every mammalian species looked at thus far. Similar granules have additionally been found in ventricular tissue of amphibian, reptilian and avian species. These granules were found to be adjacent to one and occasionally both poles of the nucleus of the myocyte, interspersed among the elements of the Golgi complex. These granules are intimately associated with the Golgi complex which consists mainly of flattened stacked, smooth surface cisternae and associated small vesicles. The cisternae have been found to be extensively fenestrated and filled with fine granular

material suggesting that atrial granules may form within the Golgi complex. These granules have been demonstrated to contain protein by ultrastructural cytochemistry (Huet et al. 1974). Yunge et al. (1979) further demonstrated that these granules can incorporate tritiated leucine and tritiated fucose, which follows the same pattern observed in several peptide secreting endocrine cell types (Jamieson et al. 1964). Cantin et al. (1982) found that these granules do not contain renin nor do they contain catecholamines or incorporate tritiated dopamine, a precursor of catecholamines.

The degree of granularity per cell is not uniform among mammalian species. In the rat for example, the number of granules may range up to 600 per cell. Their number decreases with increasing species size. For example, rats have more granules per cell than dogs, which have more granules per cell than cows. (Jamieson et al. 1964) The degree of granularity can also change in animals maintained on various salt and water intakes. In a study by Marie et al. (1976), rats were maintained on different salt and water intakes. A significant increase in the degree of granularity was noted in the right atrium as compared to the left atrium. Sodium loading the animal via adding 1% sodium chloride to the drinking water with or without the addition of DOCA significantly decreased the number of granules in the right atrium. The greatest decrease in the degree of granularity however, occurred in the rats

administered the DOCA in addition to the saline in the drinking water. Conversely, total sodium restriction for 3 weeks greatly increased the granulation in the left atrium with out any change in the granularity of the right atrium as compared to the control. Water restriction for 5 days increased the granularity only in the right atrium with out any significant change in the left atrium. Drug administration has additionally been shown to alter the degree of granularity. Administration of reserpine (Palade 1961) and atropine (Hibbs and Ferrand, 1969) has been shown to significantly reduce the number of atrial granules in the rat whereas the administration of beta-blockers has the effect of significantly increasing the number of granules (Okamato 1969). In certain diseases states, the degree of granularity has also been shown to change. The number of granules has been shown to increase in adrenal regeneration hypertension (Martinez-Palomo et al. 1966).

Atrial Extracts

In 1981, deBold and colleagues made a crucial experiment. Male Sprague Dawley rats were decapitated so that the atrial could be obtained. The hearts were rapidly removed and placed in a saline solution. They were then homogenized with the addition of 10 parts of saline to 1 part of atria by wet weight, centrifuged down at 2000 G for 20 minutes and the supernate was collected and stored frozen until it could be assayed utilizing a bioassay. A

similar preparation was made of ventricular tissue and this served as the control since this tissue does not contain granules. The bioassay consisted of an anesthetized male rat which had its femoral artery catheterized, in order to monitor arterial pressure, and femoral vein catheterized, in order to continuously infuse an inulin containing saline solution and inject the extract. The bladder was catheterized in order to collect and measure urine flow rate. The extract was injected inravenously into the rat once a stable urine flow rate had been obtained. The results were an increase in sodium excretion, urine flow rate, and potassium excretion all of which were over with in the 20 minute urine collection time period. The rise in sodium excretion was the greatest increase (17 fold) as compared to the the volume excreted (10 fold) which was still greater that the rise in potassium excretion (5 fold). A brief but consistent fall in arterial pressure was also noted. GFR was measured and was found not to significantly increase. However, it should be noted that GFR was measured in the face of a changing urine flow rate and therefore was not an accurate estimate of GFR. A similar preparation of ventricular extract exhibited no effect.

An improved bioassay for measuring the diuretic and natriuretic activity in atrial extracts was developed by Johnson (1985). Atria were collected from 100 rats and prepared in a similar fashion as stated in deBold's paper.

The extract was pooled and the amount of protein per ml was determined. Beginning with 27 ug of protein per ml per injectate and increasing up to 200 ug/ml of protein per injectate, a typical dose response curve was generated. What is significant about this study is that above the threshold dose of 200 ug/ml protein of atrial extract, there is no longer an increase in the diuretic and natriuretic activity of the extract. Thus in order to measure any differences in the potency of atrial extracts, the amount of extract injected must lie below the saturation level, but above the threshold level of the dose response curve.

Isolation of Atrial Natriuretic Peptide

A relationship of the specific granules to the natriuretic and diuretic activity was established by Garcia et al. (1982). Several fractions from rat atrial homogenates were isolated by the use of differential and sucrose gradient centrifugation. The natriuretic and diuretic activity, as measured by rat bioassays, were restricted to the fractions rich in specific granules. The material inside these granules was found to be protease sensitive as the natriuretic and diuretic activity could be abolished by such treatment.

With the aid of gel filtration it was found that the natriuretic and vasorelaxant properties of rat atrial extracts seamed to fractionate into two broad regions.

These regions were divided into a high molecular weight fraction greater than 10,000 daltons, and a low molecular weight fraction less than 10,000 daltons (Currie et al. 1983). The partially purified high molecular weight fraction was found to contain a peptide of approximately 13,000 daltons, later to be designated as pro-ANP. The low molecular weight fractions led to the discovery of a family of peptides, all of which had the same amino acid core sequence, but differed in length (Kangawa et al. 1984). These peptides were found to contain a disulfide bridge and treatment of the peptides with carboxypeptidase A established that the molecules posessed a free carboxy terminal acid and not an amide (Napier et al. 1984).

The availability of atrial peptide sequence data led to cloning of the cDNA and then of the gene for rat ANP (Yamanaka et al. 1984). Prepro atrial sequences were deduced from DNA clones complementary to atrial mRNA. Rat and human prepro atrial sequences were found to be derived from prepropolypeptides which were found to be 152 and 151 amino acids long respectively. (Maki et al. 1984; Nemer et al. 1984) Rat prepro-ANP contained a 24 amino acid amino-terminal signal sequence.

There is no direct evidence which describes prepro-ANP processing. If the ANP system is similar to many peptide hormones, it is likely that the signal peptide is removed cotranslationally by proteolytic enzymes associated with the rough endoplasmic reticulium (Docherty et al. 1982).

This initial cleavage is probably the result of an endoprotease located on the luminal side of the microsomal membrane. In many peptide systems the putative signal peptidase seems to act preferentially on amino acids having small neutral side chains. In the case of rat prepro-ANP the cleavage occurs between alanine on the signal sequence and asparagine on pro-ANP.

Once the signal peptide is removed, the resulting product is probably translocated via the endoplasmic cisternum to the Golgi complex where it is packaged into secretory granules (Rambourg et al. 1984). The cDNA coding for rat prepro-ANP indicates the presence of two carboxy-terminal arginines which have not been found on any rat ANP peptide to date (Maki et al. 1984). This pair of amino acids is probably removed during translocation through the cisternal space in the Golgi complex or an early cleavage event within the newly formed granules. The resulting 126-amino-acid peptide is referred to as pro-ANP. There is a greater diversity of systems for posttranslational proteolytic processing of propeptides, in contrast to the cotranslational processing of many prepropeptides, which appears to follow a generally similar design. Propeptides can be processed within their cells of origin or, as in the case of angiotensinogen, they can be secreted and then processed by extracellular proteases. One interesting characteristic of propeptide processing is that cleavage sites are usually marked by a pair of basic amino

acids. For example, proteolytic cleavage might take place at the arginine-arginine pair at pro-ANP 101 and 102 which would yield the biologically active carboxy-terminal fragment 103-126 (Atriopeptin III).

There is evidence that a small amount of pro ANP is released and present in the circulation. Using HPLC and an RIA to ANP, Gutkowska et al. (1984) reported the presence of a small peak of immunoreactive ANP that did not elute in the same vicinity as that of low molecular weight ANP standard. Lang et al. (1985) applied a similar technique to examine the molecular weight of the released form of ANP from isolated perfused rat hearts. They found a small amount of high molecular weight ANP in the perfusate in addition to the low molecular weight ANP. Thus in the rat, pro-ANP was the predominant form in the atria, while low molecular weight ANP was largely released from isolated perfused hearts. The existence of some pro ANP in plasma suggests that it was secreted with low molecular weight ANP.

Extra atrial conversion of high molecular weight to low molecular weight has been substantiated by two studies: Trippodo et al. (1986) found that incubation of high molecular weight ANP with rat blood or platelets in vitro resulted in the conversion to low molecular weight ANP within minutes. Platelet-induced conversion was associated with enhanced activity in relaxing aortic smooth muscles; Oshima et al. (1984) observed that injections of high

molecular weight ANP into the renal artery showed little renal vascular actions, whereas intravenous injection caused renal vascular dilation suggesting that conversion of the high to the low molecular weight ANP may occur in the circulation.

The circulating form of the peptide is speculated to contain amino acids 123 through 150, which is composed of 28 amino acids (Schwartz et al. 1985). The disulfide bridge between the two cysteines of the C-terminus has been shown to be essential for biological activity. Interestingly, the human circulating form of ANP has a methionine substituted for isoleucine at position 134 (Kangawa et al. 1984).

It is possible that during conditions of high sodium loading, presumably when production and secretion of ANP are increased, there might be an increased rate of conversion of the less active to the more active forms. Thus, despite the decreased number of visible granules, the atria could contain a greater amount of of activated ANP. The opposite could take place during water deprivation.

Release of ANP

Release of ANP has been shown to occur in vivo by atrial distension in the isolated rat heart-lung preparation (Dietz et al. 1984).

Ledsome et al. (1985) found that an increase in left atrial pressure of 11 cm water in dogs increased plasma ANP 70%. Bilateral cervical vagotomy and atenolol

administration did not prevent the increase in ANP. Pulmonary vein distension increased heart rate, but did not increase plasma ANP. It was concluded that release of ANP depends on stretch of the atrium rather than on a reflex involving left atrial receptors.

Release of ANP in vitro, as measured by an increase in radioimmunoreactivity of the medium, has been shown to occur with activation of the polyphosphoinositide system by alpha-adrenergic stimulation or stimulation of the V_1-type vasopressin receptors, and by calcium ionophores or active phorbol esters (Sonnenburg 1986).

Once ANP is secreted by atrial cardiocytes, it diffuses into atrial capillaries where it is collected by the coronary sinus thereby reaching the general circulation through the right atrium. This statement was based on the work of two investigators. Schriffin et al. (1985) obtained blood samples from the coronary sinus in six patients with various cardiac conditions. Samples obtained from the coronary sinus contained 4 times as much as those obtained from the femoral artery. Lang et al. (1985) confirmed the presence of a low molecular weight atrial peptide in the coronary venous effluent of isolated perfused hearts.

Binding of ANP in the Peripheral Tissues

Von Schroder et al. (1985) studied the distribution of ANP binding sites in rat, guinea pig and rabbit tissue utilizing autoradiography of slide mounted tissue sections

incubated with iodinated ANP. This procedure allowed for clear visualization of the ANP binding site distribution. Major binding sites of I-125 labelled ANP were found in the glomeruli and papilla of the kidney and in the aortic smooth muscle. Other regions of binding included the iliac vein, choroid plexus, anterior pituitary, lung, and adrenal zona glomerulosa. Autoradiograms from vascular, renal, adrenal and lung tissue of spontaneously hypertensive rats were not different from those of normal rats.

Using a similar process, Jacobowitz et al. (1985) found immunoreactive ANP located in the brain - specifically, the nerve fibers and cell bodies in the preoptic area, hypothalamus, mesencephalon and pons of the rat. In colchicine treated rats, a large number of immunoreactive ANP positive cell bodies were seen in the organum vasculosum of the lamina terminalis, in several hypothalamic nuclei such as the periventricular, arcuate and ventral premammillary nuclei, and in the dorsalateral tegmental nuclei of the pons. The largest accumulation of ANP containing cells was found to be located in an area of the brain known as the anteroventral third ventricle (AV3V) region. The AV3V region appears to be a critical area for the development and maintenance of experimental hypertension, as well as fluid and electrolyte balance (Hartle et al. 1984).

Natriuretic Action of ANP

The precise mechanism by which atrial natriuretic peptide exerts its natriuretic and diuretic activity is still uncertain at this time. The natriuresis is not due to a pressure natriuresis since mean arterial pressure decreases when the peptide is injected intravenously into rats. The natriuretic response is not due to inhibition of the sodium-potassium ATPase (Pollack et al. 1983b) and appears to be independent of prostaglandin formation (Keeler et al. 1982). The natriuresis induced by ANP could be potentiated by converting enzyme inhibition (Wang et al. 1985).

A redistribution of renal blood flow does not appear to be responsible for the natriuresis induced by ANP administration. Huang et al. (1985) examined the effect of synthetic ANP on single-nephron GFR (SNGFR) and total GFR. SNGFR, whether measured at the proximal tubule or the distal tubule, increased by approximately 40%. This increase almost exactly paralleled the increase in GFR strongly suggesting that ANP does not cause a redistribution of SNGFR with in the kidney.

A decrease in the hypertonicity of the medullary interstitium thereby washing out the concentration gradient needed for salt and water conservation has been suggested to account for the natriuresis noted upon ANP administration. Borenstein et al. (1983) found that a systemic bolus administration of atrial extract into the

rat was associated with an increase in both total and medullary blood flow in the kidney as measured by microsphere and albumin uptake methods. Maack et al. (1985) found a decrease in urine osmolality without a significant change in free water clearance during ANP infusion in the rat, again suggesting that medullary washout occurred. Burnett et al. (1984) confirmed the finding of Maack et al. (1985), but then noted that urine osmolarity returned to control values during the post infusion period suggesting that medullary washout did not occur.

ANP has been suggested to exert a direct tubular effect on the nephron at some distal tubular site, thereby accounting for the natriuresis and diuresis. An early micropuncture study by Briggs et al. (1982) found that the natriuresis induced by ANP extracts occurred without a significant change in glomerular filtration rate, renal plasma flow, or filtration fraction. Furthermore, a direct tubular effect of ANP in superficial nephrons segments up to the the distal tubule was not detected. Pollack et al. (1983a) concluded that sodium reabsorption must be directly inhibited at the distal tubule level because glomerular filtration rate and renal blood flow were not affected by atrial extract. The authors specifically suggested that ANP inhibits sodium reabsorption at some distal nephron site. This was based on the finding that after distal tubule blockade, in which a significant increase in sodium and chloride excretion resulted, ANP administration produced a

significant increase in GFR, and in this case, no change in the fractional excretion of either sodium or chloride was observed. Since ANP did not inhibit tubular sodium reabsorption in any other portion of the nephron in distally blocked animals, the factor must inhibit sodium distal reabsorption. However, Burnett et al. (1984) concluded that synthetic ANP infusion in the dog renal artery decreased proximal tubule reabsorption as estimated by changes in lithium excretion - a marker of proximal reabsorption.

Many of the studies presented thus far gave bolus injections into animals and then measured GFR at times when urine flow was not steady. An attempt to redefine whether or not an increase in GFR was responsible for the increase in sodium excretion upon ANP administration was performed once again, this time during the course of constant synthetic ANP infusion and when urine flow was steady. Berineth et al. (1984) infused synthetic ANP into a dog renal artery and found an initial increase in renal blood flow, GFR and filtration fraction, followed by a decrease in mean arterial pressure and renal blood flow without any change in renal vascular resistance. Urine osmolality was found to decrease without any changes in free water clearance. Burrnett et al. (1984) observed a unique renal blood flow response upon intrarenal administration of ANP. In anesthetized dogs, continued infusion of synthetic ANP first transiently increased renal blood flow followed by a

significant decrease in renal blood flow that persisted the remainder of the infusion period, with no change in total renal vascular resistance as compared to preinfusion levels. A significant increase in GFR and fractional sodium excretion were noted in addition to the traditional natriuresis and diuresis. Camarago et al. (1984) found that sustained infusion of atrial extract and synthetic peptide into the isolated perfused kidney increased GFR and renal vascular resistance, while renal blood flow decreased. This and the previous study support the notion that the increase in GFR was associated with an increase in post glomerular arteriolar resistance. Of course an increase in the glomerular capillary coefficient is a possibility, but measurements for single nephron hemodynamics are necessary to elucidate the exact mechanism of the ANP induced increase in GFR. The authors concluded that the natriuresis can be accounted for at least in part by renal hemodynamic effects rather than by the presence of a putative tubular natriuretic effect.

In some of the initial studies characterizing the renal actions of ANP, it was noted that both plasma and urinary cyclic GMP were increased after treatment (Hamet et al. 1984). Cyclic GMP levels are elevated after the administration of diuretics such as furosemide (Oswald et al. 1977). This led to the hypothesis that cGMP might be responsible for the natriuresis and diuresis seen with ANP infusion. In support of this hypothesis, Hamlet et al.

(1984) incubated rat kidney homogenates with ANP and found an increase in tissue levels of cGMP and a decrease in cGMP phosphodiesterase. Tremblay et al. (1985) noted a 50-fold increase in cyclic GMP in isolated glomeruli whereas only a slight increase in cyclic GMP was noted in the distal part of the tubule. Windquest et al. (1984) incubated LLC-PK1 cells grown in culture with varying concentrations of ANP in the presence of the phosphoesterase inhibitor, butylmethylxanthine, in order to determine whether cGMP production was enhanced in renal tubular epithelial cells. At the lowest concentration of ANP, cyclic GMP production was enhanced five fold. Maximum stimulation increased cGMP production 13-fold. Thus, this study clearly demonstrated that ANP was capable of stimulating cyclic GMP production in renal tubular epithelial cells. However, these data do not provide an answer to the question of whether an increased cGMP plays a role in ANP induced natriuresis.

An attempt to answer this question was made by Marsh et al. (1985) who infused ANP into spontaneously hypertensive and normotensive rats at a dose which produced an equal natriuresis in both groups of rats. Urinary cGMP increased dramatically in the normotensive rats, but only slightly in the hypertensive rat. Blaine et al. (1986) infused ANP into one renal artery of dogs where it was noted that urinary excretion of cGMP by both the infused and non infused kidney were equal, suggesting that urinary cGMP was not of renal origin, but derived from elevated

plasma levels. These studies clearly suggest that a dissociation exists between the natriuresis induced by ANP and urinary cyclic GMP excretion.

Vasorelaxant Properties of Atrial Natriuretic Peptide

A fall in arterial pressure in deBold's original study (1981) was noted upon intravenous administration of atrial extracts and was attributed to the loss of fluid in the urine. Shortly thereafter, this was proved to be false as the fall in arterial pressure occurred before a significant fluid loss occurred. This observation led to the hypothesis that atrial extracts relaxed smooth muscle.

Currie et al. (1983) applied atrial extract to vascular smooth muscle, precontracted with KCl, and to intestinal smooth muscle, precontracted with carbachol, where a prompt relaxation of both types of muscle was noted. Both atrial extracts (Kleinert et al. 1983) and synthetic ANP (Sugiyama et al. 1984) were additionally noted to relax vascular smooth muscle precontracted with norepinephrine, angiotensin II, and KCl. Windquest et al. (1984) demonstrated that synthetic ANP could relax rabbit facial veins but were weakly active in relaxing the phasic contractions of rat portal veins in vitro. However, rabbit facial veins resemble arterioles more closely than other venous tissue in its smooth muscle composition and intrinsic tone. Kleinert et al. (1984) noted that in order for ANP to induce any relaxation, isolated vessels must

first be precontracted with humoral or non-humoral agonists.

ANP may have a unique effect on angiotensin II induced contractility in vitro. Kleinert et al. (1984) found that high concentrations of angiotensin were unable to overcome ANP induced relaxation whereas norepinephrine did so easily. Atlas et al. (1986) additionally found that ANP antagonizes vasoconstriction induced by angiotensin II contracted vessels in vitro. Sensitivity of the blood pressure-lowering effects of ANP in vivo appeared to be enhanced in renin-dependent models of renovascular hypertension as compared with other experimental hypertensive models.

The precise mechanism of ANP induced vascular smooth muscle relaxation is unknown at this time, but cGMP has been suggested to be involved. Cyclic GMP also has been proposed as a mechanism of the relaxation of smooth muscles to compounds such as nitroglycerin. Hamet et al. (1984) found that in vivo injection of ANP increased both urinary excretion and plasma levels of cGMP. Additionally, Hirata et al. (1984) found that ANP elevated cyclic GMP levels of rat aorta smooth muscle cells in culture. Windquest et al. (1984) incubated rabbit aortic rings with ANP in the presence of a phosphodiesterase inhibitor where a significant increase in cyclic GMP levels were noted as compared to untreated tissues.

Cardiovascular Effects of Atrial Natriuretic Peptide

A fall in arterial pressure was noted by de Bold (1981) upon intravenous administration of atrial extracts. Both Maack et al. (1984) and Volpe et al. (1985) found that constant infusion of ANP produced a sustained dose dependent fall in arterial pressure in both conscious and anesthetized normotensive animals. Volpe et al. (1985) noted that the sensitivity to the blood pressure lowering effects of ANP appeared to be enhanced in renin-dependent hypertensive animals - a model which presumably exhibits elevated levels of angiotensin II. Seymour et al. (1985) reported that ANP induces an even greater fall in arterial pressure in DOCA-salt hypertensive rats, a model characterized by volume expansion and suppression of the renin-angiotensin system as compared to renin-dependent hypertensive animals.

The vasorelaxant properties of ANP led to the belief that peripheral vasodilation after ANP administration caused the fall in mean arterial pressure. Ackerman et al. (1986) reported that both bolus injections of atrial and ventricular extracts decreased peripheral vascular resistance. However, only atrial extracts caused a significant hypotension due to a cardiac inhibition which involved both bradycardia and failure of stroke volume to increase appropriately. The author stated that the observations were not due to a direct action of atrial extracts on myocytes, but were likely the result of

interactions with the cardiovascular reflex mechanism.

Atlas et al. (1986) stated that the depressor action of low, possibly physiological, doses of ANP in two-kidney, one-clip Goldblatt rats was due to a decrease in total peripheral resistance. However, high doses of ANP were found to lower cardiac output, particularly in volume expanded deoxycorticosterone-salt hypertensive rats.

Volpe et al. (1986) found that vascular resistance was involved in blood pressure-lowering effects of ANP in vasoconstricted hypertensive models. In two-kidney, one clip rats, low doses of ANP (72 ng/kg min) significantly reduced arterial pressure, which was associated with a fall in total peripheral resistance. Higher doses (1800 ng/kg min) were needed to induce a significant fall in cardiac output. In contrast, DOCA-salt hypertensive rats exhibited a dose-dependent fall in stroke volume and cardiac output, which was associated with a rise in calculated resistance. No effect on pressure was noted until the higher rates of ANP were infused. Thus, the effects of ANP infusion on systemic hemodynamics appears to be both dose dependent and dependent on the underlying hemodynamics.

A fall in cardiac output was not observed by all investigators. For example, Koike et al. (1984) did not observe a reduction in cardiac output after an intravenous bolus injection of ANP in conscious rats. Cardiac output in this study was measured using microspheres and it is possible that the mode of administration could affect the

cardiovascular actions of the extract.

Lappe et al. (1985) concluded that a fall in cardiac output was due to a decrease in venous pressure rather than to a direct cardiac depressant action of ANP. In their study, the cardiovascular actions of a constant infusion of ANP (250 ng/kg min) in conscious rats was examined where it was found to decrease mean arterial pressure, and increase total peripheral resistance. Infusion of the peptide into spontaneously hypertensive rats resulted in the same results in addition to an increase in heart rate, and a decrease in cardiac output, stroke volume, and central venous pressure. The fall in cardiac output during ANP was concluded to be due to a decrease in venous pressure, and not due to a direct cardiac depressant action of ANP.

However, recent evidence suggests that ANP lowers arterial pressure by mechanisms other than peripheral vasodilation. Breuhaus et al. (1986) confirmed that ANP infusion (300 ng/kg min) lowers arterial pressure, stroke volume and right atrial pressure in addition to lowering cardiac output. Heart rate and total peripheral resistance increased reflexively, because partial ganglionic blockade was able to prevent both the heart rate increase and the peripheral resistance increase. Ganglionic blockade alone caused a reduction in right atrial pressure. However, ANP caused a greater fall in stroke volume than ganglionic blockade and it was suggested that the fall in cardiac output may be due to coronary vasoconstriction. Coronary

vasoconstriction was confirmed in an isolated perfused heart preparation by Wangler et al. (1985).

Relationship Between Atrial Distension and ANP: Dissociation Between the Hemodynamic and the Natriuretic Response to ANP

It has already been mentioned that an increase in left atrial pressure increases plasma ANP. This led to the hypothesis that the noted natriuresis and diuresis which accompanies atrial distension might be due to ANP release. If this were true, then the response to ANP infusion and left atrial distension should be the same. Goetz et al. (1986) compared both renal function and systemic hemodynamics after infusion of 3,000 ng/kg min of synthetic ANP, and after left atrial distension. Both atriopeptin infusion and left atrial distension caused a significant increase in urine flow and sodium excretion and a decrease in renal blood flow. However, the pattern of systemic hemodynamic response to atriopeptin infusion was different from that elicited by left atrial distension. Specifically, left atrial distension did not change cardiac output or total peripheral resistance, but increased heart rate, pulmonary arterial pressure, aortic pressure, and decreased stroke volume. The authors concluded that the renal response to left atrial distension was mediated by the release of ANP whereas the pattern of systemic hemodynamic responses were not.

The previous study does not rule out the influence of a neurally mediated natriuretic response after left atrial distension. Goetz et al. (1986) found that both right and left atrial distension increased plasma ANP in conscious dogs, but sodium excretion increased only during left atrial distension. After cardiac denervation, an increase in left atrial pressure increased ANP, but did not increase sodium excretion. Thus, the increase in plasma ANP during atrial distension appears to be incapable of independently increasing salt and water excretion.

Luft et al. (1986) preformed a dose response curve in rats weighing between 250 and 275 grams. Synthetic ANP was administered intravenously in incremental doses of 25, 50, 100, 250 and 1000 ng bolus injections. A significant influence on blood pressure was identified at 25 ng while a significant influence on sodium excretion was not identified until the 100 ng dose. Assuming a plasma volume of 4% of the total body weight, and 25 ng and 100 ng distributed evenly in the plasma, this would yield plasma concentrations of 833 fmoles/ml and 3,333 fmoles/ml respectively. Thus, a much lower dose of ANP was required in order to observe the hemodynamic responses as compared to the much larger dose required to observe the natriuretic effects.

Other Actions of ANP in Terms of Salt and Water Homeostasis

ANP has been shown to affect hormones which alter salt and water regulation such as the renin-angiotensin system, aldosterone, and vasopressin. Thus, ANP has a potentially important interaction with these systems, which suggests a role for ANP in the homeostatic control of arterial pressure as well as extracellular fluid volume.

1. Renin-Angiotensin System

Constant infusion of ANP causes a marked inhibition of renin release despite a fall in arterial pressure (Maack et al. 1984). This inhibition of renin release occurred even in the face of a fall in renal plasma flow. There is evidence that the acute effect of ANP on renin secretion was in large part dependent on the renal hemodynamic action. A possible hypothesis suggested by the authors was that the increase in GFR and in filtered solute load led to an increased supply of sodium chloride to the macula densa.

Volpe et al. (1986) suggested that the inhibitory effect on renin release was most apparent when basal secretion rate is elevated, but this inhibitory effect requires normal renal perfusion. ANP may induce slight stimulation of plasma renin in animals with unilateral renal artery constriction, possibly due to further decrease in renal perfusion pressure. However, ANP was able to suppress plasma renin in sodium-depleted one-kidney, one-clip rats possibly suggesting an enhanced sensitivity

of the macula densa mechanism after sodium depletion, so that even slight increases in distal sodium chloride could turn off secretion.

Dosa et al. (1986) found that acute constriction of one renal artery, which provokes unilateral renin secretion from the clipped kidney, prevented ANP induced increases in GFR and sodium excretion and blocked the ability of ANP to lower plasma renin levels. Instead, ANP tended to increase plasma renin slightly in this situation. Volpe et al. (1985) found that ANP did not lower plasma renin acutely in chronic two-kidney, one clip Glodblatt rats, but rather caused a significant increase (Volpe et al. 1985).

It is possible that ANP may have a direct effect on renin release on the juxtaglomerular cells, but it appears that the renal hemodynamic effects of ANP are responsible for the acute inhibition of renin release observed when renal perfusion is not decreased.

Antonipillai et al. (1986) noted that ANP by itself had no effect on renin release from rat cortical slices but potentiated angiotensin II inhibition of renin secretion. ANP also potentiated the inhibitory effect of angiotensin on a substance which altered intracellular calcium and a substance which blocks calmodulin's effects on renin secretion. The authors concluded that since angiotensin II's action on renin release was associated with increases in intracellular calcium, ANP acted by altering the intracellular calcium-calmodulin mediated steps of

angiotensin II action and not via cAMP (Antonipillai et al. 1986).

2.Aldosterone Secretion

Evidence for a direct adrenal effect of ANP in vivo is provided by the observation that ANP induces a significant and reversible fall in plasma aldosterone in dogs (Maack et al. 1984) and induces profound reductions in plasma aldosterone in renin-dependent two-kidney, one-clip hypertensive rats despite a concurrent increase in plasma renin activity (Volpe et al. 1985).

Synthetic ANP inhibits basal aldosterone production from bovine or rat adrenal cells and also antagonizes the stimulation of aldosterone by agonists such as angiotensin II, ACTH, dibutyryl cyclic AMP, and potassium (Atarashi et al. 1984; Kudo et al. 1984). Goodfriend et al. (1984) further studied the inhibitory action of ANP on aldosterone production. Under basal conditions, ANP was found to inhibit aldosterone synthesis at an early portion of the steroidogenic pathway at a point before mitochondrial uptake and metabolism of cholesterol.

The effect of ANP appears to be specific for the outer zone of the adrenal cortex, because there is no effect on glucocorticoid production by isolated rat fasciculata-reticularis cells or on plasma cortisol (Atarashi et al. 1984).

3. Vasopressin Release

In vitro, ANP has been shown to stimulate the release of vasopressin from isolated posterior lobes of the rat hypophysis. At physiological concentration, ANP was found to bind to a receptor where it was three times more effective than 60 mM KCl at eliciting vasopressin release (Januszewicz et al. 1985). Although the present study indicated that ANP has a direct releasing effect of vasopressin on the isolated posterior lobe, the overall effect of vasopressin release by the intact hypothalamic - hypophyseal system is modulated by a host of nervous reflexes.

In vivo, Samson (1985) found that ANP inhibited dehydration and hemorrhage-induced vasopressin release in the rat. Intravenous ANP, as low as 20 pmol in 100 ul of a bolus dose, significantly inhibited dehydration and hemorrhage induced vasopressin release in the rat. Thus, a possible role exists for cardiac peptides in the control of vasopressin release as well as the existence of a counter regulatory peptidergic system for maintenance of fluid and electrolyte homeostasis. Manning et al. (1985) found that pharmacological doses of vasopressin infused into rats can stimulate ANP release, presumably by activation of the V_1 type receptors. This finding completes the evidence for a negative feedback endocrine loop whereby vasopressin stimulates ANP release, which in turn suppresses vasopressin release. These opposing hormones may interact

in the regulation of fluid and electrolyte homeostasis.

Degradation of Atrial Natriuretic Peptide

In vitro degradation of ANP has been shown to be protease sensitive (Garcia et al. 1982). Veress et al. (1985) found that incubation of atrial extracts in vitro with blood before intravenous injection into bioassay rat reduced the natriuretic activity of the factor. Specifically, inactivation was associated with a white cell / platelet fraction, indicating that these blood elements may play a physiological role in the metabolism of ANP. Tang et al. (1984) found that synthetic ANP was degraded by tissue homogenates, the relative activities being: kidney > liver > lung > plasma > heart. Smaller peptide fragments were formed after incubation with kidney homogenates. The degradation of synthetic ANP was inhibited by an aminopeptidase inhibitor and a carboxypeptidse inhibitor. Briggs et al. (1984) stated that inactivation of ANP by renal kallikrein may be a potential mechanism for in vivo regulation of natriuretic activity.

An in vivo study by Tang et al. (1984) found that the disappearance of iodinated ANP from urethane anesthetized rat plasma was rapid with an estimated half-life of 2.5 minutes.

Pathophysiological Implications of ANP

Pathophysiological implications of ANP in experimental hypertension, congestive heart failure and after expansion of plasma volume have been suggested. A half natriuretic dose of ANP, which only produced 50% of the maximal natriuretic response, induced a fall in arterial pressure in both two-kidney, one-clip hypertension, and in one-kidney, one-clip hypertension of 61 mmHg and 45 mmHg respectively. In normotensive rats, the same dose only produced a 29 mmHg fall in arterial pressure (Garcia et al. 1985).

Chronic infusion of ANP into conscious two-kidney, one- clip hypertensive rats for 7 days was studied by Garcia et al. (1985). A reduction in arterial pressure from 183 mmHg to 116 mmHg after the fifth day of infusion was noted in addition to a fall in plasma renin activity.

Chronic infusion of ANP into conscious spontaneously hypertensive rats (SHR) and Wistar Kyoto controls (WKY) for 7 days was studied by Garcia et al. (1985). A reduction in arterial pressure from 177 mmHg to 133 mmHg was noted after the fifth day in the hypertensive rats, while no change in arterial pressure was noted in the control group at the dose used. The authors further noted that there was no change in natriuresis and diuresis between the two groups. The authors concluded that the fall in arterial pressure was the result of a fall in total peripheral resistance.

Plasma ANP levels in hypertensive SHR rats are

significantly elevated over those of control Spraque Dawley rats. Blood pressure was measured to be 195 mmHg in 16 week old SHR rats as compared to 116 mmHg for age matched controls. Plasma ANP levels measured 203 pg/ml in SHR rats vs. 128 pg/ml in control rats. It was additionally noted that SHR rats had significantly greater amounts of ANP in the left atrium as compared to control rats (Gutkowska et al. 1985).

Yasujima et al. (1985) evaluated the effect of ANP infusion into rats made hypertensive by infusion of norepinephrine. Administration of ANP into the jugular vein via osmotic minipumps at a dose of 150 ug/kg, which did not induce any changes in systolic blood pressure, urine volume or sodium excretion in control rats, was found to return arterial pressure from 146 mmHg to 120 mmHg.

It has been speculated that ANP may play a role in the fluid accumulation noted in congestive heart failure. Chimoskey et al. (1984) first reported that hamsters with familial cardiomyopathy and congestive heart failure have an apparent deficiency in ANP. Burnett et al. (1986) reported that plasma ANP levels were elevated in patients with congestive heart failure. Thus, the sodium retention and edema formation characteristic of heart failure occur despite increased circulating ANP levels.

ANP has been speculated to play a role in blood volume expansion. Expansion of blood volume in rats with .9% sodium chloride has been shown to produce a large diuresis

and natriuresis which can be inhibited by 50% if ANP antisera is injected at the beginning of the infusion (Cantin 1985). Right atrial appendectomy in rats reduced the natriuresis and diuresis by 50% after blood volume expansion in iso-oncotic Ringers-albumin solution (Veress et al. 1986).

In two patients who underwent rapid atrial pacing a two fold increase in ANP was observed after beginning treatment. Significant increases were additionally found in patients with paroxysmal tachycardia (Schriffin et al. 1985b).

Reduced Renal Mass

An Overview

Reduction of renal mass, either experimentally or during the course of chronic renal disease, places a substantial demand on the remaining nephrons to maintain the bodily fluids compatible with life. Each remaining nephron must assume an increasing share of the excretory function provided there is not a concomitant decrease in the amount of substances which require excretion by the kidneys. Uremic animals do maintain external sodium, potassium and magnesium balance until the end stage of chronic renal disease. A very small percentage of the original population of nephrons can often maintain life without major dietary alterations. These accomplishments of excretory function occur in all forms of chronic renal disease and are therefore largely independent of any structural change which may occur. Two unique processes are responsible for these remarkable feats which occur with a reduction in renal mass (Peters 1963). The first, compensatory adaptation, occurs in the early stages after the loss of functional renal tissue. This is a unique process in that the compensatory adaptive response involves a decrease in the energy-consuming transport activity. Compensatory adaptation is followed by compensatory hyperfunction which is often accompanied or preceded by an

increase in blood flow. In the kidney, the most conspicuous functional aspect of compensatory hyperfunction is an increase in GFR.

In spite of compensatory adaptation and compensatory hyperfunction, abnormalities in solute transport do exist in advanced chronic renal disease. These abnormalities in chronic renal disease include pathological changes such as scarred and damaged renal parenchyma which in turn can impair the function of the surviving nephrons. For example, chronic uremic patients cannot concentrate or dilute their urine normally (Harrington et al. 1973). Both acidification and alkalinization of the urine may be defective precipitating acid-base disturbances (MacLean et al. 1980). Uremic patients can not conserve sodium maximally on a low sodium diet, (Polak et al. 1971) and often exhibit a reduced ability to reabsorb glucose and bicarbonate (Shankel et al. 1967).

The Intact Nephron Hypothesis

Despite the fact that diseased kidneys are not the same as normal kidneys, a remarkable degree of integration remains. This is often referred to as the "intact nephron hypothesis" (Bricker 1969). That is, the residual nephrons in chronic renal disease appear to function as an organized group in the sense that the remaining nephrons exibit glomerular tubular balance. Glomerular tubular balance is often used to indicate a relationship between GFR and

The Intact Nephron Hypothesis

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tubular function of the remaining nephrons regardless of the segment or segments of the tubule where the tubular lesion occurs (Wesson 1973). The degree of homogeneity of glomerular tubular balance exists if the ratios between single nephron GFR and the rate of tubular transport of the reference material are closely comparable in all the nephrons in the kidney, irrespective of the absolute value for SNGFR in the nephron tested. The intact nephron hypothesis is important in that it states that the remaining nephrons do not excrete solutes by "falling apart".

Several techniques have been employed to evaluate the homogeneity of glomerular tubular balance in chronic renal disease. The first involves comparing the ratio of GFR to the value for a tubular function, in this case ammonia secretion, in the diseased kidney and comparing this ratio to the value measured in a normal kidney (Bricker et al. 1971). It was found that for any given volume of glomerular filtrate, the tubules of the diseased kidney secrete exactly the same number of molecules of ammonia as do the tubules of the normal kidney. That is, the equality of the clearance ratios between the two kidneys of any animal with chronic renal disease, whether unilateral or bilateral, establishes the fact that the relationship between the mean rate of tubular transport of the reference marker and the mean rate of SNGFR is exactly the same in either kidney whether or not it is damaged.

The next technique utilizes a method known as the glucose filtration test, which is also a clearance technique. This involves elevating the concentration of glucose in the plasma in a step wise fashion from fasting levels to levels exceeding the transport maximal rate of glucose reabsorption. If the concentration of glucose is identical at any given time in the filtrates of all functioning glomeruli, the amount of glucose filtered by each nephron will be determined by its value for SNGFR. The transport maximum of glucose in any nephron will therefore depend upon the balance between SNGFR and glucose reabsorptive capacity of the proximal tubule from the kidney with chronic renal disease. A normal kidney will essentially reabsorb all the filtered glucose until the transport maximum is reached. The titration curve for the whole kidney represents the composite of the individual titration curves for all of its functioning nephrons, hence any nephron in which glucose reabsorption is reduced out of proportion to glomerular function would excrete glucose into the urine at a low blood glucose levels. Experimental results have shown that the titration curve is not abnormal (ie the transport maximum for glucose is the same) nor is the splay in the titration curve for the diseased kidney greater than for a normal kidney, nor is the splay zone increased (Bricker et al. 1960).

The final technique utilizes the study of SNGFR and the tubular transport of a reference solute in a group of

nephrons studied individually in a kidney with chronic renal disease, with the use of micropuncture techniques in both glomerular and non glomerular lesions. When the fractional fluid reabsorption is plotted against the percentage length of proximal tubule at which the tubular fluid is sampled, the function is closely comparable to that obtained from nephrons of normal kidneys (Mazumdar et al. 1975). When absolute sodium reabsorption along the the proximal tubule is plotted against SNGFR for the same nephron, similar evidence for homogeneity of glomerular tubular balance is found (Maddox et al. 1975). When single nephron glucose reabsorption is plotted against SNGFR in animals with glomerulonephritis, homogeneity of glomerular tubular balance is preserved (Kawamura 1977). Thus, the homogeneity of glomerular tubular balance in the chronically diseased kidney exists despite an increased value of SNGFR in healthy nephrons.

Regulation of Solute Balance

The previous section alluded to the fact that an organized pattern of function among residual nephrons in diseased kidneys occurs. This in itself does not explain how these nephrons can continue to maintain external balance for the many solutes the kidney is required to regulate. Thus, continuous solute specific adaptation must occur in the remaining nephrons. That is, for any given solute, the maintenance of external balance requires that

the rate of excretion remain equal to the net amount entering the bodily fluids on a continuing basis. In order to accomplish this external balance, the level of glomerular tubular balance must be repeatedly reset for every solute under renal regulation. The impact of a reduction in nephron population occurs in two stages. First, there is a decrease in solute and water excretion initially retaining in these unexcreted amounts. Then, restoration of the external balance occurs to return the bodily fluids to their previous steady state. (Bricker and Fine 1981). This stage requires that the rate of excretion per nephron, or per unit of glomerular filtration rate, must increase in inverse proportion to the number of surviving nephrons and their composite GFR. Several patterns of adaptation occur which enable the the kidney to handle the effects of a reduction in renal mass. These major patterns of adaptation which occur in the residual nephrons include: no regulation, regulation with limitation, and complete regulation.

1. No Regulation

No regulation represents those solutes whose excretion rate is controlled by the glomerular filtration rate. The solutes are not not excreted by any type of active transport system which is responsive to changes in the serum concentration of that solute. An example of this type of solute is urea and creatinine. (Smith 1951). For urea a

fraction of the filtered load is excreted. The remainder is reabsorbed but not in a way that is regulated in order to render the plasma concentration constant (Shannon and Smith 1935). The fraction of the filtered load of urea that is excreted is primarily determined by the simultaneous excretion of water. Because the fractional excretion of water increased in chronic renal disease, the fractional excretion of urea similarly increases. Creatinine additionally exhibits the same characteristics of urea except that at elevated plasma concentrations, creatinine can be secreted (Shannon 1935). However, this secretory mechanism in itself is not sufficient to meet the excretory rates which would be necessary in order to maintain normal plasma levels. For both of these solutes, as nephron loss occurs, a rise in the plasma concentration occurs, and the inverse relationship between the plasma concentration of urea or creatinine and GFR is sufficiently predictable for either of the two solutes to serve as a rough clinical index of GFR as long as the net rate of acquisition remains constant. In general, for each 50% reduction in GFR, the plasma levels of urea and creatinine double (Kopple et al. 1974).

2. Regulation with Limitations

Regulation with limitations applies to those solutes that are filtered and actively reabsorbed or secreted by the tubule. A reduction in GFR leads to the retention of

solutes thereby raising the plasma levels of those solutes. The retention of those solutes initiates a series of adaptive events which increase the rate of tubular reabsorption or secretion, and the result is to increase the excretion rate per nephron of the remaining nephrons. This adaptation takes place only through part of the progression of chronic renal disease after which plasma levels of those solutes increase. That is, once the limitation of adaptation is reached, each further reduction in nephron population leads to an increase in plasma levels of those solutes. Two solutes which fall into this category include phosphate and urate. Serum phosphate levels tend to remain normal until GFR is reduced by approximately 75%; after which they rise in parallel to that of urea and creatinine (Goldman et al. 1954). The limitation of adaptation is slightly reduced with urate and thus plasma levels may be elevated earlier in the course of the disease.

3. Complete Regulation

Complete regulation refers to those solutes whose excretion rate exhibits complete adaptive increase in excretion as nephron population falls. Those solutes include sodium (Bricker 1967), potassium (Schultze et al. 1971) and magnesium (Coburn et al. 1969). For sodium and magnesium, the fractional reabsorption decreases and GFR falls. For potassium, the major adaptation consists of an

increase in tubular secretion.

It is well known that with chronic renal disease, animals are still able to maintain sodium balance on an average salt intake despite a progressive decrease in nephron population. This regulation is often referred to as the magnification phenomenon and is defined as follows: "For any given perturbation of bodily fluids occasioned by the entry of any given amount of solutes into the extracellular fluid, the excretory response per nephron must increase as GFR decreases" (Bricker et al. 1978). The magnification phenomenon in chronic renal disease is best illustrated with an example of sodium handling by the kidney (Fine et al. 1976). A person in health may ingest 120 mEq of sodium per day and have a GFR of 120 ml/min. That same person will excrete 1 out of every 200 sodium ions filtered. In contrast a person with chronic renal disease ingesting the same amount of sodium may have a GFR of only 2 ml/min. The remaining nephrons will have to excrete 32 out of every 200 sodium ions filtered. To further extend this example, if the normal person ingests half the original sodium intake, or 60 mEq of sodium per day, then 1 out of every 400 sodium ions filtered will be excreted. The person with chronic renal disease on the same sodium intake will excrete 120 out of 400 sodium ions. The magnification phenomenon applies additionally to those solutes falling into the category of "complete regulation" and "regulation with limitation".

Afferent Mechanisms in the Regulation of Sodium Balance in Reduced Renal Mass

The question now remaining is "how is the kidney able to sense purturbations in the extracellular fluid compartment?" This question was discussed in detail in the section "Regulation of the Effective Circulating Volume" where it was stated that the upper portions of the body and the thorax appear to be a possible site for these dector elements. For example when a person is immersed in a tank of water to the neck, a translocation of the extracellular fluid occurs from the lower extremities to the central portion of the body. A modest degree of natriuresis follows (Epstein et al. 1972). When patients with varying degrees of chronic renal disease are similarily immersed, the same translocation of the extracellular fluid occurs as in the normal person, and a modest degree of natriuresis occurs. There is an inverse relationship between the magnitude of the rise in the fractional sodium excretion and the GFR (Bricker et al. 1978). Thus it appears that some detector signal resides in the intrathoracic cavity even though the the extracellular fluid volume under goes contraction in the extremities. Another experimental condition leads to similar conclusions. When patients with chronic renal disease are expanded with approximately 1.5 liters of saline, again the fractional excretion of sodium occurs, and again, the magnitude is inversely related to the steady

state GFR (Schultze et al. 1969). If at the peak of the natriuretic response resistance to venous return is increased by inflating a cuff around both thighs to pressures just below the diastolic pressure, which presumably results in sequestration of fluid in the lower extremities and decreases central blood volume, the natriuresis is halted and the fractional sodium excretion returns to the preinfusion levels (Slatopolsky et al. 1968).

Efferent Mechanisms in the Regulation of Sodium Balance in Reduced Renal Mass

There are several factors which may influence the rate of sodium excretion in chronic renal disease as noted in the section entitled "Regulation of the Effective Volume". An increase in arterial pressure has been suggested to account for the increases in sodium excretion. Other mechanisms include an increase in GFR and a change in aldosterone levels, which have long been considered the primary factors responsible for the regulation of sodium excretion. Alterations in the so called "physical factors", withdrawal of nerve activity and an osmotic diuresis have additionally been suggested. Finally, the existence of some natriuretic hormone or hormones may influence the rate of sodium excretion.

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Increase in Arterial Pressure

A study by Cole et al. (1968) noted a 10 - 15 mm Hg rise in arterial pressure upon uninephrectomy. This increase in arterial pressure could conceivably lead to a pressure natriuresis thereby accounting for the noted increase in sodium excretion. However, the natriuresis could not be abolished by reducing the renal perfusion pressure with an aortic ligature to below control values. Thus, the natriuresis per nephron does not appear to be associated with the rise in arterial pressure noted upon reduction of renal mass.

Increase in GFR

A change in SNGFR to the most outer cortical nephrons could conceivably result in an increased fractional sodium excretion. Weber et al. (1975) found that animals with chronic renal disease exibited SNGFR's that were two or more times greater than control values. This increase does support a role for an increased SNGFR to increase sodium excretion in those nephrons involved. Additionally, Peters et al. (1963) has shown that GFR does increase upon a reduction in renal mass and continues to do so until 70 to 80 % of the original GRF is attained. Despite the fact that an increase in GFR occurs, and hence the filtered load of sodium, this can not completely account for the sodium excretion seen in reduced renal mass. Schultze et al. (1969) evaluated the affects of the increase in GFR per

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nephron on the natriuresis in uremic animals. In their study, the adaptive increase in filtration rate was reversed by constricting the renal arteries of the experimental kidney. When the renal artery was constricted, GFR and the filtered load of sodium fell below prenephrectomy levels. Despite these maneuvers, the natriuresis persisted as did the fractional sodium excretion. Additionally, sodium retention did not occur and sodium balance was maintained. Another experiment by Rocha et al. (1973) using animals with glomerulonephritis has shown that no change or a decrease in SNGFR is seen. Additionally, balance studies have shown that these animals can maintain external sodium balance as long as they are not nephrotic.

Aldosterone Levels

Aldosterone, a mineralocorticoid secreted from the zona glomerulosa cells of the adrenal cortex, is known to promote sodium retention. Thus, a decrease in aldosterone levels would be expected to promote sodium excretion. Studies by Berl et al. (1978) have actually shown that aldosterone levels may be elevated in patients with advanced chronic renal disease. Additionally, Schultze et al. (1969) found that the natriuretic response upon transition to a remnant kidney occurred even though the animals were maintained at maximal levels of synthetic aldosterone. Furthermore, another group of adrenalectomized

animals with chronic renal disease were still able to maintain sodium balance. Thus, it seams unlikely that aldosterone is the regulator of the fine tuning of sodium balance in chronic renal disease.

Redistribution of Renal Blood Flow

A redistribution of blood flow from the outer cortical nephrons to the inner cortical nephrons could decrease sodium excretion. Carrier et al. (1973) found such a redistribution of blood flow in both acute and chronic reduction of renal mass. Changes in the reabsorptive capacity of the nephron were studied by the viewing the intrarenal blood flow changes utilizing krypton 85 disappearance curves, autoradiograms, and silicone rubber injections. Outer cortical blood flow decreased while a progressive vasodilation of the inner medullary blood vessels was observed in addition to a marked decrease in proximal and distal sodium reabsorption both after partial reduction of renal mass and complete removal of one kidney after partial removal of the other. These hemodynamic changes cannot be explained by changes in arterial pressure or by uremia per se since the animals in the partial reduction of renal mass were presented with the same vascular changes as the other group.

Renal Nerve Activity

The natriuresis noted in reduced renal mass is not caused by the withdrawal of renal nerve activity. Klein and Gittes (1973) reduced the renal mass of animals and compared the resultant sodium excretion and fractional sodium excretion. It was found that the resulting natriuresis was not dependent on the presence of renal nerves.

"Physical Factors"

"Physical factors" represent those factors which can influence net fluid reabsorption in the renal tubule as previously discussed in "Control of Sodium Excretion". Maddox et al. (1975) pointed out that the noted decrease in proximal tubule sodium reabsorption which occurs in chronic renal disease could be the result of an alteration in physical factors. A change in hematocrit has been shown to alter proximal tubule reabsorption, a decrease increasing sodium excretion and an increase decreasing sodium excretion. (Bahlmann et al. 1967). Consistant with the hypothesis that a decrease in hematocrit might increase the fractional sodium excretion has been the finding that many uremic patients are anemic. However, correction of anemia does not obliviate the increased fractional sodium excretion. Additionally, Weber et al. (1975) has found that large changes in proximal sodium reabsorption have little influence on sodium excretion in both normal animals and

"Physical [illegible]

nerves.

[illegible] [illegible] [illegible]

equium [illegible]

[illegible]

[illegible]

[illegible]

does not [illegible]

[illegible]

[illegible]

hypothesis [illegible]

[illegible]

sodium [illegible]

[illegible]

[illegible]

physical [illegible]

renal [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

animals with chronic renal disease. Furthermore, nephrotic uremic animals should exhibit an inhibition of fluid reabsorption in the proximal tubule due to the profound hypoalbuminemia. In this particular group, external sodium balance is not preserved and sodium retention occurs (Bourgoignie et al. 1974). Most of the data collected in the literature thus far has suggested that the influence of physical factors is on distal tubule sodium reabsorption. Distal tubule sodium reabsorption is the location of the so called "fine tuning of sodium transport" (Stein et al. 1973). As a final point, it is difficult to conceive how the noted rise in the fractional excretion of sodium from approximately .5% to 40 or 50% in chronic renal disease is the result of physical factors.

Osmotic Diuresis per Nephron

It could be hypothesized that an increased osmotic diuresis per nephron could account for the increased sodium excretion per nephron in reduced renal mass. Two studies appear to rule out this hypothesis. Schultze et al. (1966) studied animals within 24 hours of nephrectomy - an interval too short to allow for appreciable retention of poorly reabsorbed solutes. The increase in sodium excretion rates and the decrease in fractional sodium reabsorption were present within 14 hours after nephrectomy. Schultze et al. (1966) studied animals maintained on a low salt diet. After completion of the control study, the normal kidney

was removed and sufficient time was allowed for uremia to develop. During this post-nephrectomy time, the filtered load of impermanent solutes increased substantially yet sodium excretion increased only slightly and the fractional sodium reabsorption fell by less than one percent. That is, when dietary sodium intake was restricted, the solute diuresis of uremia did not evoke an appreciable natriuretic response. Thus, a major role of solute diuresis in the genesis of the noted natriuresis seems unlikely.

Natriuretic Hormone

There is an increasing amount of evidence that some type or types of natriuretic hormone may serve as a modulator of sodium excretion in chronic renal disease. Homer Smith in 1936 first proposed the existence of a natriuretic substance modulating sodium excretion in uremia. However, it was not until 1968 that Bricker et al. first reported the existence of a natriuretic substance present in serum fractions from uremic patients. This humoral substance was found to be a low molecular weight substance which inhibits the uptake of para-amino hippurate (PAH) by rabbit kidney cortical slices in vitro. PAH uptake by kidney slices is a sodium dependent process inferring the presence of an inhibitor of sodium transport in uremic serum.

Again in 1970, Bricker et al. reported the existence of a humoral natriuretic substance present in uremic serum

fractions of patients. This humoral substance was found to decrease short circuit current of an isolated toad bladder when it was added to the serosal surface of the chamber, suggesting that sodium transport was inhibited. Interestingly, serum fractions from patients with acute renal failure failed to show the existence of this substance yet these patients are still able to maintain sodium balance. This suggests that some other natriuretic substance must be involved in the regulation of sodium excretion in this instance.

Bourgoignie et al. (1971) additionally found that the same fraction could decrease short circuit current of frog skin when added to the serosal side, again suggesting that sodium transport was inhibited. This study and the previously mentioned two studies suggest that an inhibitor of sodium transport exists in the serum of patients with chronic uremia and there exists a potential role of this material in the regulation of sodium excretion in uremia.

The next experiment by Bourgoignie et al. (1974) was designed to examine whether or not that same serum fraction, which inhibited PAH uptake in rabbit cortical kidney slices and inhibited short circuit current in frog skin and toad bladder, could elicit a natriuretic response when injected intravenously into an assay rat with a reduced nephron population. The rationale for using such an assay animal was that if the circulating inhibitor in the uremic patient is a natriuretic hormone, the assay animal

should have an increased endogenous concentration of that factor. Thus, the response to additional circulating inhibitor should be decreased. However, there is evidence which suggests that the sensitivity of the control system governing sodium excretion increases as nephron population decreases (Slatopolsky et al. 1968). That is, the smaller the number of nephrons contributing to sodium excretion, the greater the response of each nephron to a given load of sodium. Thus, an increased responsiveness could be an enhanced sensitivity of the nephrons to a circulating natriuretic factor. The result of injecting such a fraction into an assay rat was an increase in sodium excretion and fractional sodium excretion of the normal serum fractions. The uremic serum fractions, on the other hand, produced a substantially and significantly greater increase in both functional parameters. The natriuresis in the rat could not be explained by the associated changes in GFR, PAH clearance, filtration fraction hematocrit or blood pressure. The authors stated that the fraction which inhibited sodium reabsorption is identical to the inhibitor of PAH uptake by kidney slices and the inhibitor of transepithelial sodium transport in frog skin and toad bladder.

Natriuretic hormone has been found in the urine. Bourgoignie et al. (1972) again found the same natriuretic fraction noted in plasma, in the urine of patients with chronic uremia. When the urine fraction from uremic

patients was injected into similarly prepared bioassay rats, a marked increase in sodium excretion and fractional sodium excretion occurred as compared to normal urine fractions.

The presence of a natriuretic substance in uremia is not caused by its retention in the plasma from failure of excretion, but rather depends on the salt intake of the animal. A study by Schmidt et al. (1974) compared two groups of animals: one group maintained on a constant salt intake; the other group subjected to a proportional reduction of sodium intake which involved the reduction of sodium intake in exact proportion to the decline in GFR. Serum fractions were obtained in both groups. It was found that the serum fractions from the animals in the group which received a constant salt intake produced a significant increase in both sodium excretion and fractional sodium excretion when injected into an assay rat, whereas the same serum fraction from animals maintained on the proportional reduction of sodium failed to produce a significant increase in sodium excretion or fractional sodium excretion. Thus, the increased rate of sodium excretion per nephron in chronic uremia is due to a physiological adaptation as fractional sodium excretion per nephron differed markedly between the two groups of animals. Furthermore, the circulating natriuretic factor plays a role in mediating the natriuretic response as there is a demonstrable difference in the natriuretic response

between the two groups, both of which are equally uremic, one with a high, the other with a low rate of sodium excretion per nephron.

Hypertension in Chronic Renal Disease

Throughout the course of renal disease, abnormalities of the cardiovascular system occur with hypertension accounting for the predominant malady (Lazarus et al. 1974). Several mechanisms have been suggested as the underlying etiology of hypertension in patients with renal failure. The more common include expansion of the extracellular fluid volume, altered activity of the renin-angiotensin system and hyperactivity of the sympathetic nervous system activity.

Extracellular Fluid Volume Expansion

Expansion of the extracellular fluid volume secondarily to sodium retention has been implicated as the most important factor leading to the development of hypertension (Bricker 1982). Blumberg et al. (1967) found that patients with chronic renal failure exhibit an increase in total body sodium and water. Wilkinson et al. (1970) found a significant correlation between an increase in total body sodium and diastolic pressure. Vertes et al (1969) studied 40 hypertensive patients with chronic renal failure and found that dialysis to remove excess fluid reduced arterial pressure to normal in 35 individuals. Thus, volume expansion is the principal factor determining hypertension in most patients with renal failure.

The exact mechanism by which increased extracellular fluid volume produces hypertension is unclear. Mean arterial pressure can increase due to an increase in cardiac output or an increase in total peripheral resistance.

Kim et al. (1975) found that cardiac index was similar in normotensive and hypertensive uremic patients, but higher than normal individuals. The elevation in cardiac index appeared to be related to anemia, a common occurrence in chronic renal failure, as correction of the anemia decreased cardiac index and produced further increments in arterial pressure.

Frohlich et al. (1969) found that hypertensive uremic patients have an increased peripheral resistance as compared to normotensive uremic patients or normotensive healthy controls. The precise mechanism by which volume expansion increases total peripheral resistance is uncertain, but several hypotheses have been put forth.

The first hypothesis, proposed by Guyton et al. (1980), was termed whole body auto regulation. They proposed that an acute increase in salt and water retention increases extracellular fluid volume, blood volume, and cardiac filling pressure. An increased cardiac output would result in an initial rise in arterial pressure. If volume expansion was maintained, total peripheral vasoconstriction would occur in order to return cardiac output to lower levels. They presumed that vasoconstriction was an adaptive

autoregulatory response to decrease tissue perfusion back towards normal. Thus in the acute phase, hypertension was maintained by an elevation in cardiac output, whereas in the chronic phase, hypertension was maintained by an increased peripheral resistance. Evidence favoring this hypothesis was obtained by Coleman et al (1970) in anephric patients. However, Kim et al. (1980) were not able to confirm the findings of Coleman et al. (1970).

Another hypothesis to explain how increased extracellular fluid volume could increase total peripheral resistance and lead to the development and maintenance of hypertension was put forth by Borst et al. (1963). They hypothesized that the diseased kidneys have a limited ability to excrete the daily sodium load resulting in an increase in the extracellular fluid volume and hypertension. The increase in arterial pressure is speculated to increase GFR and decrease proximal tubular sodium reabsorption so that sodium balance is achieved. Sodium balance can be achieved, but at the expense of elevated arterial pressure.

An increased reactivity of vascular smooth muscle cells was another hypothesis first suggested by Tobian et al. (1952) to account for the increased total peripheral resistance. Several suggestions have been put forth to explain how increased vascular reactivity might occur. DeChamplain et al. (1968) demonstrated that sodium loading facilitates sympathetic nervous transmission and the

release of norepinephrine from adrenergic granules which in turn could increase vascular reactivity.

"Natriuretic hormone" has been implicated as a possible cause for an increase in vascular reactivity. This hormone, speculated to be produced in the brain, has been shown to exist in response to volume expansion (Kaloyanides et al. 1971). Natriuretic hormone has been shown to inhibit sodium reabsorption in the distal tubule (Clarkson et al. 1972) via inhibition of the sodium potassium ATPase (Poston et al. 1981). Natriuretic hormone has been shown to affect other transmembrane transport processes and has been thought to explain the increase in intracellular sodium content of muscle (Bilbrey et al. 1973), red blood cells (Cole et al. 1973), and leukocytes (Edmonson et al. 1973) in patients with uremia. These cells show a decrease in sodium efflux and ouabain sensitive ATPase activity (Cole et al. 1968). The sodium-potassium ATPase is coupled with the sodium-calcium exchange system in muscle cells, and an inhibition of the former system has been shown to change intracellular electrolyte concentrations raising basal tone and contractility (Sweadner et al. 1980). Thus, an increase in contractility of smooth muscles of the arterioles due to an increase in natriuretic hormone could explain the increased peripheral resistance (Blaustein et al. 1977) Evidence in support of this hypothesis comes from observations that hemodialysis reduces intracellular sodium and lowers arterial pressure (Cole et al. 1968).

Altered Activity of the Renin-Angiotensin System

Increased activity of the renin-angiotensin system has been implicated in the developement of hypertension. In the study of Vertes et al (1969), it was stated that of the 40 patients with hypertension, 35 had normal arterial pressures after dialysis. The remaining 5 patients remained hypertensive despite hemodialysis. In these 5 patients, plasma renin activity was 10 times higher than in the volume responsive group. Thus, an alteration in the activity of the renin-angiotensin system occurs in the maintenance of hypertension.

Total nephrectomy was an early treatment of hypertension not corrected by dialysis. DelGreco et al. (1975) performed bilateral nephrectomies in 145 hypertensive patients. Hypertension improved dramatically in 115 (79%) and remained unchanged in 30 (21%). Hypertension was corrected in 74 of 79 patients with high plasma renin activity and in only 13 of 29 patients with normal plasma renin activity. Renin was not measured in the remaining 17. Thus, the kidney was shown to contribute to hypertension in approximately 80% of the patients and nephrectomy had an effect even in patients without hyperreninemia. Kim et al. (1975) studied severely hypertensive patients and measured exchangeable sodium both before and after nephrectomy. The fall in arterial pressure was attributed to a decrease in peripheral resistance since cardiac index remained constant. Bianchi et al. (1972)

studied 5 patients whose hypertension was controlled by fluid removal with dialysis and low salt intake. Bilateral nephrectomy decreased diastolic pressure further without producing any change in extracellular volume or exchangeable sodium. This study demonstrates an important role for the renal pressor system not only in patients with uncontrollable hypertension but also those who respond to volume contraction.

The renin-angiotensin system preserves its functional integrity in renal failure even in the most advanced stages (Leehen et al. 1977). Patients appear to respond to sodium intake by altering plasma renin activity (Kahn et al. 1975), and the normal response of plasma renin activity to orthostasis was found to be well preserved (Leehen et al. 1977). Fadem et al. (1979) showed that blocking of angiotensin II by saralasin was associated with increased renin secretion.

Although the renin angiotensin system preserves its function, an abnormal relationship between renin and volume in hypertension of chronic renal failure exists. Warren et al. (1970) studied the response of plasma renin activity to volume changes in hypertensive patients with chronic renal failure. They concluded that renin secretion was inappropriate for the volume status because saline infusion failed to suppress renin whereas in control subjects, a smaller fluid load completely inhibited renin secretion. Weidman et al. (1976) demonstrated an inverse correlation

between plasma renin activity and exchangeable sodium for both normotensive and hypertensive patients with chronic renal failure. Arterial pressure correlated significantly with the product of exchangeable sodium times the log of plasma renin activity, and the duration of the pervious hypertension.

The angiotensin antagonist, saralasin, and the use of the converting enzyme inhibitor, captopril, has been shown to diagnose renin dependent hypertension (Wilson et al. 1977). Results obtained with angiotensin antagonists suggest that renin does contribute to hypertension in some patients with chronic renal disease, such as those with high renin activity. Results obtained with converting enzyme inhibitor suggest that blocking the renin-angiotensin system has a more important antihypertensive effect than does correcting volume expansion. This was concluded by Acosta et al. (1982) who evaluated the relative participation of volume expansion and inappropriate renin secretion in the hypertension of chronic renal failure by comparing the effect of volume contraction with that of converting enzyme inhibition. After a control period, volume depletion was induced by hemodialysis which was followed a few days later by captopril administration. The investigators found that volume depletion did not significantly change blood pressure in the patients they studied. However, captopril administration reduced arterial pressure to the normal

range in both groups and increased plasma renin activity further. The authors concluded blocking the renin angiotensin system has a far more potent antihypertensive effect than does correcting volume expansion.

Excess aldosterone elevates arterial pressure in normal patients due to salt and water retention. Thus, it is possible that excess aldosterone secretion participates in the generation and maintenance of hypertension in chronic renal failure (Berl et al. 1978). Evidence favoring this hypothesis is that salt and water retention occur in patients with renal failure. However, tubular reabsorption of sodium is decreased, not increased, and Vetter et al. (1976) found that plasma aldosterone levels are actually decreased in patients with renal failure and hypertension .

Hyperactivity of the Sympathetic Nervous System

The sympathetic nervous system has also been implicated as the cause of hypertension in chronic renal failure. Elevated plasma concentration of catecholamines has been reported in patients with uremia and hypertension (Atuk et al. 1975). Baroreceptor activity in hypertensive uremic patients was found to be altered, compared to normal patients (Lazarus et al. 1973). The decreased sensitivity was attributed to impaired autonomic nervous system activity. This and an elevation in plasma catecholamines could lead to an increased peripheral vasoconstriction and hence hypertension.

MATERIALS AND METHODS

Animal Anesthesia

All surgical procedures were performed under either halothane (5% induction followed by a maintenance dose of 2%) or sodium pentobarbital (50 mg/kg) anesthesia. The plane of anesthesia was assessed by periodic testing of both the pupillary reflex and the paw pinch reflex.

Animal Surgery and Experimental Protocol

Male Sprague Dawley rats weighing approximately 280 to 350 grams were used in the study. They appeared to be in excellent health. The first surgical procedure was the first part of a two stage reduction of renal mass (RRM). The fist stage involved partial removal of the rat's left kidney. This procedure involved making a left flank incision of approximately 3 cm in length through the skin and underlying muscle tissue in order to expose the kidney. The fat around the kidney was removed as was the capsule. The renal artery and vein were exposed and the nearby tissue dissected away so that both could be clamped off with a small bulldog clamp. Once the renal vessels were clamped off, both poles of the kidney were excised with

a scissors. The total amount of tissue removed from the kidney was approximately 0.5 gram. of kidney wet weight or 2/3 of the kidney. The exposed poles were then cauterized to prevent bleeding when the clamp on the renal artery and vein was removed. Cauterizing both ends had the effect of rendering more renal tissue non-functional. The clamp was removed and the ends of the kidney checked for additional bleeding. The kidney was clamped off for no longer than 3 minutes. The kidney was then placed back inside the abdominal cavity, the muscle layer sutured up using 000 silk suture followed by suturing up the skin with 00 silk suture. The animal was placed back in its cage to recover. Some rats underwent a sham operation which included clamping the renal vessels but they did not have any renal tissue removed or destroyed.

Five days after the operation to partially reduce renal mass, the rats had catheters placed in their femoral artery and femoral vein. The femoral artery catheter was constructed of Tygon tubing, the end of which was attached to 2 to 3 cm of silastic tubing to be inserted into the femoral artery. The femoral vein catheter was constructed of Tygon tubing, but of a different diameter, again with 2 to 3 cm of silastic tubing on the end which was inserted into the femoral vein. After catheterization of the vessels the catheters were tunneled under the skin to exit on top of the head. The catheters were then encased in a metal spring which protected them from being chewed by the rats.

The metal spring was fixed to the rat with dental cement on top of the rat's skull. The dental cement was held to the skull by placing two screws on the upper surface of the skull. The other end of the metal spring was attached to a swivel. The swivel was constructed of plastic syringe parts, stainless steel tubing, and 16 gauge hypodermic needles. The swivel enabled the rat to turn around in its cage with out becoming tangled while maintaining a leak proof route of infusion into the animal. The initial infusion solution was a saline solution infused at a rate of 3.93 ml per day to give a total of 1 mEq of sodium per day. The swivel was located at the top of the metabolism cage. The metabolism cage enabled the measurement of water intake and urine volume. Inside the cage, the rat had free access to sodium free food and distilled water.

The animals were allowed two days to recover from the catheter surgery before the first control measurements were obtained (C1). These included the daily measurements of water intake and urine volume, which continued for the remainder of the experiment. A sample of the urine was collected from each urine collection container and frozen for subsequent analysis of sodium and potassium. Heart rate and mean arterial pressure were recorded by a Statham transducer attached to a Gould polygraph. This measurement was made every other day and continued until the end of the experiment. On the third control day (C3), an arterial sample of approximately 800 ul was obtained in a cooled

heparinized syringe for the measurement of plasma atrial natriuretic peptide, plasma sodium, plasma potassium, blood urea nitrogen and plasma osmolality.

The following day the other kidney was removed which marked the first experimental day (E1). The other kidney, the right kidney, was removed by the following procedure. Again under halothane or pentobarbital anesthesia, a mid-line abdominal incision was made from the tip of the sternum, down the skin to 1 cm above the pubis. The underlying muscle was cut down the linea alba, and separated to expose the right kidney. The tissue around the kidney was dissected away and a ligature was placed around both the renal artery and renal vein. The ligature was tied tightly and the kidney was excised. Thus, the rats underwent a total of 5/6 nephrectomy. The muscle layer was sutured followed by the closure of the skin. The sham operated rats under went the same procedure except that the kidney was not removed. The following experimental day (E2) another blood sample of approximately 800 ul was obtained in the fashion previously mentioned. Blood samples were additionally obtained on the fourth and sixth experimental day (E4 and E6 respectively).

On the seventh experimental day, both the sham operated and the nephrectomized rats were divided into two groups; thus, there were four groups in all. One sham group and one nephrectomized group continued to receive 1 mEq of sodium per day while one sham group and one nephrectomized

group were switched to 6 mEq of sodium per day. These four groups are designated 1 mEq Sham, 1 mEq RRM, 6 mEq Sham and 6 mEq RRM. Blood samples were additionally obtained on the tenth and fourteenth day of the experiment (E10 and E14 respectively). The fourteenth day of the experiment was the last experimental day, and the animals were sacrificed after data collection.

Procedure for Measurement of Plasma Atrial Natriuretic Peptide

Determination of rat plasma ANP was carried out utilizing a radioimmunoassay. The antisera, unlabeled antigen (standards) and labeled antigen were obtained from a commercially available kit generously donated by the Upjohn Company. The antisera in the kit contained both Rat Atriopeptin III antisera, which was raised against purified synthetic Rat Atriopeptin III in rabbits, and goat anti-rabbit sera. The kit additionally contained unlabeled antigen (Rat Atriopeptin III) used for standards, as well as labeled antigen (I^{125}-Rat Atriopeptin III).

A non-equlibrium method of incubation was used. Antisera and either unlabeled antigen (standards) or samples were incubated at 4°C for 15 hours, after which iodinated Atriopeptin III was added to the mixture. The testube, containing antisera, labeled and unlabeled antigen, was incubated at 4°C for another 4 hours. The antibody-antigen complex then formed a precipitate which

separated the bound peptide from the unbound peptide. Two milliliters of saline were added to each tube before centrifugation at 2000g for 20 minutes. The supernatant was decanted by batch method and the pellet counted in a gamma counter. A standard curve was generated by holding a fixed concentration of antisera and labeled Atriopeptin III, while increasing the concentration of unlabeled antigen. ANP in an experimental sample was quantified by comparing its displacement of the labeled Atriopeptin III with the standard curve. Standards were run with each assay. The sensitivity of the assay is approximately 30 fmoles/ml and our 50% binding point is approximately 280 fmoles/ml, with an inter-assay variation of less than 10%.

Determination of Plasma Blood Urea Nitrogen

Plasma blood urea nitrogen (BUN) was determined by the modified method of Fawcett and Scott (1960). Briefly, 0.5 ml of urease was added to 10 ul of plasma. The enzyme was allowed to hydrolyze the urea to NH_3 for 30 minutes. After this time 0.5 ml of Phenol and 0.5 ml of Sodium Nitroprusside were added to each tube where they were allowed to react for another 30 minutes. After this time the tubes were vortexed, the solution placed in plastic cuvettes, and read in a spectrophotometer at 320 nm. Appropriate standards were run with each assay.

Analysis of Plasma Sodium and Potassium

Plasma sodium and potassium were measured utilizing either an ion electrode (Beckman Ion Electrode) specific for sodium or a flame photometer (Instrumentation Laboratory). Both machines were calibrated before use using standards manufactured by either Instrumentation laboratory or Beckman for the flame photometer and ion electrode respectively. All plasma samples were usually run in duplicate.

Analysis of Plasma Osmolality

Plasma osmolality was measured by freezing point depression using a Beckman osmometer. The machine was calibrated using standards obtained from the company. All samples were run in duplicate and often a third or fourth time if the initial osmolality readings differed by more than 3 mosmoles.

Analysis of Urine Sodium and Potassium

Urine for sodium and potassium was stored frozen until ready for analysis. On the day of analysis, it was thawed, and then centrifuged to remove particles in suspension. Urine sodium and potassium were measured using a flame photometer (Instrumentation Laboratory) or an ion electrode (Beckman). Again the machine was calibrated before each use with pre-made standards and all samples were run in duplicate.

Statistical Analysis

Data were entered into an IBM PC computer using the data handling software package Lotus and then uploaded to an IBM 4381 ("Mainframe") computer. Statistical analysis of the data was performed using the statistical software package, Statistical Analysis System (SAS). A one-way ANOVA with 4 levels was used to calculate significant F values at the .05 level. Differences among the groups were tested for significance with the Student-Neuman-Keuls test.

RESULTS

Data are presented for 4 groups of rats: 1) 8 sham operated rats maintained on 1 mEq of sodium per day the entire experimental period ("1 mEq Sham"); 2) 9 rats with a reduction of renal mass maintained on 1 mEq of sodium per day the entire experimental period ("1 mEq RRM"); 3) 12 sham operated rats switched from 1 mEq of sodium per day to 6 mEq of sodium per day at the end of day E7 ("6 mEq Sham"); and 4) 8 rats with a reduction of renal mass switched from 1 mEq of sodium per day to 6 mEq of sodium per day at the end of day E7 ("6 mEq RRM").

Blood Urea Nitrogen

Blood urea nitrogen (BUN) measurements for days C3, E2, E4, E6, E10, and E14 are presented in table 1 and plotted in figure 1. On day C3, BUN in the 1 mEq RRM group, which measured 24 mg%, was significantly elevated with respect to the 1 mEq Sham group, which measured 19 mg%, the 6 mEq RRM group, which measured 17 mg%, and the 6 mEq Sham group, which measured 16 mg%. None of the other group comparisons were found to be significantly different from each other on day C3.

BUN from days E2 through E14 was found to be significantly elevated in both the 1 mEq RRM group and the 6 mEq RRM group, compared to both the 1 mEq Sham group and the 6 mEq Sham group. There were no significant differences in BUN between the 1 mEq Sham group and the 6 mEq Sham group from day E2 through E14. The means of these two groups averaged 19 mg%, 18 mg%, 18 mg%, 17 mg%, and 17 mg% for days E2, E4, E6, E10 and E14 respectively. There were no significant differences in BUN between the 1 mEq RRM group and the 6 mEq RRM group from day E2 through E14. The means of these two groups averaged 46, 49 mg%, 50 mg%, 56 mg%, and 58 mg% for days E2, E4, E6, E10 and E14 respectively.

Plasma Sodium

Plasma sodium measurements for days C3, E2, E4, E6, E10 and E14 are presented in table 2 and plotted in figure 2. Although significant differences were noted from day to day, none of the experimental values were outside the physiological range for plasma sodium and there did not appear to be any systematic relationship to RRM or sodium intake. The means of the 4 groups from day C3 through E14 averaged 142 mEq/l, 142 mEq/l, 143 mEq/l, 143 mEq/l, 144 mEq/l and 144 mEq/l respectively.

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Plasma Potassium

Plasma potassium measurements for days C3, E2, E4, E6, E10 and E14 are presented in table 3 and plotted in figure 3. There were no significant differences in plasma potassium among groups from day C1 through E14, except on day E2. The means of the 4 non-significantly different groups averaged 4.6 mEq/l, 4.7 mEq/l, 4.9 mEq/l, 4.7 mEq/l and 4.9 mEq/l on days C1, E4, E6, E10 and E14 respectively. On day E2, plasma potassium was noted to be significantly elevated in the 6 mEq Sham group measuring 5.2 mEq/l as compared to the 6 mEq RRM group, which measured 4.5 mEq/l. Both the 1 mEq Sham group and 1 mEq RRM group were not significantly different measuring 4.6 mEq/l and 4.8 mEq/l respectively from the 6 mEq Sham group. All other group comparisons were not significantly different from each other.

Plasma Osmolality

Plasma osmolality measurements for days C3, E2, E4, E6, E10 and E14 are presented in table 4 and plotted in figure 4.

On day C3, plasma osmolality was significantly elevated in the 1 mEq RRM group, measuring 303 mosmoles/kg, as compared to the 6 mEq Sham group, which measured 288 mosmoles/kg, and the 6 mEq RRM group, which measured 286 mosmoles/kg. The 1 mEq Sham group, measuring 293 mosmoles/kg, was found not to be significantly different

from the the 1 mEq RRM group. All other group comparisons were found to be not significant.

On day E2, plasma osmolality was significantly elevated in the 1 mEq RRM group, measuring 310 mosmoles/kg, as compared to the 1 mEq Sham group, which measured 294 mosmoles/kg and the 6 mEq Sham group, which measured 288 mosmoles/kg. The 6 mEq RRM group, which measured 306 mosmoles/kg was significantly elevated from the 6 mEq Sham group. All other group comparisons were not significantly different.

On day E4, plasma osmolality in the 1 mEq RRM group, measuring 311 mosmoles/kg, was significantly elevated from the 1 mEq Sham group, which measured 295 mosmoles/kg and the 6 mEq Sham group, which measured 285 mosmoles/kg. The 6 mEq RRM group, measuring 310 mosmoles/kg, was significantly elevated from the both the 1 mEq Sham group and the 6 mEq Sham group. All other group comparisons were not significantly different.

On day E6, plasma osmolality was significantly elevated in the 1 mEq RRM group, measuring 320 mosmoles/kg, as compared to the 6 mEq RRM group, which measured 304 mosmoles/kg, the 1 mEq Sham group, which measured 304 mosmoles/kg, and the 6 mEq Sham group, which measured 288 mosmoles/kg. All other group comparisons were not significantly different.

On day E10, the 1 mEq RRM group, measuring 327 mosmoles/kg was significantly elevated from the 1 mEq Sham

group, which measured 304 molmoles/kg, and the 6 mEq Sham group, which measured 288 mosmoles/kg. Both the 6 mEq RRM group, measuring 313 mosmoles/kg, and the 1 mEq Sham group were significantly elevated from the 6 mEq Sham group. All other group comparisons were found to be not significant.

On day E14, the 1 mEq RRM group, measuring 328 mosmoles/kg was found to be significantly higher than the 1 mEq Sham group, which measured 300 mosmoles/kg, and the 6 mEq Sham group, which measured 290 mosmoles/kg. All other group comparisons were not significantly different.

Mean Arterial Pressure

Mean arterial pressure measurements for days C1, C3, E2, E4, E6, E8, E10, E12 and E14 are presented in table 5 and plotted in figure 5. There were no significant differences in mean arterial pressure among groups from day C1 through E2. The means of the 4 groups averaged 112 mmHg, 106 mmHg and 110 mmHg on days C1, C3, and E2 respectively.

On day E4, the 1mEq Sham and the 6 mEq RRM measured 111 mmHg and 113 mmHg respectively. The 1 mEq RRM group was significantly elevated to 119 mmHg as compared to the 6 mEq Sham group, which measured 108 mmHg. All other comparisons were not significantly different from each other.

On day E6, none of the groups were significantly different from each other. The means of the 4 groups averaged 114 mmHg.

On day E8, the 6 mEq RRM group, measuring 135 mmHg, was significantly elevated from the 6 mEq Sham group, measuring 112 mmHg, and the 1 mEq Sham group, measuring 103 mmHg. The 1 mEq RRM, measuring 124 mmHg, group was significantly elevated from the 1 mEq Sham group. All other group comparisons were found to be not significant.

On day E10, the 6 mEq RRM group, measuring 137 mmHg, was significantly different from the 6 mEq Sham group, measuring 114 mmHg, and the 1 mEq Sham group, measuring 107 mmHg. The 1 mEq RRM group measured 122 mmHg and was not significantly different from the 6 mEq group. All other comparisons were not significant.

On day E12, the 6 mEq RRM group, measuring 152 mmHg, was significantly different from the 1 mEq RRM group, which measured 123 mmHg, the 6 mEq Sham group, which measured 117 mmHg, and the 1 mEq Sham which measured 102 mmHg. The 1 mEq RRM group was found to be significantly different from the 1 mEq Sham operated group. All other group comparisons were not significant.

On day E14, the 6 mEq RRM group, measuring 149 mmHg, was significantly elevated above all groups. The 1 mEq Sham group measured 108 mmHg, the 6 mEq Sham group measured 118 mmHg, and the 1 mEq RRM group measured 124 mmHg. All other group comparisons were not significant.

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Heart Rate

Heart rate measurements for days C1, C3, E2, E4, E6, E8, E10, E12 and E14 are presented in table 6 and plotted in figure 6. There were no significant differences in heart rate among groups from day C1 through E10 and E14, and the means of the 4 groups averaged 381 beats/min, 399 beats/min, 422 beats/min, 413 beats/min, 403 beats/min, 395 beats/min, 399 beats/min, and 399 beats/min respectively. On day E12, heart rate was found to be significantly different in the 6 mEq Sham group, measuring 413 beats/min, as compared to the 1 mEq Sham group, which measured 360 beats/min. The 1 mEq RRM group, measuring 368 beats/min, and the 6 mEq RRM group, measuring 403 beats/min, was found not to be significantly different from the 6 mEq RRM group or the 1 mEq Sham group. All other group comparisons were found not to be significantly different.

Water Intake

Water intake for days C1 through C3, and E1 through E14 are presented in table 7 and plotted in figure 7. There were no significant differences in water intake among groups from day C1 through E1. The means of the 4 groups averaged 32 ml/day, 31 ml/day, 26 ml/day and 24 ml/day on days C1, C2, C3 and E1 respectively.

On day E2, water intake was significantly elevated in the 6 mEq RRM group, measuring 44 ml/day, as compared to the 1 mEq RRM group, which measured 31 ml/day, the 6 mEq

Sham group, which measured 27 ml/day and the 1 mEq Sham group, which measured 22 ml/day. All other group comparisons were not significantly different.

On day E3, water intake was significantly elevated in the 6 mEq RRM group, measuring 47 ml/day, as compared to the 1 mEq RRM group, which measured 34 ml/day, the 6 mEq Sham group, which measured 29 ml/day, and the 1 mEq Sham group, which measured 20 ml/day. The 1 mEq RRM group was also found to be significantly different from the the 1 mEq Sham group. All other group comparisons were found to be not significant.

On day E4, water intake was significantly elevated in the 6 mEq RRM group, measuring 46 ml/day, as compared to the 1 mEq RRM group, which measured 34 ml/day, the 6 mEq Sham group, which measured 31 ml/day, and the 1 mEq Sham group, which measured 21 ml/day. Both the 1 mEq RRM group and the 6 mEq Sham group was found to be significantly different from the the 1 mEq Sham group All other group comparisons were found to be not significant.

On day E5, water intake was significantly elevated in the 6 mEq RRM group, measuring 42 ml/day, as compared to the 6 mEq Sham group, which measured 27 ml/day, and the 1 mEq Sham group, which measured 22 ml/day. The 1 mEq RRM group, measuring 32 ml/day, was found not to be significantly different from the the 6 mEq RRM group. All other group comparisons were found to be not significant.

On day E6, water intake was significantly elevated in

the 6 mEq RRM group, measuring 42 ml/day, as compared to the 1 mEq Sham group, which measured 21 ml/day. The 1 mEq RRM group, which measured 28 ml/day was significantly elevated from the 1 mEq Sham group. The 6 mEq Sham group, measuring 31 ml/day was not significantly different from any of the groups. All other group comparisons were not significantly different.

On day E7, water intake was significantly elevated in the 6 mEq RRM group, measuring 44 ml/day, as compared to the 6 mEq Sham group, which measured 29 ml/day and the 1 mEq Sham group, which measured 25 ml/day. The 1 mEq RRM group, which measured 38 ml/day was not significantly different from any of the groups. All other group comparisons were not significantly different.

On day E8, water intake was significantly elevated in the 6 mEq RRM group, measuring 58 ml/day, as compared to the 6 mEq Sham group, which measured 42 ml/day, the 1 mEq RRM group, which measured 35 ml/day, and the 1 mEq Sham group, which measured 22 ml/day. Both the 6 mEq Sham group and the 1 mEq RRM group was significantly different from the 1 mEq Sham group. All other group comparisons were not significantly different.

On day E9, water intake was significantly elevated in the 6 mEq RRM group, measuring 64 ml/day, as compared to the 6 mEq Sham group, which measured 41 ml/day, the 1 mEq RRM group, which measured 34 ml/day, and the 1 mEq Sham group, which measured 25 ml/day. The 6 mEq Sham group was

significantly different from the 1 mEq Sham group. All other group comparisons were not significantly different.

On day E10, water intake was significantly elevated in the 6 mEq RRM group, measuring 74 ml/day, as compared to the 6 mEq Sham group, which measured 43 ml/day, the 1 mEq RRM group, which measured 37 ml/day, and the 1 mEq Sham group, which measured 27 ml/day. All other group comparisons were not significantly different.

On day E11, water intake was significantly elevated in the 6 mEq RRM group, measuring 69 ml/day, as compared to the 6 mEq Sham group, which measured 41 ml/day, the 1 mEq RRM group, which measured 38 ml/day, and the 1 mEq Sham group, which measured 24 ml/day. Both the 6 mEq Sham group and the 1 mEq RRM group was significantly different from the 1 mEq Sham group. All other group comparisons were not significantly different.

The same patterns of significance were noted on days E12 through E14 as in day E11. Only the absolute numbers on each day were different. These values can be found in table 7.

Sodium Balance Ratio

The sodium balance ratio for days C1 through C3, and E1 through E14 are presented in table 8 and plotted in figure 8. There were no significant differences in sodium balance among groups from day C1 through E1 and the means of the 4 groups averaged .56, .78, .87 and .89

respectively.

On day E2, the 6 mEq RRM group, calculated to be 1.30, was found to be significantly elevated from the 1 mEq RRM group, which was calculated to be .92 and the 1 mEq Sham group, which was calculated to be .60, but was not significantly different from the 6 mEq Sham group, which was calculated to be 1.20. Both the 6 mEq Sham group and the 1 mEq RRM group was found to be significantly different from the 1 mEq Sham group. All other group comparisons were found to be not significant.

On days E3 through E7, there were no significant differences in sodium balance among groups. The means of the 4 groups averaged .87, .87, 1.04, 1.06 and .98 on days E3, E4, E5, E6 and E7 respectively.

On day E8, the 6 mEq Sham group, calculated to be .72, and the 6 mEq RRM group, calculated to be .68, was noted to be significantly lower than the 1 mEq Sham group, which was calculated to be .97, and the 1 mEq RRM group, which was calculated to be 1.01. All other group comparisons were found to be not significant.

On days E9 and E10, there were no significant differences in sodium balance among groups. The means of the 4 groups averaged .95, and .89 on days E9 and E10 respectively.

On day E11, the 1 mEq Sham group was found to be significantly different from the 1 mEq RRM group, which was calculated to be .96, the 6 mEq Sham group, which was

calculated to be .97, and the 6 mEq RRM group, which was calculated to be .99. All other group comparisons were found to be not significantly different from each other.

On days E12 through E14, there were no significant differences in sodium balance among groups. The means of the 4 groups averaged .96, .87 and .88 on days E12, E13 and E14 respectively.

Plasma Atrial Natriuretic Peptide

Plasma atrial natriuretic peptide (ANP) measurements for days C3, E2, E4, E6, E10 and E14 are presented in table 9 and plotted in figure 9.

On day C3, ANP was found to be significantly reduced in the 6 mEq RRM group, measuring 156 fmoles/ml, as compared to the 1 mEq Sham group, which measured 301 fmoles/ml. The 1 mEq RRM group, measuring 236 fmoles/ml, and the 6 mEq Sham group, measuring 215 fmoles/ml, were not significantly different from each other or any other group. All other group comparisons were not significantly different.

On day E2, there were no significant differences in ANP among groups the mean of the 4 groups averaged 230 fmoles/ml.

On day E4, ANP was found to be significantly reduced in the 6 mEq RRM group, measuring 189 fmoles/ml, as compared to the 1 mEq Sham group, which measured 279 fmoles/ml. The 1 mEq RRM group, measuring 241 fmoles/ml,

and the 6 mEq Sham group, measuring 239 fmoles/ml, were not significantly different from each other or any other group. All other group comparisons were not significantly different.

On day E6, there was no significant differences in ANP among groups and the mean of the 4 groups averaged 230 fmoles/ml.

On day E10, ANP was again found to significantly elevated in the 6 mEq RRM group, measuring 417 fmoles/ml, as compared to the 1 mEq Sham group, which measured 241 fmoles/ml, the 1 mEq RRM group, which measured 221 fmoles/ml, and the 6 mEq Sham group, which measured 287 fmoles/ml. All other group comparisons were not significantly different.

On day E14, ANP was again found to significantly elevated in the 6 mEq RRM group, measuring 444 fmoles/ml, as compared to the 1 mEq Sham group, which measured 282 fmoles/ml, the 1 mEq RRM group, which measured 255 fmoles/ml, and the 6 mEq Sham group, which measured 287 fmoles/ml. All other group comparisons were not significantly different.

Correlation Data

Atrial natriuretic peptide and mean arterial pressure were correlated. The correlation coefficient for plasma ANP and MAP is $r = 0.800$. The equation for the regression of ANP on MAP is $y = 5.66x - 48.175$. ANP and water intake were

also correlated. The correlation coefficient for plasma ANP and water intake is r = 0.751. The equation for the regression of ANP on water intake is y = 5.936x -36.986. Finally, MAP and water intake were correlated. The correlation coefficient for MAP and water intake is r = 0.630. The equation for the regression of MAP on water intake is y = .7036x + 11.722.

TABLE 1

Blood Urea Nitrogen (mg%)

		Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 10	Expt 14
1 mEq Sham	$\bar{X}$	19.59 +	20.11	20.49	19.75	18.70	18.29
	SEM	1.56	1.68	1.71	1.63	1.65	1.95
1 mEq RRM	$\bar{X}$	24.14	42.22 #o	49.53 #o	50.37 #o	55.82 #o	61.86 #o
	SEM	1.27	2.28	5.66	7.54	12.85	14.87
6 mEq Sham	$\bar{X}$	15.79 +	17.50	15.68	16.68	15.32	16.17
	SEM	1.11	1.09	1.29	1.10	1.00	1.31
6 mEq RRM	$\bar{X}$	17.09 +	49.08 #o	47.00 #o	50.44 #o	56.81 #o	53.24 #o
	SEM	1.81	6.05	5.82	11.22	12.03	8.27

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\+ = significantly different ($p < .05$) from the 1 mEq RRM group.

\# = significantly different ($p < .05$) from the 6 mEq Sham group.

Figure 1. Blood Urea Nitrogen (BUN) measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C3, E2, E4, E6, E10 and E14.

Symbols next to the squares or triangles represent the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

δ = significantly different ($p < .05$) from the 1 mEq RRM group.

Ω = significantly different ($p < .05$) from the 6 mEq Sham group.

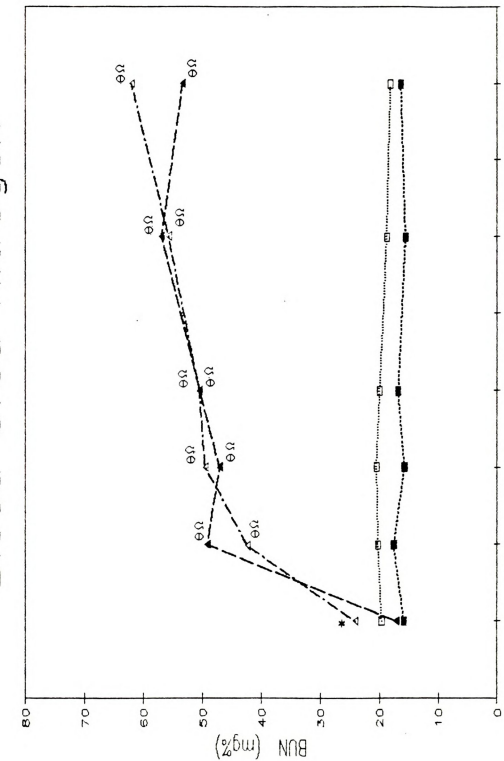

Blood Urea Nitrogen
BUN (mg%)
80
70
60
50
40
30
20
10
0
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 2

Plasma Sodium (mEq/l)

		Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 10	Expt 14
1 mEq Sham	$\bar{x}$	146.18	144.06	143.88	142.68	145.76	144.89
	SEM	1.54	1.32	1.03	0.71	1.96	2.42
1 mEq RRM	$\bar{x}$	142.23	144.09	146.28	144.72	145.84	144.01
	SEM	2.05	1.55	1.05	0.87	0.89	0.55
6 mEq Sham	$\bar{x}$	141.00	140.16	140.03	139.55	141.21	141.35
	SEM	1.65	0.99	0.81	0.68	1.04	1.19
6 mEq RRM	$\bar{x}$	139.89	141.18	141.84	142.96	144.87	144.80
	SEM	0.99	1.16	0.96	1.87	1.66	1.60

There were no significant differences amoung groups.

Figure 2. Plasma Sodium concentration measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C3, E2, E4, E6, E10 and E14.

There were no significant differences ($p > .05$) amoung groups.

Figure 5.

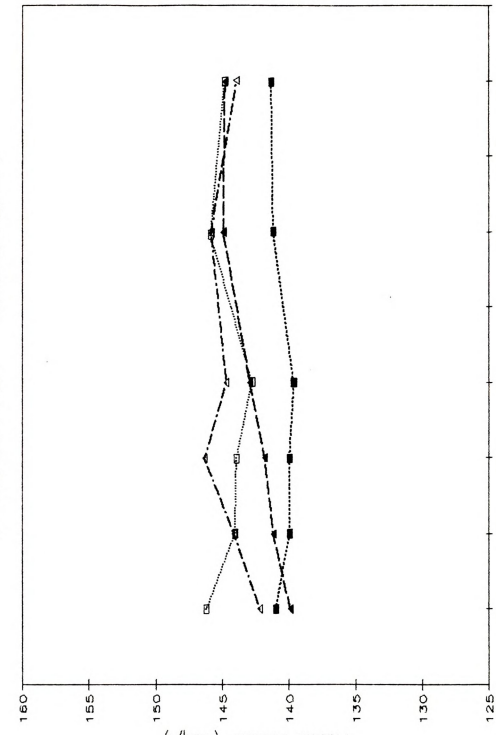

Plasma Sodium
Plasma Sodium (mEq/l)
160
155
150
145
140
135
130
125
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 3

Plasma Potassium (mEq/l)

		Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 10	Expt 14
1 mEq Sham	$\bar{X}$	4.70	4.69	4.54	4.97	4.62	4.71
	SEM	0.03	0.04	0.04	0.05	0.03	0.04
1 mEq RRM	$\bar{X}$	4.45	4.78	4.63	4.85	4.71	4.75
	SEM	0.02	0.02	0.04	0.05	0.06	0.06
6 mEq Sham	$\bar{X}$	4.91	5.18 +	5.00	4.92	4.59	5.10
	SEM	0.09	0.07	0.05	0.05	0.07	0.08
6 mEq RRM	$\bar{X}$	4.31	4.46	4.76	4.83	4.86	5.08
	SEM	0.06	0.05	0.07	0.09	0.10	0.10

Symbols to the right of the mean represent the following:

+ = significantly different ($p < .05$) from the 6 mEq RRM group.

Figure 3. Plasma Potassium measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C3, E2, E4, E6, E10 and E14.

Symbols next to the filled square represents the following:

δ = significantly different ($p < .05$) from the 1 mEq RRM group.

Figure 3.

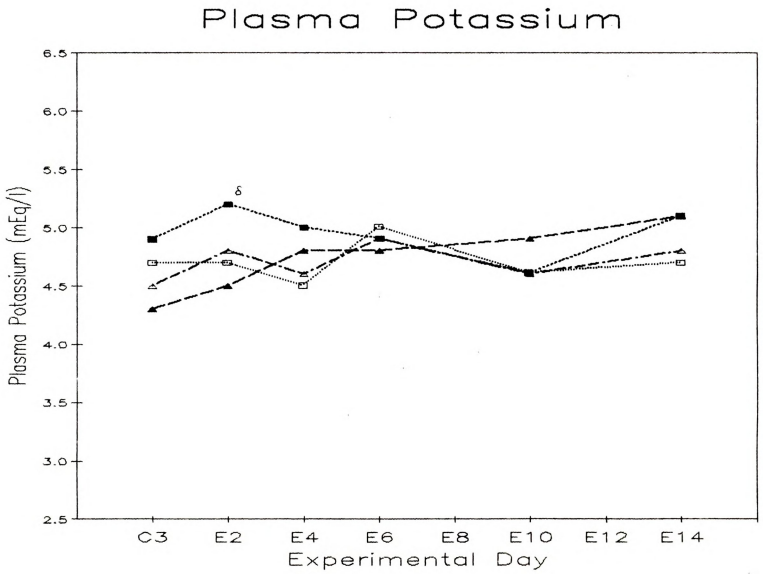

Plasma Potassium
Plasma Potassium (mEq/l)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
δ
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 4

Plasma Osmolality (mosmoles/kg water)

		Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 10	Expt 14
1 mEq Sham	$\bar{X}$	293.14	293.71	295.25	300.50	303.50 #	300.00
	SEM	1.10	1.55	0.74	2.87	1.62	1.77
1 mEq RRM	$\bar{X}$	303.44 #+	310.11 #o	310.78 #o	320.38 *	326.67 #o	327.67 #o
	SEM	7.37	7.51	7.45	8.72	7.85	8.13
6 mEq Sham	$\bar{X}$	288.55	288.00	285.33	286.17	288.55	290.00
	SEM	0.48	0.30	0.43	0.60	0.52	0.58
6 mEq RRM	$\bar{X}$	286.13	306.25 #	309.63 #o	303.63	313.25 #	311.14
	SEM	0.87	2.68	2.55	1.79	2.88	2.65

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\# = significantly different ($p < .05$) from the 6 mEq Sham group.

\+ = significantly different ($p < .05$) from the 6 mEq RRM group.

* = significantly different ($p < .05$) from all groups.

Figure 4. Plasma Osmolality measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C3, E2, E4, E6, E10 and E14.

Symbols next to the filled square represents the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

δ = significantly different ($p < .05$) from the 1 mEq RRM group.

Ω = significantly different ($p < .05$) from the 6 mEq Sham group.

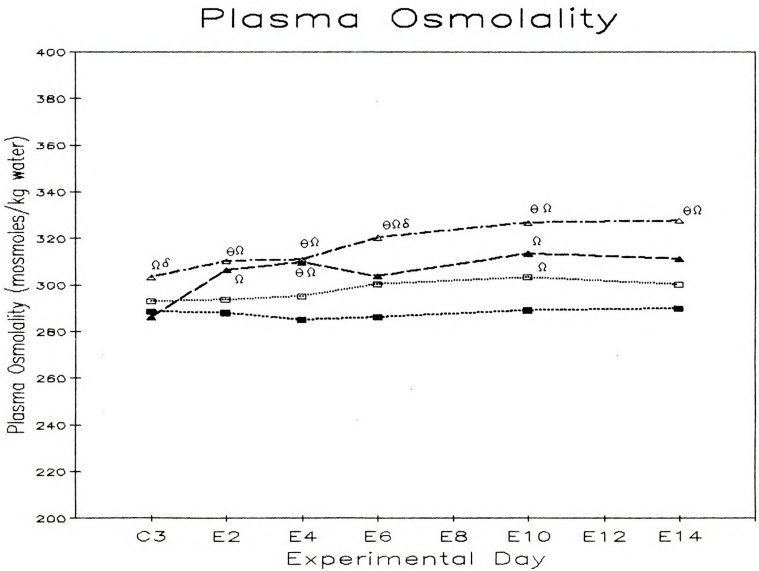

Plasma Osmolality
Plasma Osmolality (mosmoles/kg water)
400
380
360
340
320
300
280
260
240
220
200
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day
ΩΔ
ΘΩ
ΘΩ
ΘΩΔ
Θ Ω
ΘΩ
Ω
ΘΩ
Ω
Ω

TABLE 5

Mean Arterial Prssure (mmHg)

		Cntrl 1	Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 8	Expt 10	Expt 12	Expt 14
1 mEq Sham	$\bar{X}$	115.25	106.63	106.75	111.13	106.50	103.88	106.88	101.75	108.63
	SEM	2.67	3.44	1.56	3.11	2.03	2.72	2.50	2.63	2.90
1 mEq RRM	$\bar{X}$	114.00	109.33	113.78	119.33	121.33	124.38 o	122.33	123.33 o	124.44
	SEM	5.56	2.05	2.91	2.26	4.00	3.16	3.18	3.90	4.13
6 mEq Sham	$\bar{X}$	109.17	106.17	110.33	107.92 +	111.58	111.67	114.00	113.83	117.92
	SEM	3.44	2.16	3.11	2.74	3.70	4.91	4.10	5.80	6.15
6 mEq RRM	$\bar{X}$	108.00	102.13	111.38	113.25	117.38	135.38 #o	135.50 #o	152.50 *	149.42 *
	SEM	5.72	2.77	3.26	3.13	5.08	8.82	8.71	4.95	6.37

Symbols to the right of the mean represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

+ = significantly different ($p < .05$) from the 1 mEq RRM group.

= significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

Figure 5. Mean Arterial Pressure measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C1, C3, E2, E4, E6, E8, E10 E12 and E14.

Symbol next to the squares and triangles represent the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

δ = significantly different ($p < .05$) from the 1 mEq RRM group.

Ω = significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

Figure 5. Mean Alternative Pressure measurements [illegible]
[illegible] Med Sham group. Represented by [illegible]
squares. The [illegible] Med RAM group. Represented [illegible]
an open triangle. The 6 Mid Jaw [illegible]
represented by a filled square. And th[illegible]
Med RAM group. Represented by [illegible]
triangle. On days 11, 12, 25, and [illegible]
ELS and ELA.

Symbol next to the squares and [illegible]
represent the following:

* = significantly different [illegible]
the 6 Med Sham group.

$ = significantly different [illegible]
the 1 Mid RAM group.

@ = significantly different [illegible]
the 1 Med Sham group.

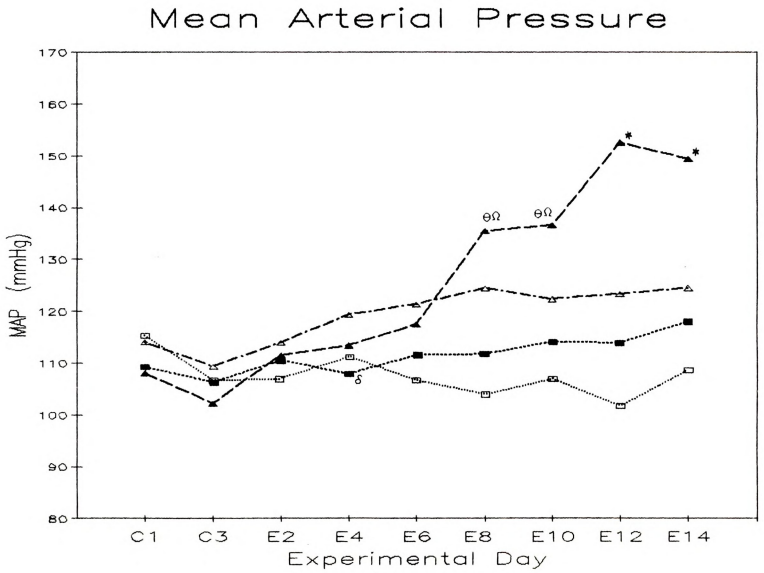

Mean Arterial Pressure
MAP (mmHg)
170
160
150
140
130
120
110
100
90
80
θΩ
θΩ
*
*
C1
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 6

Heart Rate (beats/min)

		Cntrl 1	Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 8	Expt 10	Expt 12	Expt 14
1 mEq Sham	$\bar{X}$	386.25	380.00	421.88	420.00	408.75	388.13	403.13	360.00	397.50
	SEM	16.22	13.63	3.40	9.82	10.12	12.82	13.13	9.40	15.53
1 mEq RRM	$\bar{X}$	370.00	410.00	433.33	406.67	416.67	380.63	396.67	368.33	387.78
	SEM	14.14	15.81	7.26	9.82	10.54	13.58	8.33	11.49	6.51
6 mEq Sham	$\bar{X}$	382.00	419.50	415.67	413.50	400.00	410.25 +o	403.25	412.50	408.50
	SEM	11.71	8.17	11.81	9.27	14.12	9.92	8.50	12.03	10.03
6 mEq RRM	$\bar{X}$	385.88	386.25	417.00	411.00	388.50	400.13	394.50	391.13	402.86
	SEM	9.12	13.32	15.34	9.55	14.64	11.93	10.74	14.14	15.01

Symbols to the right of the mean represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\+ = significantly different ($p < .05$) from the 1 mEq RRM group.

Figure 6. Heart Rate measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C1, C3, E2, E4, E6, E8, E10, E12 and E14.

Symbols next to the filled square represent the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

δ = significantly different ($p < .05$) from the 1 mEq Sham group.

Figure 6. Heart rate measurements [illegible]

```
                                    the 1 med Shaw Qrond.

6 = minifficaulk different (h
                                    the 1 med Shaw Qrond.

                                    the following:

                                    Symbole next to the filled square
                                    (1, 3, E5, E4, E9, E8, E10, E11 and E12
                                    represented by a filled triangle.
                                    by a filled square, and the 6 med Shaw
                                    triangle. the 6 med Shaw Qrond, represented
                                    med RHW Qrond, represented by an
                                    Qrond, represented by an Qnen
```

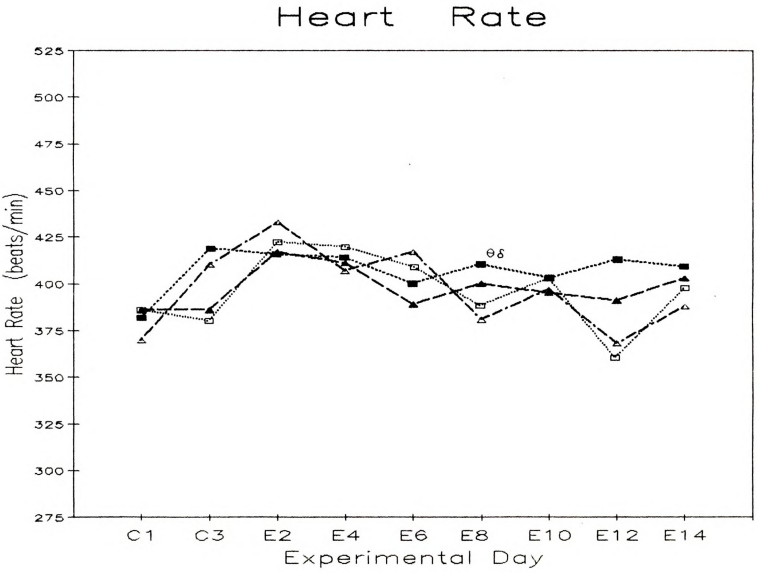

Heart Rate
Heart Rate (beats/min)
525
500
475
450
425
400
375
350
325
300
275
θδ
C1
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 7

Water Intake (ml/day)

		Cntrl 1	Cntrl 2	Cntrl 3	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7
1 mEq Sham	$\bar{X}$	25.60	23.90	21.62	22.89	22.20	20.17	21.41 *	21.76	20.50	25.27
	SEM	4.18	3.56	2.14	4.26	2.82	2.65	1.96	1.34	1.43	2.84
1 mEq RRM	$\bar{X}$	26.22	26.30	22.44	20.17	30.52	33.90 o	34.27	32.86	38.15 o	35.51
	SEM	3.10	3.07	1.99	1.94	4.55	2.96	3.66	3.65	3.71	2.91
6 mEq Sham	$\bar{X}$	39.60	35.76	27.49	24.14	27.13	29.49	31.81	27.31	31.34	28.57
	SEM	4.34	4.63	2.62	1.79	2.41	2.84	2.95	3.02	4.05	3.99
6 mEq RRM	$\bar{X}$	37.72	36.58	32.65	30.15	45.57	49.05 *	47.74 *	43.72 o	46.09 o	44.86 o
	SEM	4.94	4.41	2.93	3.09	3.93	6.46	4.98	4.57 #	4.52	4.14 #

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\# = significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

TABLE 7 (continued)

Water Intake (ml/day)

		Expt 8	Expt 9	Expt 10	Expt 11	Expt 12	Expt 13	Expt 14
1 mEq Sham	$\bar{X}$	22.02 *	25.16	27.71	24.09 *	23.02 *	23.07 *	21.75 *
	SEM	3.18	2.83	4.96	1.95	1.98	2.20	2.45
1 mEq RRM	$\bar{X}$	35.32	34.12	37.15	38.10	34.50	35.55 o	36.02
	SEM	3.48	2.76	3.38	2.50	2.61	3.35	2.67
6 mEq Sham	$\bar{X}$	42.49	41.59 o	43.53	40.66	41.69	38.66	44.39
	SEM	3.16	5.33	4.39	3.76	3.66	4.37	4.69
6 mEq RRM	$\bar{X}$	60.03 *	71.50 *	77.32 *	69.87 *	71.30 *	72.20 *	74.35 *
	SEM	5.96	4.49	5.72	4.62	4.97	5.67	5.08

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\# = significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

Figure 7. Water Intake measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C1, C2, C3, E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13 and E14.

Symbols next to the squares or triangles represent the following:

Θ = significantly different ($p < .05$) from the 1 mEq Sham group.

Ω= significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

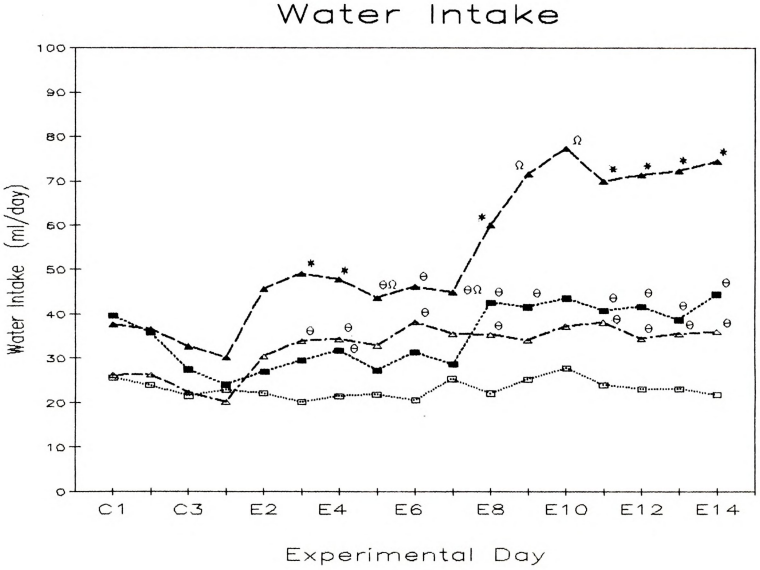

Water Intake
Water Intake (ml/day)
100
90
80
70
60
50
40
30
20
10
0
C1
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 8

Sodium Balance Ratio

		Cntrl 1	Cntrl 2	Cntrl 3	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7
1 mEq Sham	$\bar{X}$	0.55	0.95	0.84	0.84	0.60 *	1.07	0.83	0.97	0.94	1.00
	SEM	0.11	0.14	0.08	0.10	0.10	0.10	0.11	0.12	0.08	0.13
1 mEq RRM	$\bar{X}$	0.43	0.66	0.84	0.82	0.92	0.72	0.81	1.10	1.13	0.98
	SEM	0.13	0.16	0.19	0.10	0.09	0.10	0.05	0.09	0.09	0.08
6 mEq Sham	$\bar{X}$	0.48	0.83	1.07	1.03	1.20	0.91	0.81	1.04	1.03	0.96
	SEM	0.09	0.12	0.12	0.16	0.10	0.13	0.10	0.08	0.09	0.08
6 mEq RRM	$\bar{X}$	0.76	0.68	0.72	0.87	1.30 +o	0.80	1.04	1.05	1.16	1.00
	SEM	0.24	0.15	0.16	0.21	0.12	0.13	0.07	0.09	0.12	0.14

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

\+ = significantly different ($p < .05$) from the 1 mEq RRM group.

* = significantly different ($p < .05$) from all groups.

TABLE 8 (continued)

Sodium Balance Ratio

		Expt 8	Expt 9	Expt 10	Expt 11	Expt 12	Expt 13	Expt 14
1 mEq Sham	$\bar{X}$	0.97	0.93	0.86	0.64 *	1.03	0.91	0.87
	SEM	0.12	0.06	0.13	0.10	0.18	0.11	0.13
1 mEq RRM	$\bar{X}$	1.01	1.06	0.77	0.96	0.79	0.81	0.91
	SEM	0.05	0.10	0.10	0.14	0.08	0.07	0.18
6 mEq Sham	$\bar{X}$	0.72 +o	0.89	0.94	0.96	0.99	0.89	0.92
	SEM	0.06	0.04	0.04	0.03	0.04	0.04	0.03
6 mEq RRM	$\bar{X}$	0.68 +o	0.91	1.03	0.99	1.02	0.89	0.74
	SEM	0.08	0.08	0.07	0.03	0.04	0.04	0.16

Symbols to the right of the means represent the following:

o = significantly different ($p < .05$) from the 1 mEq Sham group.

+ = significantly different ($p < .05$) from the 1 mEq RRM group.

* = significantly different ($p < .05$) from all groups.

Figure 8. Sodium Balance calculations of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C1, C2, C3, E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13 and E14.

Symbols next to the squares or triangles represent the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

δ = significantly different ($p < .05$) from the 1 mEq RRM group.

Ω = significantly different ($p < .05$) from the 6 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

Figure 8. Sodium Balance calculations at the [illegible] drop, represented by an open square, the 1 med RRM drop, represented by an open triangle, the 6 med Shem drop, represented by a filled square, and the 5 med RRM drop, represented by a filled triangle. [illegible] C1, C2, C3, E1, E2, E3, F4, F5, F6, F7, F8, F9, E10, E11, E12, E13 and E14.

Symbols next to the squares or triangles represent the following:

θ = significantly different (p < .05) from the 1 med Shem drop.

δ = significantly different (p < .05) from the 1 med RRM drop.

Ω = significantly different (p < .05) from the 6 med Shem drop.

* = significantly different (p < .05) from all drops.

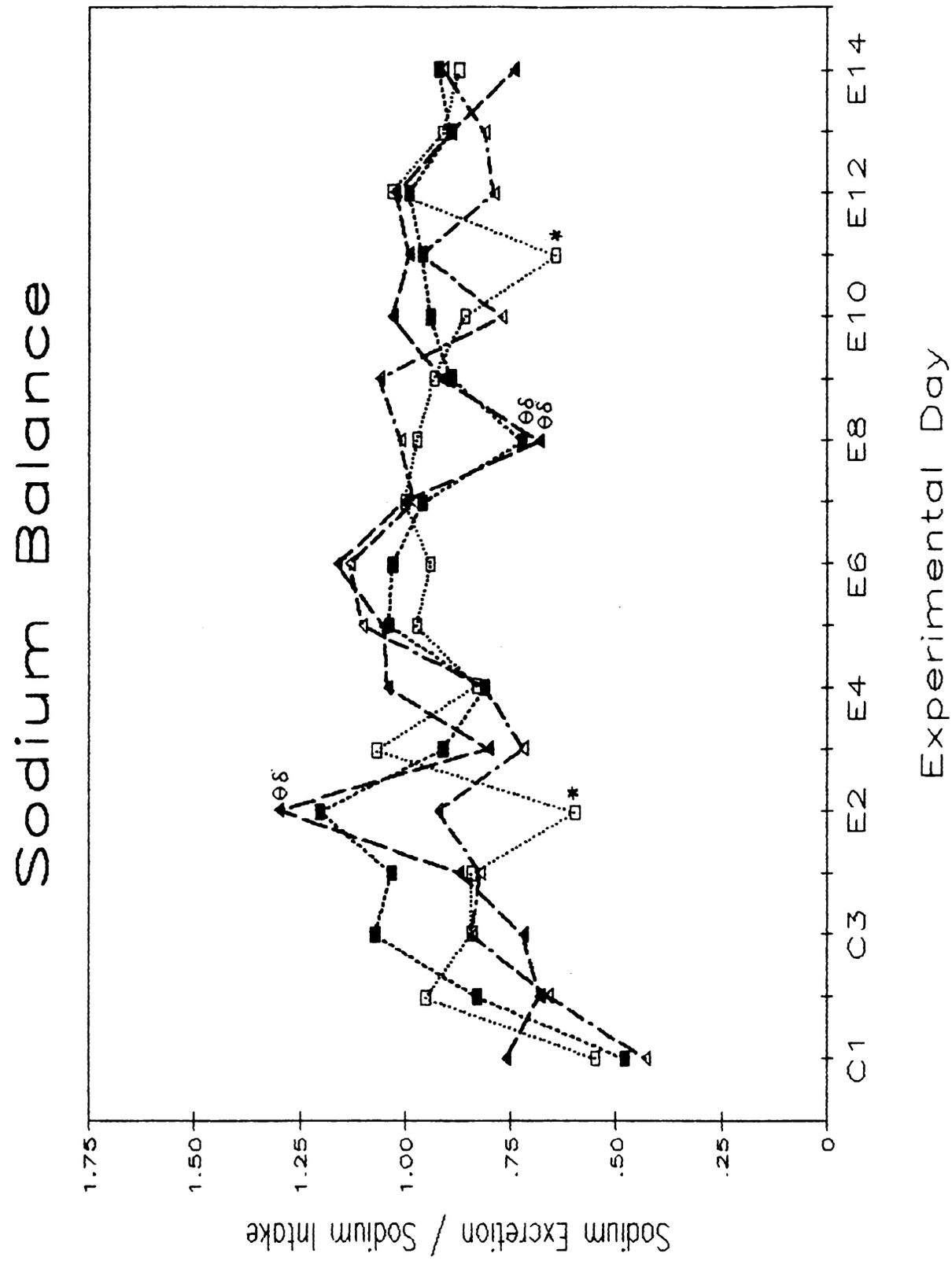

Sodium Balance
Sodium Excretion / Sodium Intake
1.75
1.50
1.25
1.00
.75
.50
.25
0
C1
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day

TABLE 9

Plasma ANP (fmoles/ml)

		Cntrl 3	Expt 2	Expt 4	Expt 6	Expt 10	Expt 14
1 mEq Sham	$\bar{X}$	300.93	259.68	293.70	335.95	240.80	293.30
	SEM	41.27	25.16	28.97	53.63	26.34	29.93
1 mEq RRM	$\bar{X}$	235.82	256.12	241.12	247.52	221.40	254.57
	SEM	36.55	35.12	22.72	31.25	19.53	34.50
6 mEq Sham	$\bar{X}$	215.08	224.94	239.07	258.04	286.75	293.36
	SEM	15.16	27.24	14.99	20.87	33.44	30.13
6 mEq RRM	$\bar{X}$	156.13 o	183.10	189.02 o	216.88	417.42 *	439.16 *
	SEM	20.62	26.16	22.16	25.22	44.10	34.22

Symbols to the right of the mean represent the following:

o = significntly different ($p < .05$) from the 1 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

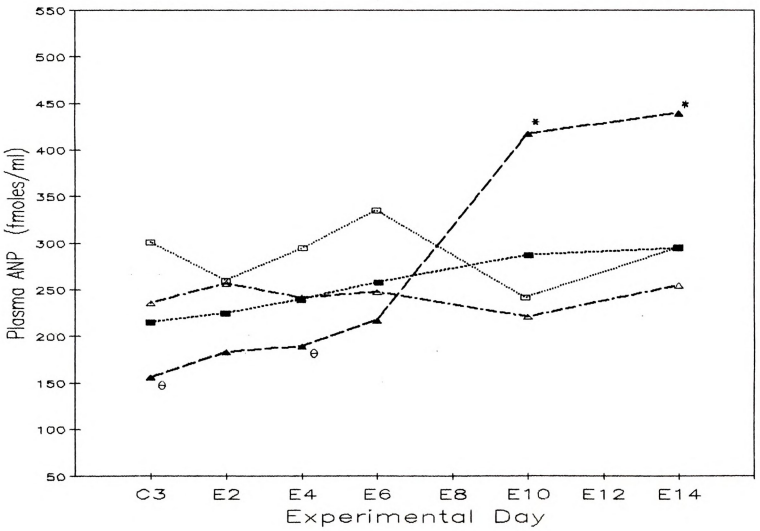
Figure 9. Plasma Atrial Natriuretic Peptide measurements of the 1 mEq Sham group, represented by an open square, the 1 mEq RRM group, represented by an open triangle, the 6 mEq Sham group, represented by a filled square, and the 6 mEq RRM group, represented by a filled triangle, on days C3, E2, E4, E6, E10 and E14.

Symbols next to the squares or triangles represent the following:

θ = significantly different ($p < .05$) from the 1 mEq Sham group.

* = significantly different ($p < .05$) from all groups.

Figure 5.


Plasma Atrial Natriuretic Peptide
Plasma ANP (fmoles/ml)
550
500
450
400
350
300
250
200
150
100
50
C3
E2
E4
E6
E8
E10
E12
E14
Experimental Day
*
*
θ
θ

DISCUSSION

The purpose of the study was to ascertain whether or not ANP might play a role in the control of sodium excretion in animals with experimentally induced reduced renal mass. It was hypothesized that ANP might be markedly elevated in the animals with reduced renal mass thereby aiding in the control of sodium excretion.

It was first established that the animals with reduced renal mass were indeed uremic. Blood urea nitrogen was elevated in both the 1 mEq RRM group and the 6 mEq RRM group as compared to the 1 mEq Sham group and the 6 mEq Sham group. There was no significance difference between the BUN of the 1 mEq RRM group and the 6 mEq RRM group indicating both groups were equally uremic. Interestingly, the 1 mEq RRM group on day C3 had significantly elevated BUN levels as compared to the other groups on this day. This finding implies that more renal mass may have been removed as compared to the 6 mEq RRM group during the operation to reduce renal mass. Thus, it can be concluded that the procedure used to make rats uremic was a valid procedure. Others have confirmed the presence of an elevated BUN when renal function is compromised. (See

review by Hayslett et al. 1979)

Plasma ANP was sampled from chronically catheterized conscious rats instead of acutely anesthetized or guillotined rats. A conscious chronic preparation was deemed necessary for two reasons: First, repeated blood samples could be obtained from the same animal over time. This enabled the following of an individual rat over time for the various parameters measured. Second, Gutkowska et al. (1984) showed that certain anesthetics were capable of raising plasma ANP levels. Specifically, morphine was a potent stimulus for ANP release raising plasma levels more than 20 fold. Ether and ketamine cholorhydrate were shown to raise plasma ANP levels more than 8 fold and 2 fold respectively. Restraint stress was another stimulus capable of increasing ANP release. Conversely, sodium pentobarbital was shown to decrease plasma ANP levels. Thus, certain anesthetics and stress are capable of altering plasma ANP levels.

Differences in the absolute amount of ANP measured in rat plasma vary from laboratory to laboratory. Various investigators have reported plasma ANP concentrations between 10 and 283 fmoles/ml. For example, this study reported a mean ANP level from sham operated rats of 240 fmoles/ml. Luft et al. (1986) reported 10 fmoles/ml in anesthetized rats. Smith et al. (1986) reported a mean ANP level of 57 fmoles/ml in rats maintained on 1 to 3 mEq of sodium per day . Tanka et al. (1984) reported plasma levels

from conscious rats of 156 fmoles/ml. Finally, Manning et al. (1984) reported a mean basal plasma level of 283 fmoles/ml. Reasons for such differences could reside in the antibody used in the determination of plasma ANP levels, whether or not the animals were anesthetized, and if so the type of anesthetic used, and whether or not the plasma was extracted. The antibody used in this study and the antibody used in the above mentioned studies came from different sources. Hence this could account for the noted differences in plasma concentration. Additionally, we did not extract our plasma as many of the other investigators did. The procedure of extraction was deemed unnecessary since both serial dilutions of plasma, and additions of atriopeptin III to plasma yielded results parallel to the standard curve. Additionally, extraction yields between 50% to 80 or 90% of the original peptide level (Chard 1982). Furthermore, a multitude of errors can occur during an extraction procedure. Finally, we obtained plasma from conscious rats in metabolism cages.

ANP was significantly elevated on day 10 and 14 of the 6 mEq RRM group, but not on experimental days C3, E2, E4 or E6. ANP was not significantly elevated in the 1 mEq Sham group, the 1 mEq RRM group or the 6 mEq Sham group. The presence of ANP in uremic rats maintained on 6 mEq of sodium per day is not caused by its retention in the plasma due to failure of excretion. If this were so, then rats maintained on 1 mEq of sodium per day the entire time would

be expected to have elevated ANP levels as well. Thus, ANP does not appear to be the circulating sodium transport inhibitor suggested to account for the increased fractional sodium excretion seen in reduced renal mass.

A proportional reduction in sodium intake to match the decline in glomerular filtration rate has been shown to obviate the need for a circulating inhibitor of sodium transport (Schmidt et al. 1974). Although fractional sodium excretion was not measured, the 1 mEq Sham group most likely has a fractional sodium excretion of approximately .9% (Valtin 1983). The 1 mEq RRM group, which had 5/6 of their kidney removed, must have increased their fractional sodium excretion well above .9% to maintain sodium balance. Because ANP levels were not elevated in this group, and were similar to the 1 mEq Sham group, it can be concluded that some other factor must have been responsible for the increased fractional sodium excretion.

The elevation of ANP in the RRM rats maintained on 6 mEq of sodium per day appears to be related to the sudden increase in sodium intake. The natriuresis per nephron governed by natriuretic hormone may suffice to maintain the extracellular volume at normal levels, but with an elevation in sodium intake, their exists a need for an additional natriuresis per nephron. The sudden increase in sodium intake could conceivably increase extracellular fluid volume. The 6 mEq Sham group was able to compensate for the sudden increase in sodium intake and volume; the 6

mEq RRM group was unable to compensate for the sudden increase, and therefore exhibit an increased extracellular fluid volume. Although extracellular volume was not measured in any of the animals, we can say for certain that extracellular fluid volume was elevated. Thus, the possibility exists that ANP is released and plays a role in extracellular fluid homeostasis in chronic renal failure in situations of sodium overload.

Elevated plasma ANP has been reported in animals with renal failure. Smith et al. (1986) noted an increase in plasma ANP in rats maintained on 3 - 5 mEq of sodium per day compared to those rats maintained on 1 - 3 mEq of sodium per day. Elevated plasma ANP has also been observed in volume expanded children with renal failure prior to dialysis treatment (Rascher et al. 1986).

Luft et al. (1986) did not observe an increase in plasma ANP in rats with experimentally induced reduced renal mass. If the sodium excretion data were an accurate reflection of the sodium intake, the rats on the "high sodium" diet were ingesting 1 mEq of sodium per day per 100 gm body weight. In comparison, the RRM group maintained on 6 mEq of sodium per day in the present study had a sodium intake of 2 mEq of sodium per 100 gm body weight. Thus, an explanation of why plasma ANP was increased in observed in the study of Luft et al. (1986) may be that the rats received only half the sodium intake administered in this study. Supporting the hypothesis that the rats of Luft et

al. (1986) did not receive enough salt to raise plasma ANP, Smith et al. (1986) also found no increase in plasma ANP in RRM rats ingesting approximately 1.5 mEq of sodium per day per 100 gm body weight.

A strong possibility exists that the increase in arterial pressure was responsible for the elevation of ANP. Mean arterial pressure significantly increased 20 mmHg after day E10 and another 20 mmHg after day E12 in the 6 mEq RRM group. Rats with RRM maintained on 1 mEq of sodium per day had a modest increase in arterial pressure of 10 mmHg during part of this same time. Although plasma ANP did not increase significantly in this latter group, the correlation coefficient for arterial pressure and plasma ANP for all rats with RRM on both 1 and 6 mEq of sodium per day is $r = 0.800$, which suggests that they are a related phenomena. Support for this hypothesis comes from the work of Manning et al. (1985) who determined that an acute increase in arterial pressure of stimulated ANP release. Rats were treated with the vasoconstrictors vasopressin, phenylephrine, angiotensin II and dAVP at doses which produced comparable increases in arterial pressure, but differed in their duration. The dependence of ANP release on changes in arterial pressure was consistent with the observation that neither the non-pressor analog of dDAVP nor AVP in the presence of a specific AVP anti-pressor antagonist caused ANP release. Kohno et al. (1986) determined that chronic elevations of arterial pressure

were capable of raising plasma ANP. Plasma ANP in spontaneously hypertensive rats, which had a mean arterial pressure of 184 mmHg, were found to contain twice as much ANP in their plasma as compared to normotensive control rats

Systemic hypertension constitutes an increased afterload to the left ventricle which subsequently decreases stroke volume. The decreased stroke volume increased left ventricular end-diastolic volume which in turn increases left atrial stretch (Leadsome et al. 1985). Thus, it appears that RRM raised plasma ANP by increasing arterial pressure which increases left ventricular afterload and atrial stretch.

A remaining question is whether or not the elevation in ANP in the 6 mEq RRM group was enough to elicit a natriuresis. Goetz et al. (1986) compared the effects of infusing different amounts of synthetic ANP in conscious hydrated dogs, and the resulting increases in plasma ANP and sodium excretion they produced. Beginning an infusion rate of 25 ng/kg min, plasma ANP increased 3 fold from a control of 28 fmoles/ml. Even at doses of 50 and 100 ng/kg min, which raised plasma ANP to 217 fmoles/ml and 487 fmoles/ml respectively, there was no increase in sodium excretion. Thus a 16 fold increase in plasma levels of ANP was not sufficient to produce a natriuresis in dogs. In another study, Goetz et al. (1986) confirmed that a much higher dose of 3,000 ng/kg min produced a natriuresis and

diuresis in conscious dogs. This dose may not necessarily reflect the lowest dose able to induce a natriuretic response as the study was not designed to be a dose response study. The rat may behave similarly. Luft et al. (1986) performed a dose response curve in rats weighing between 250 and 275 grams. Synthetic ANP was administered in incremental doses of 20, 50, 100 and 250 ng bolus injections. A significant influence of ANP on sodium excretion was not identified until the 100 ng bolus dose. Assume for calculation purposes a plasma volume of 4% of the total body weight, and the bolus dose of 100 ng distributed evenly in the plasma. This would yield a plasma concentration of 3,333 fmoles/ml. Yasujima et al. (1985) infused synthetic ANP at a dose of 104 ng/kg min and found it did not induce any changes in urine flow or sodium excretion when administered intravenously for 3 days. In this study, it is possible that the elevation of plasma ANP seen in the RRM rats maintained on 6 mEq of sodium per day was not enough to increase sodium excretion. It may however, be enough to alter some of the other parameters of salt and water homeostasis ANP has been shown to affect. For example, vasopressin release may be altered; renin release may be altered; aldosterone release may be altered.

As an alternative hypothesis, the remaining nephrons in rats with reduced renal mass may be hypersensitive to circulating ANP. Cole et al. (1985) showed that remaining glomeruli in rats with reduced renal mass were

hypersensitive to intravenous ANP administration. Cole et al. (1985) additionally found that 200 ng/kg min was enough to elicit a 9 to 12 fold increase in sodium excretion in rats which underwent 5/6 nephrectomy 4 weeks before. Even smaller doses of 60 ng/kg min were able to elicit a 3 fold increase in sodium excretion. This compares to the study of Yasujima et al. (1985) where 104 ng/kg min of ANP was unable to induce a change in sodium excretion in normal rats, and the study of Goetz et al. (1986) where 50 and 100 ng/kg min of ANP infusion were unable to induce any changes in sodium excretion in dogs. ANP may exert an additional tubular effect in rats with reduced renal mass. Support of this hypothesis again comes from the work of Cole et al. (1985) who infused 60 ng/kg min of ANP in urethane anesthetized rats with reduced renal mass. It was found that a natriuresis and diuresis occurred at a time when urine flow was stable, yet total GFR was not significantly different from pre-infusion controls.

Heart rate was not significantly different when differences in ANP were found. Thus, changes in heart rate were not responsible for the elevated ANP levels.

The general increase in arterial pressure in the animals maintained on 6 mEq of sodium per day is similar to what others have noted upon a reduction in renal mass, and is often used as a model to study hypertension. The mechanism of induction of this hypertension appears to be volume dependent. This statement is based on the work of

Kazada et al. (1971) who found that upon a 70% reduction of renal mass, intravascular volume increased. Thus, reduction of renal tissue favors the retention of sodium and consequently expansion of the extracellular and intravascular spaces thereby producing hypertension.

Hypertension was not as pronounced in the rats maintained on 1 mEq of sodium after day 7 as compared to the rats maintained on 6 mEq of sodium per day. This finding is consistent with the work of Miksche et al. (1970) who found that renal hypertension induced by the clamping of one renal artery, leaving the other kidney untouched, was either prevented or could not be maintained when rats were placed on a sodium deficient diet.

A strong argument against a direct effect of the renin-angiotensin system on the development and maintenance of hypertension in the model used in this study was the finding that in unilateral nephrectomized rats, neither plasma renin activity nor renin concentrations in the kidney were elevated above normal values (Miksche et al. 1970).

Water intake significantly increased in the 6 mEq RRM group after the animals were placed on 6 mEq of sodium per day. An increase in sodium intake increases osmolality. The associated increase in osmolality is met with an increase in thirst in order to maintain plasma osmolality within a normal range (Fitzsimons 1976).

Sodium excretion was measured as urinary sodium

excretion only and did not take into account fecal sodium excretion. Mohring et al. (1972) have shown that fecal sodium only accounts for for approximately 3 to 4% of the total excretory sodium when the sodium is ingested in the diet. The rats in this study did not receive any sodium in their food and it would be expected that their fecal sodium content would be negligible. The ratio of sodium excretion divided by sodium intake is called the balance ratio (Mohring et al. 1972). Overall, the balance ratio appeared to be close to one. Some of the reason why a perfect balance ratio was not achieved include the observation of salt crystals on the swivels and on the syringe luers due to leakage. This sodium could not be taken into account in the determination of sodium intake measurements and thus, an artificial lowering of the balance ratio occasionally appeared. An attempt was made to correct for sodium losses which may have occurred due to swivels and infusion lines leaking, and any additional sodium gains which might have occurred in the form of medications. Any sodium from the urine which did not wash down into the collection flask was also not taken into account for and hence that amount of sodium not analyzed which may also artifically lower the balance ratio. Despite the statements regarding the sodium error of the system, the total amount of unaccountable salt only accounts for approximately 5% of the total balance ratio. Exceptions to this were found on days C1, C2, E1, and E2 for all groups of rats. During days C1 and C2, the

animals were still recovering from the catheter surgery performed two days ago and were not in sodium balance yet. On day E1, all groups of rats were noted to be out of balance as the rats were recovering either from the removal of a kidney or its appropriate sham operation. Another period of time when the the balance ratio is considerably below one, or a time of positive sodium balance, occurs on day E7 only in the rats switched to 6 mEq of sodium per day. The rats in this group do come back into balance by E8.

Plasma sodium was not significantly different in the four groups of rats even in the rats with reduced renal mass. This is in agreement with what others have found. That is, sodium is a solute that is completely regulated by the kidney in uremia (Hayslett 1979).

Plasma potassium was significantly elevated on days E4, E6 and E10 of the 6 mEq Sham group as noted in table 7 of the results section. The increase in plasm potassium does not appear to follow any pattern and it is unknown as to why this group might have elevated levels as compared to the rest of the groups. A possible explanation could be hemolysis of the red blood cells thereby artificially elevating the plasma potassium levels.

Plasma osmolality was significantly different from one group to another. Plasma osmolality was the last measurement to be made, and the possibility exists that the wide range of osmolalities measured could be the result of

a variable degree of broken-down plasma proteins which may artificially increase plasma osmolality in some cases. A consistent increase in plasma osmolality appeared to parallel the increase in BUN only in the 1 mEq RRM group. Because plasma osmolality was measured by freezing point depression, urea was measured as an osmotically active particle even though it is an ineffective osmotically active particle able to permeate the cell. It is unknown why plasma osmolality did not increase in the 6 mEq RRM group. It has already been mentioned that plasma urea contributes to the net osmolality as measured by freezing point depression. The plasma in the 6 mEq RRM group may have sat at room temperature for a longer period of time than the 1 mEq RRM group. Urea in plasma can be converted to ammonia by bacterial decomposition (Caraway 1962). If this were the case, the true osmolality would be less.

CONCLUSIONS

1. The outlined procedure to reduce renal mass was a valid procedure to produce chronic renal failure as assessed by the elevation in BUN.

2. ANP only increased in the 6 mEq RRM group, but not in the 1 mEq RRM group or the 6 mEq Sham group.

3. The increase in ANP in the 6 mEq RRM group can not be due to either a reduction of renal mass or a sodium intake of 6 mEq per day because neither the 1 mEq RRM group or the 6 mEq Sham group had increased concentrations of ANP.

4. Therefore, the increase in ANP must be an effect of both RRM plus 6 mEq of sodium.

5. The increase in ANP could be due to an increase in extracellular fluid volume in the 6 mEq RRM group.
   The only relevant evidence, the balance data, do not support this possibility.
   However, animals could be volume expanded and still be in sodium balance.

6. The increase in ANP could be the result of a sustained increase in arterial pressure, seen only in the 6 mEq RRM group, because both acute increases of arterial pressure with vasoconstrictors or chronic increases in arterial pressure in other disease stated cause plasma ANP to increase.

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