SODIUM REPLACEMENT BEHAVIOR OF SODIUM DEFICIENT RATS

BEHAVIORAL AND PHYSIOLOGICAL ASPECTS OF SODIUM REPLACEMENT IN SODIUM DEFICIENT RATS

by

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

July, 1971.

DOCTOR OF PHILOSOPHY (1971) MCMASTER UNIVERSITY
(Psychology) Hamilton, Ontario

TITLE: Behavioral and Physiological Aspects of Sodium Replacement in Sodium Deficient Rats

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NUMBER OF PAGES: vii, 108

SCOPE AND CONTENTS:

The relationship between sodium deficiency and consequent sodium appetite was examined in rats experiencing high endogenous levels of aldosterone prior to diuretic-induced sodium loss and in rats incapable of secreting aldosterone because of adrenalectomy. Sodium appetite of rats with high aldosterone secretion was characterized by marked over-compensation for sodium deficits while salt intake of comparably deficient rats not experiencing high aldosterone secretion until after natriuresis was precisely sufficient to replace sodium loss. It is proposed that enhanced aldosterone secretion potentiates other natrorexigenic effects of sodium deficiency in the elicitation of sodium appetite, thereby resulting in salt intake in excess of need. Adrenalectomized rats also overcompensated for deficits incurred during sodium deprivation, but this is ascribed to repeated experience with sodium deficiency and replacement.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to E. M. Stricker who has guided me as advisor and friend throughout graduate school, to George Wolf who nurtured my initial interests in research and has been a great help ever since, and to Harold Chapman and G. R. Morrison whose advice and criticism were always helpful. I would also like to acknowledge the friendship and aid of Nancy Wilson, Richard Porter, Steve Black and Janice Burke who provided a comfortable and stimulating environment in which to work. Many others have helped me during my graduate career in ways too numerous to mention. However, no one deserves my gratitude more than my wife, Mary. Without her patience, understanding, and enthusiastic support, this work would never have been completed, and perhaps never begun.

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GENERAL INTRODUCTION

The homeostasis of the body fluid compartments depends on the proper maintenance of the sodium concentration of the extracellular fluid. Sodium is the major extracellular electrolyte and along with chloride accounts for most of the osmotic activity of the extracellular fluid. Thus, the amount of sodium in the extracellular fluid usually dictates the volume of the extracellular compartment including the intravascular fluid (blood). In addition, because the intra- and extracellular fluids must remain in osmotic equilibrium and because sodium is actively excluded from cells, the extracellular sodium concentration also determines the volume of cells (Leaf, 1962).

Alterations in the sodium concentration of the extracellular fluid can occur as the result of changes in the amount of sodium or the amount of water. In either case, pathological conditions often result. For example, increased sodium concentration in the absence of adequate fluid intake results in cellular dehydration, but with normal fluid intake may lead to hypertension and edema formation (Dahl & Schackow, 1964; Ross & Christie, 1969). On the other hand, decreased sodium concentration due either to dilution or absolute reduction of sodium causes over-hydration of cells (water intoxication).

If the reduction in sodium concentration is absolute, the volume of the intravascular fluid may also be reduced (hypovolemia) leading to circulatory difficulties (Leaf, 1962).

Total body sodium is not uniformly distributed throughout the tissues. The adult rat contains approximately 15 to 20 milli-equivalents (mEq) of sodium. Over 90 per cent of the total is distributed equally between the extracellular fluid and bone. Cells, which actively exchange sodium for potassium are responsible for the remainder (Bergstrom, 1955; Nichols & Nichols, 1956). Bone appears to act as a mineral reservoir for many electrolytes (Bergstrom, 1956) and as such contains sodium in higher concentration than the surrounding extracellular fluid. This is due to the fact that half of the sodium in bone is relatively fixed in the crystal phase while the remainder (20-25 per cent of the total body sodium) is contained in a fluid phase. The sodium in the bone fluid fraction can be rapidly exchanged with sodium in the rest of the body (Bergstrom & Wallace, 1954) and is readily contributed to the extracellular fluid during acute sodium depletion (Bergstrom, 1955; Nichols & Nichols, 1956).

In view of its vital role in maintaining the stability of body fluid volume and the integrity of the circulatory system, it is not surprising that elaborate mechanisms exist for the regulation of body sodium levels. Regulation implies that the intake and output of sodium interact in such a way as to provide a stable sodium balance adequate for life support. The more stable the balance despite fluctuations in intake and output, the more effective the regulation.

Regulation does not imply that intake and output are exactly equal nor that the relationship between them remains static since growth or illness might alter sodium balance requirements. The remarkable stability of the extracellular sodium concentration in man and most mammals at 145.0 to 150.0 mEq/1 plasma water attests to the efficiency of the regulatory mechanisms for sodium balance.

Control of sodium balance requires the interaction of both physiological and behavioral mechanisms. For example, an animal experiencing a sodium loss must limit further losses as well as replace the amount of sodium that has already escaped. Physiological mechanisms insure maximal sodium retention during times of need while behaviour is of paramount importance for replacement of unavoidable deficits. The remainder of this introduction will describe the behavioral and physiological mechanisms involved in the regulation of sodium balance. I will begin with the physiological aspects since an understanding of them is a prerequisite for understanding sodium acquisition behavior.

Section I: Physiological Factors in the Regulation of Sodium Balance

Kidney:

As is the case with many of the constituents of the extracellular fluid, the kidneys are responsible for precise regulation of sodium balance. The entire contents of the extracellular fluid, excluding protein, fats and blood cells, are filtered through the renal tubules and reabsorbed about 15 times each day allowing ample opportunity for regulation. Approximately 85 per cent of the filtered sodium load moves

out of the proximal renal tubules down an electrochemical gradient into the tubular cells and is actively expelled into the interstitial space. The reabsorbate is then taken up by the capillaries in response to peritubular oncotic forces and returned to the general circulation (Windhager, Lewy & Spitzer, 1969; Davis & Knox, 1970). Sodium reabsorption in the proximal tubule is obligatory and does not appear to be responsive to body sodium requirements. However, the amount of sodium reabsorbed appears to be directly related to peritubular oncotic concentration (Brenner, Falchuk, Keimowitz & Berliner, 1969). Expansion of the extracellular fluid volume reduces peritubular concentration and by slowing proximal reabsorption augments sodium excretion (Knox, Howards, Wright, Davis & Berliner, 1968). Under normal circumstances, the amount of sodium that remains in the proximal tubule passes through the loop of Henle and the distal tubule essentially unchanged and is excreted in the urine.

Sodium retention requires a more elaborate mechanism involving at least three interacting factors (Binnion, 1969). First, intrarenal mechanisms exist that can decrease blood flow to the glomeruli. Decreases in renal arterial pressure result in a reduced glomerular filtration rate and enhanced sodium reabsorption from the proximal tubule (Landwehr, Schnermann, Klose & Giebisch, 1968). Together, these effects reduce sodium excretion (Thompson & Pitts, 1952). Secondly, the renal blood supply can be redistributed from the superficial cortical glomeruli to the more effective nephrons in the renal medulla (Pomeranz, Birtch, & Barger, 1968; Horster & Thurau, 1968). Finally, distal tubular sodium

reabsorption can be increased by the presence of the adrenal mineralocorticoid hormone, aldosterone. This latter factor appears to be of primary importance to the fine control of sodium excretion even though aldosterone affects only a small fraction of the filtered sodium, i.e., 2-5 per cent (Roemmelt, Sartorius & Pitts, 1949). Animals lacking aldosterone invariably die from chronic sodium loss and consequent circulatory collapse unless replacement is instituted (Tepperman, 1968).

Aldosterone:

The sodium retaining effects of aldosterone were first described by Simpson and Tait (1952) and later by Barger, Berlin and Tulenko (1958). Micropuncture studies have demonstrated that the hormone acts almost exclusively on the distal tubules to enhance the exchange of sodium for potassium (Hierholzer, Weiderholt, Holzgreve, Giebisch, Klose & Windhager, 1965; Cortney, 1969). Aldosterone decreases the sodium to potassium ratio in the final urine since more sodium is reabsorbed and more potassium is excreted. This effect provides a simple albeit indirect test for aldosterone secretion (Simpson & Tait, 1952; Johnson, 1954).

The secretion of aldosterone — the most potent mineralocorticoid produced by the adrenal cortex — is under multifactor control. The pituitary exercises some control over aldosterone secretion but the mechanisms remain controversial. In rats, removal of the pituitary gland results in marked atrophy of all adrenal cortical zones except the aldosterone producing zona glomerulosa (Miller, 1965). Nevertheless, decreased aldosterone secretion has been reported immediately after

hypophysectomy (Eilers & Peterson, 1964) and hypophysectomy prevents the normal increase in aldosterone secretion during prolonged dietary sodium deficiency (Marieb & Mulrow, 1965; Palmore & Mulrow, 1967). Thus, despite contrary histological evidence, the pituitary may be involved in the control of aldosterone secretion especially during sodium deficiency. The pituitary factor responsible for potentiation of aldosterone secretion has not been identified. Adrenocorticotrophin (ACTH) injection does not restore aldosterone secretion in hypophysectomized rats (Palmore, Anderson & Mulrow, 1968). Instead, somatotrophin has been tentatively identified as the non-ACTH factor in in vitro studies (Lee & DeWied, 1968), although in vivo experiments would seem to exclude this hormone as an important factor (Palmore & Mulrow, 1967). Recent data suggests that both ACTH and somatotrophin may be necessary for aldosterone secretion (Palkovits, DeJong, VanderWal & DeWied, 1970). No other central nervous system structure other than the pituitary appears to have any serious role in the regulation of aldosterone secretion (Mulrow, 1966).

In addition to pituitary factors, aldosterone secretion can be augmented by alterations in plasma electrolyte levels and plasma volume. Decreases in plasma sodium concentration with or without concurrent increases in plasma potassium act directly on the adrenal cortex to increase aldosterone in dogs and sheep (Davis, Urquhart & Higgins, 1963; Blair-West, Coghlan, Denton, Goding, Wintour & Wright, 1963), although evidence in the rat is unclear (Palmore, Marieb & Mulrow, 1969). There have been reports that increases in plasma potassium stimulate

aldosterone secretion (Blair-West, Coghlan, Denton, Goding, Munro, Peterson & Wintour, 1962; Eilers & Peterson, 1964), but negative findings exist as well (Singer, 1960). Hypovolemia also appears to be an adequate stimulus for aldosterone secretion (Solyom, Kotra, Salamon & Sturcz, 1963) with hypervolemia acutely depressing secretion (Spat, Sturcz & Solyom, 1968). It is perhaps not surprising that secretion of the sodium retaining hormone is responsive to the extracellular conditions prevailing during sodium deficiency.

The renin-angiotensin system:

The most important regulatory mechanism for control of aldosterone secretion is the renin-angiotensin system. Renin is a hormone produced by the juxtaglomerular apparatus of the kidney and released into the circulatory system (Vander, 1967). Renin acts on a plasma substrate — angiotensinogen — to produce a decapeptide (angiotensin I) which is further hydrolysed by a converting enzyme in the lungs to angiotensin II (Vane, 1969). This final octapeptide, as well as being the most powerful endogenous vasoconstrictor, functions as the primary stimulus for the release of aldosterone (Mulrow, 1966; Binnion, 1969).

A relationship between the juxtaglomerular apparatus and aldosterone secretion has long been suspected on the basis of histological evidence. Injection of kidney extract containing renin leads to enlargement of the adrenal zona glomerulosa (Deane, Shaw & Greep, 1948). This enlargement is evidence for hypersecretion of aldosterone. Hartroft and Hartroft (1955) have reported that granulation (renin

production and storage) of the juxtaglomerular apparatus was positively correlated with zona glomerulosa size. These renin related changes in the adrenal cortex are mediated by angiotensin II (Marx, Deane, Mowles & Sheppard, 1963).

Renin-angiotensin mediation of aldosterone secretion is well understood in man and dog (Mulrow, 1966; Davis, 1967; Fraser, Brown, Chinn, Lever & Robertson, 1969), but is less clear with regard to the rat. Alterations in aldosterone secretion are closely related to activity of the renin-angiotensin system (Gross, Brunner & Ziegler, 1965). However, injections of renin or angiotensin have only a minor effect on aldosterone secretion (Eilers & Peterson, 1964; Marieb & Mulrow, 1965; Cade & Perenich, 1965) unless the doses are large (Singer, Losito & Salmon, 1964; Dufau & Kliman, 1968; Masson & Travis, 1968). The effect of angiotensin on aldosterone may be severely limited by normally high sodium turnover (Dufau, Crawford & Kliman, 1969). Thus, the importance of renin-angiotensin in increasing aldosterone in rats may be demonstrable only during sodium deprivation (Kinson & Singer, 1968). Accordingly, the loss of renin following nephrectomy interferes with the normal increase in aldosterone seen during sodium depletion (Palmore, Marieb & Mulrow, 1969) and after acute constriction of the vena cava (Eilers & Peterson, 1964). The fact that aldosterone secretion can be demonstrated in the absence of the renin-angiotensin system (Palmore, Marieb & Mulrow, 1969) does not preclude an important supportive role for the latter in aldosterone secretion.

The gross stimuli for increased activity of the renin-angiotensin

system are remarkably similar to the already mentioned stimuli for aldosterone secretion. For example, treatment with ACTH appears to stimulate renin secretion in the rat (Hauger-Klevene, Brown & Fleischer, 1969). In addition, decreases in blood volume due either to hemorrhage or acute sodium depletion are also effective (Ziegler & Gross, 1964; Gross, Brunner & Ziegler, 1965) while hypervolemia reduces renin secretion (Gordon & Sullivan, 1969). However, local renal conditions resulting from sodium deficiency and hypovolemia influence renin secretion more directly. Two mutually compatible mechanisms for renin secretion have been proposed (Vander, 1967). One conception is that changes in renal perfusion pressure are monitored by a receptor near the pre-glomerular arteriole. Thus, decreases in perfusion pressure as would accompany hypovolemia and sodium deficiency would increase renin secretion and ultimately, through angiotensin II and aldosterone, lead to enhanced sodium retention. A second hypothesis relates renin release to delivery of sodium to the macula densa area of the distal tubule adjacent to the afferent arteriole (Nash, Rostorfer, Baile, Wathen & Schneider, 1968). A decreased sodium load at the macula densa would enhance renin secretion (Vander & Miller, 1964). In addition to its effect on sodium retention via aldosterone, the renin-angiotensin system may also act at the single nephron level to alter sodium excretion (Davis & Knox, 1970).

Central control of sodium secretion:

Recent reports have suggested that receptors sensitive to sodium exist in the periventricular area of the diencephalon (Mouw & Vander, 1970; Dorn & Porter, 1970). Such receptors have long been suspected on the basis of reports of altered renal sodium handling following human brain damage (Cort, 1954). Lesion studies in rats (Keeler, 1959; Dorn & Rothballer, 1968) have implicated the hypothalamus in control of sodium excretion. In addition, hyperosmotic solutions infused into the third ventricle augment sodium excretion but only if they contain sodium (Andersson, Dallman & Olsson, 1969; Dorn & Porter, 1970). On the other hand, hypo-osmotic or iso-osmotic solutions depress sodium excretion (Mouw & Vander, 1970). These alterations of renal sodium handling occurred in the absence of significant changes in filtration rate, renal perfusion pressure, blood pressure or renal plasma flow. However, low sodium perfusions did enhance renin secretion (Mouw & Vander, 1970). Thus, the diencephalic influence on sodium retention may be partially mediated by the renin-angiotensin-aldosterone mechanism.

Summary:

In summary, physiological regulation of sodium metabolism involves renal, hormonal and central mechanisms. Over 90 per cent of the sodium filtered through the kidneys is reabsorbed from the proximal tubules in response to arterial pressure, glomerular filtration and peritubular oncotic forces. This reabsorption is generally unresponsive to body sodium requirements and is considered obligatory. Fine control of sodium excretion occurs in the distal tubules in response to the mineralocorticoid hormone, aldosterone. Although aldosterone affects only a small

fraction (2-5 per cent) of the filtered sodium, it is of vital importance to sodium balance regulation. Animals lacking aldosterone invariably die from chronic sodium loss unless sodium or hormone replacement is instituted.

Aldosterone secretion is under multifactor control. Increased production and release of aldosterone can be elicited by decreased extracellular sodium concentration, increased extracellular potassium concentration, increased ACTH or somatotrophin and increased renin-angiotensin activity. The latter factor is of primary importance for aldosterone secretion. In addition, many of the extracellular changes that augment aldosterone secretion also increase renin-angiotensin activity suggesting that they act both directly on the adrenal cortex and indirectly via the renin-angiotensin system to enhance aldosterone. It is important to emphasize that aldosterone secretion and renin-angiotensin activity are sensitive to body sodium requirements.

In addition to these renal and hormonal mechanisms, recent evidence suggests that central neural receptors for sodium concentration may be involved in regulation of sodium retention and excretion. These receptors have been located in the hypothalamus and may act via the renin-angiotensin system to alter renal sodium handling. However, central areas, with the exception of the pituitary gland, are not considered important in the direct regulation of aldosterone secretion.

It should be clear that physiological regulation of sodium balance involves the complex interaction of many factors and a multi-level sensitivity to body sodium needs.

Section II: Behavioral Factors in the Regulation of Sodium Balance

In this section, the behavioral aspect of sodium regulation and its physiological basis will be described. Since sodium deficits unavoidably occur during an organism's life, regulation of sodium implies ingestion of sodium. The physiological mechanisms just described function to minimize sodium loss during times of need, but behavior is required to replace sodium losses. Normal ingestion of sodium requires no specialized responses or control mechanisms since it will accompany every day food intake. Moreover, the rat and many other mammals, especially those whose diet precludes constant sodium availability, actually prefer moderately salty solutions to water. Thus, given the ability to detect salt and an innate saline preference, a rat should never experience a sodium deficit in a sodium rich environment. However, when losses occur, regulation requires a mechanism for recovery. It is at this point that sodium appetite becomes essential to sodium balance regulation. In order to distinguish sodium appetite from ingestion due to saline preference, appetite is defined as the ingestion of concentrated salt solutions normally avoided by the rat. Further, since increased drive would be necessary for ingestion of aversive solutions, sodium appetite is likely evoked under conditions of increased sodium need.

Existence of sodium appetite:

The significance of sodium appetite for the regulation of sodium balance was first demonstrated in rats deprived of the aldosterone-renal

sodium retention mechanism through bilateral adrenalectomy (Richter, 1936). These rats suffer continuous loss of sodium in urine and will die if large amounts of salt are not added to their diet. Thus, regulation of sodium balance by the adrenalectomized rat depends solely on the increased ingestion of sodium. In fact, increased salt appetite in the adrenalectomized rat is well documented (Bare, 1949; Epstein & Stellar, 1955; Nachman, 1962). In addition, sodium deficient rats will perform an operant act for sodium chloride solutions at rates that are proportional to the degree of depletion, demonstrating that sodium need has potent motivating properties (Lewis, 1960; Quartermain, Miller & Wolf, 1967). These rats will also ingest greater volumes of unpalatable, highly concentrated sodium chloride solutions that normal rats will not approach (Nachman, 1962).

Evidence that a net loss of body sodium elicits an increased appetite for sodium solutions also comes from the work of Denton and Sabine (1961; 1963). They have demonstrated that sheep experiencing a loss of sodium in saliva manifest an increased ingestion of sodium solutions. Intraperitoneal dialysis against glucose also results in a net loss of extracellular sodium when the volume of the dialysing fluid is removed a few hours after injection by paracentesis (Darrow & Yannet, 1935). This treatment has similarly been shown to result in an increased appetite for sodium chloride solutions (Falk, 1965).

Taste and sodium appetite:

Although it is abundantly clear that sodium deficiency elicits sodium appetite, the underlying physiological mediation for the response

remains unclear. Early investigators examined the possibility that sodium deficiency altered the gustatory sensitivity for salt. Richter (1956) noted that adrenalectomized rats ingested very dilute salt solutions that normal rats seemed to ignore when water was available. He concluded that the rats' salt taste threshold had been lowered by sodium deficiency enabling them to detect salt at lower concentrations than normal animals. It is now clear that Richter was describing a decreased preference threshold rather than a decreased sensory threshold since by his method the taste threshold was the lowest salt concentration preferred to water. It should be noted that the preferred salt concentration is not necessarily related to the actual taste threshold since a rat may detect salt at a concentration much lower than that preferred (Koh & Teitelbaum, 1961). In contrast to Richter's early findings, electrophysiological recording of taste sensitivity indicates that sodium deficiency does not alter sensory thresholds (Pfaffmann & Bare, 1950).

Although peripheral changes in the gustatory system do not occur during sodium deficiency, Pfaffmann (1964) has hypothesized that changes in nervous excitability in the central gustatory areas could result in an enhanced sensitivity to salt solutions. Recent studies of the importance of central gustatory areas for sodium appetite have yielded inconclusive results. Destruction of the sub-cortical taste relay severely attenuates sodium appetite in the sodium deficient rat (Wolf, 1968). However, lesions in the higher cortical projection areas have little effect on sodium appetite (Wolf, DiCara & Braun, 1970).

Regardless of the final resolution of this problem, it should be clear that taste plays a major role in sodium appetite since it aids the rat in selection of salt solutions. Total denervation of the peripheral taste receptors eliminates sodium appetite in adrenalectomized rats (Richter, 1939; 1956).

Hormones and sodium appetite:

With regard to a possible hormonal mediator of sodium appetite, Rice and Richter (1943) found that normal rats increase their salt intake in response to treatment with desoxycorticosterone, an intermediate in the biosynthesis of aldosterone. More recent work (Wolf & Handal, 1966) seems to support the hypothesis that endogenous aldosterone functions in the elicitation of sodium appetite in sodium deficient rats, perhaps by potentiating the effects of the deficiency. In addition, mineralocorticoid elicited sodium appetite appears to be as motivating as that induced by adrenalectomy (Quartermain & Wolf, 1967). However, even though an increased level of aldosterone may stimulate sodium appetite in normal and sodium deficient rats, it obviously cannot account for the sodium appetite following adrenalectomy.

The renin-angiotensin system has also been implicated in the elicitation of sodium appetite. As described in the previous section, sodium deficiency results in increased circulating levels of renin and angiotensin. An early report demonstrated that renin injections would increase sodium intake in normal rats (Braun-Menendez, 1953). However, it is possible that the renin injections elicited sodium appetite by

increasing aldosterone secretion. Alternatively, renin treatment may have induced sodium deficiency, since it is a strong diuretic and natriuretic in the rat (Gross, Brunner & Ziegler, 1965), thereby augmenting sodium intake. However, reduction of renin secretion in sodium deficient sheep by nephrectomy does not interfere with sodium appetite unless recovery from surgery is complicated by general behavioral depression (Bott, Denton & Weller, 1967a). The same study also demonstrated that angiotensin infusions slightly depressed sodium appetite despite increased aldosterone levels. This effect was probably not due to the angiotensin pressor effect since hypertension did not significantly modify salt appetite. Finally, a recent study by Stricker (1971b) suggests that neither sodium appetite nor saline preference will appear after sodium deficiency when renin-angiotensin activity is increased unless aldosterone secretion is also augmented. Thus, the renin-angiotensin system may be involved in sodium appetite only indirectly through its effects on aldosterone.

In view of its supportive role in the regulation of aldosterone secretion (see Section I), the pituitary might also be important for sodium appetite. However, hypophysectomized rats are as efficient as intact animals in restoring sodium balance following acute sodium deficiency (Jalowiec, Stricker and Wolf, 1970). In addition, hypophysectomy does not interfere with the development of a saline preference following sodium deficiency (Fregly, Galindo & Cook, 1961).

Hyponatremia and hypovolemia as stimuli for sodium appetite:

Recent studies of the factors involved in the mediation of sodium

appetite have demonstrated that a net loss of body sodium is not a prerequisite. Instead, internal redistributions of fluids and electrolytes that result in the intravascular concomitants of sodium deficiency (hyponatremia and/or hypovolemia) can also elicit sodium appetite. For example, subcutaneous injection of formalin destroys local capillaries allowing intravascular protein, sodium and plasma to leak into a local interstitial edema. This results in hypovolemia, hyponatremia, increased aldosterone secretion and, as might be expected, increased sodium appetite (Wolf & Steinbaum, 1965; Jalowiec & Stricker, 1970a). However, the increased aldosterone secretion is unnecessary for the appetite since formalin also enhances salt intake in adrenalectomized rats (Wolf & Steinbaum, 1965).

The hyponatremia and hypovolemia induced by sodium deficiency are potent stimuli for aldosterone secretion (Denton, 1965; see also Section I) and may be more directly involved in the elicitation of sodium appetite than actual sodium loss or increased aldosterone. Richter (1956) postulated that hyponatremia is the important stimulus for sodium appetite. However, although prolonged dilutional hyponatremia may elicit increased appetite (Tosteson, DeFriez, Abrams, Gottschalk & Landis, 1951), acute hyponatremia induced by gastric water loading has no effect on sodium intake (Stricker & Wolf, 1966). Working with sheep, Beilharz and Kay (1963) concluded that it is unlikely that the concentration of plasma sodium, by itself, is an important stimulus since a rapid fall in concentration induced by glucose infusion did not stimulate sodium appetite. Moreover, sodium deficient sheep continued

to ingest salt despite a relative increase in plasma sodium concentration produced by concurrent water deficit (Beilharz, Denton & Sabine, 1962) or hypertonic saline infusion into the cerebral arteries (Beilharz, Bott, Denton & Sabine, 1965). However, intravenous infusions of large saline loads did proportionately decrease sodium ingestion. In addition, a recent study (Jalowiec & Stricker, 1970b) clearly establishes a role for hyponatremia in the potentiation of sodium appetite by demonstrating that formalin treated rats that remain hyponatremic ingest more salt solution than rats recovered from the acute vascular effects of the treatment.

Recent evidence has also pointed out that sodium appetite may appear after simple decreases in blood volume in the absence of any alteration of plasma sodium concentration. For example, Stricker and Wolf (1966) demonstrated that subcutaneous injection of polyethelene glycol (PG) which decreases plasma volume isosmotically (Stricker, 1966), elicits salt appetite within twenty-four hours after treatment. However, with continuous access to salt and water, sodium appetite may be potentiated by hyponatremia, which develops slowly following increased renal retention of ingested water (Stricker & Jalowiec, 1970) in contrast to its immediate appearance after formalin injection (Jalowiec & Stricker, 1970a). Sodium appetite also appears gradually after PG treatment if water and salt are available immediately (Stricker & Jalowiec, 1970), whereas it is apparent within three hours after formalin (Jalowiec & Stricker, 1970a). If fluids are withheld for eight hours after injection, PG treated rats do not show a sodium appetite despite decreased

blood volume, but formalin treated rats which are hypovolemic and hyponatremic have a pronounced appetite (Stricker & Wolf, 1966). Finally, it is important to note that hypovolemia can elicit sodium appetite in the absence of aldosterone secretion (Wolf & Stricker, 1967).

Reservoir hypothesis:

At this point it would appear that increased aldosterone secretion, hyponatremia and hypovolemia are all involved in the elicitation of sodium appetite although no single factor is crucial. In order to account for this situation, Stricker and Wolf (1969) have developed the concept of a sodium reservoir receptor. The postulated receptor would initiate sodium appetite in response to reservoir depletion with hyponatremia and hypovolemia acting either directly or indirectly via aldosterone (aldosterone potentiates active sodium transport in many tissues). Although the site of the proposed reservoir is unknown, it could conceivably be located in bone (Bergstrom, 1955; see Section I).

Neuroanatomical substrates:

Rats with lesions in the lateral hypothalamic feeding area show impaired or absent sodium appetite responses to sodium deficiency or desoxycorticosterone treatment (Wolf, 1967). In addition, large lesions in the ventromedial hypothalamus at the same rostrocaudal level disrupt sodium appetite and saline preference following sodium deficiency (Novakova & Cort, 1966; Quartermain, Wolf & Keselica, 1969). These findings would suggest that mechanisms essential for sodium appetite may exist in both the lateral and medial hypothalamus. Alternatively,

ventromedial lesions may disrupt sodium appetite by severing important pathways between the lateral hypothalamus and the pituitary (Quartermain, Wolf & Keselica, 1969). However, the pituitary does not appear to be substantially involved in sodium appetite since hypophysectomized rats can effectively restore sodium balance following acute sodium deficiency (Jalowiec, Stricker & Wolf, 1970). It should be noted that hypophysectomized rats also retain the sodium they ingest suggesting that the pituitary is not vital to sodium metabolism at either a behavioral or physiological level.

Thirst and sodium appetite:

It is interesting to note the relationship between sodium appetite and thirst as behavioral regulators of intravascular fluid volume. Restoration of plasma volume requires the addition of both sodium and water. Accordingly, Falk (1965) has shown that dialysed rats not only increase their intake of hypertonic saline but also exhibit enhanced water ingestion. Formalin treated rats also substantially increase their water intake when ingesting concentrated salt solutions (Jalowiec & Stricker, 1970a).

Thirst in rats made hypovolemic by polyethelene glycol injection appears to be inhibited if only water is available and this inhibition is probably the consequence of a reduction of effective osmolality (Stricker, 1969, 1971a). However, no inhibition of thirst is evident if salt solutions are present in addition to water (Stricker and Jalowiec, 1970). As mentioned previously, polyethylene glycol treated rats initially drink water and sodium appetite appears after substantial water intake and

extracellular dilution have occurred. Thus, the appearance of sodium appetite is important for the continued ingestion of fluids and the final restoration of plasma volume. This relationship might be expected since restoration requires the addition of water and salt in isotonic quantities. In fact, the drinking behavior of rats after formalin injection suggests that they alternately ingest concentrated salt solution and water so that the final concentration of ingesta is isotonic (Jalowiec & Stricker, 1970a).

Interaction of behavioral and physiological regulation:

Hyponatremia and hypovolemia have been shown to result in increased circulating levels of aldosterone (Solyom, Kotra, Salamon & Sturcz, 1963; see Section I), and hypovolemia enhances anti-diuretic hormone secretion (Ginsburg & Brown, 1956). Thus, the hyponatremia and hypovolemia resulting from sodium deficiency are not only effective stimuli for sodium appetite and thirst but also for salt and water retention. Recently, Jalowiec and Stricker (1970a) have shown that the sodium deficient rat uses both the behavioral and physiological regulatory mechanisms to restore plasma volume and sodium concentration. Sodium appetite and thirst as well as renal retention of salt and water appear concurrently following the induction of acute sodium deficiency and continue until plasma volume and sodium concentration have been restored. When restoration is complete, renal retention ceases, but curiously, sodium appetite and thirst continue for some time. The sodium and water ingested in excess of need are rapidly excreted. This uncoupling of the

behavioral and physiological responses to acute sodium deficiency suggests that sodium appetite may not be as sensitive to sodium requirements as renal retention.

Clearly, the close interaction between behavioral and physiological regulatory mechanisms is necessary since the inactivation of either mechanism prohibits efficient restoration of sodium deficits. For example, the adrenalectomized rat deprived of the renal retention mechanism exhibits a chronically increased sodium intake (Richter, 1936). On the other hand, if only water or no fluids are available after acute sodium deficiency, renal retention of salt and water are evident until the salt solution finally becomes available (Jalowiec & Stricker, 1970b). Thus, the interference with either the behavioral or physiological responses severely limits the rat's ability to deal with sodium deficiency.

Specificity and learned vs. innate considerations:

The increased sodium appetite in sodium deficient rats appears to be specific to the sodium ion. Richter and Eckert (1938) tested adrenalectomized rats in two-bottle, 24 hour preference tests and found that the sodium deficient rats showed marked preferences for most sodium salts but not for non-sodium solutions. These results have been confirmed and extended to other salt solutions by Fregly (1958) using 24 hour tests and by Nachman (1962) in brief access tests. The specificity of sodium appetite seems to be based, at least in part, on the taste of salt. Nachman (1963) has shown that adrenalectomized rats will ingest lithium chloride which, although tasting like sodium chloride, is extremely

toxic and does not ameliorate the sodium deficiency. Finally, Rodgers (1967) has demonstrated that the sodium deficient rat's preference for salty foods is not a response to the novelty characteristic of a new diet.

The rapid appearance of sodium appetite in sodium deficient rats has led many investigators to question the role of learning in development of the response (Epstein & Stellar, 1955; Nachman, 1962; Handal, 1965). The possibility of a learned basis for sodium appetite has been minimized on the basis of the following data. First, sodium deficient rats usually show immediate preferences for sodium solutions (Nachman, 1962; Handal, 1965). Secondly, Mook (1969) has shown that esophagostomized, adrenalectomized rats increase hypertonic saline intake even though no relief of deficiency occurs. Finally, sodium deficient sheep replace their sodium deficits so rapidly that post-absorptive need reduction does not occur until after drinking is complete (Bott, Denton & Weller, 1967b). All of these data suggests that sodium appetite does not depend on post-absorptive relief of sodium deficiency, i.e., reinforcement via need-reduction.

Of course the possibility remains that animals experience some degree of sodium deficiency during their pre-experimental life and have the opportunity to relate satisfaction of this deficiency with salt intake. This factor is minimized by the more than adequate supply of sodium in laboratory animal diets. It is also possible that salt solutions are selected on a random basis with the rapid acceptance and ingestion resulting from reinforcing taste stimulation (Denton & Sabine, 1963; Richter, 1956). Thus, the immediate acceptance of salt solutions might be based on concurrent reinforcement and learning could be inferred.

However, the only major suggestion that a relationship between sodium preference and repair of sodium deficits is learned rather than innate is the finding of Smith, Pool and Weinberg (1958). They esophagostomized rats so that ingested fluids could not reach the stomach. When these rats were later made sodium deficient, they showed no salt preference. The authors concluded that salt appetite only occurs as a consequence of salt reaching the gut. Most investigators now accept that an innate mechanism, probably based on some alteration of the animal's attitude toward salt, perhaps because of changes in the gustatory system, is responsible for mediating salt appetite during sodium deficiency.

The learned versus innate controversy has recently taken on a new dimension. The fact that sodium deficient rats will show an immediate preference and appetite for sodium, even in the absence of post-absorptive feedback, suggests that salt selection and appetite are preceded by directed motivation toward salt. Evidence for this notion requires that the drive state be demonstrated without providing salt as reinforcement. Krieckhaus and Wolf (1968) accomplished this by training thirsty rats to bar press for sodium or non-sodium solutions. When later made sodium deficient and tested without fluid reinforcement, the rats trained with sodium solutions pressed more than those trained with non-sodium fluids. In addition, Krieckhaus (1970) has been able to show that rats can learn the location of sodium in a T-maze when thirsty and utilize the information when subsequently sodium depleted for the first time. Additional evidence that inexperienced sodium deficient rats seek salty tasting fluids comes from studies by Stricker and Wilson (1970). Rather

than train their rats in a situation in which salty fluids were neutral (as in Krieckhaus & Wolf, 1968), they used a learned aversion paradigm (Revusky & Garcia, 1970) so that their rats completely avoided salty fluids. Nevertheless, following sodium deficiency, the rats resumed intake of the salty fluids suggesting that the drive was powerful enough to overcome past negative experiences with salt.

Thus, awareness of the required substance seems to precede and thereby probably directs the animal's behavior toward sodium acquisition. According to a recent summary by Wolf (1969), the innate sodium appetite system might work in the following manner....

> "When the sodium drive is activated, the taste of sodium is charged with reinforcement value and stimuli associated with previous sodium ingestion gain secondary reinforcement value....sequential secondary reinforcements, increasing in intensity as the goal is approached, guide the sodium deficient rat toward places or responses which previously yielded the primary reinforcer—sodium."

Summary:

Sodium deficiency elicits responding on both a physiological and behavioral level. Sodium appetite is essential to the amelioration of sodium deficiency since the physiological renal response can only insure that further losses do not occur and that ingested sodium is retained.

The fact that sodium deficient rats will show an immediate

preference and appetite for sodium, even in the absence of post-absorptive feedback, suggests that salt selection and appetite are preceded by directed motivation toward salt. It appears that the sodium deficient rat is innately aware of the characteristics of the substance that will restore its deficiency. The sense of taste probably plays an important role in this unlearned response by helping the rat identify and select salt solutions. In its absence, adrenalectomized rats die without ingesting sodium. Nevertheless, sodium deficiency does not alter peripheral taste sensitivity for salty solutions although it may change nervous excitability of central gustatory areas.

Many factors have been postulated as mediators of sodium appetite. Actual sodium loss is not essential since internal redistributions of sodium and plasma elicit sodium appetite. Instead, the effects of sodium deficiency on aldosterone secretion, plasma sodium concentration and plasma volume appear to be more directly involved in the elicitation of sodium appetite. However, none of these factors alone would seem to be essential. Increased aldosterone can elicit sodium appetite in normal animals, but adrenalectomized rats show an appetite in the absence of aldosterone. The importance of hyponatremia is controversial although it does appear to potentiate sodium appetite. However, sodium appetite can also be elicited by hypovolemia unaccompanied by either hyponatremia or increased aldosterone secretion. An attempt has been made to link all of these factors to a sodium reservoir receptor that would be sensitive to hyponatremia and hypovolemia perhaps via aldosterone and would initiate sodium appetite in response to depletion of the reservoir.

Sodium appetite and thirst are often combined as behavioral regulators of intravascular fluid volume since restoration of plasma deficits requires the ingestion of both salt and water. In addition, recent research has demonstrated that the behavioral responses to sodium deficiency interact with physiological renal responses to restore plasma volume and sodium concentration as efficiently as possible. This close interaction is to be expected since intravascular effects of sodium deficiency are potent stimuli for aldosterone and anti-diuretic hormone secretion as well as sodium appetite and thirst. Interference with either the behavioral or physiological responses makes regulation of body sodium difficult and highly dependent on the remaining response mechanism.

EXPERIMENT 1

SODIUM APPETITE FOLLOWING FUROSEMIDE-INDUCED SODIUM DEFICIENCY: POTENTIATION BY ALDOSTERONE

Proper assessment of the relationship between sodium appetite and sodium need requires measures of the degree of sodium deficiency as well as consequent intake. Until recently, few attempts have been made to assess external sodium loss prior to salt intake in rats. Falk (1965) and Wolf (1964) reported that salt ingestion greatly exceeded sodium loss from intraperitoneal dialysis. Similar findings have also been reported for rats losing sodium in urine during chronic treatment with a diuretic (Novakova & Cort, 1966). This apparent behavioral over-compensation for sodium need has also been demonstrated in the absence of external sodium loss. Subcutaneous injections of either formalin or polyethylene glycol elicit a sodium appetite via an internal redistribution of body fluids which also results in marked sodium and water retention (Jalowiec & Stricker, 1970a; Stricker & Jalowiec, 1970). Following these treatments, sodium need (internal loss) could only be estimated as the amount of ingested sodium that was retained. These estimates appeared valid since sodium retention ceased when plasma volume and sodium concentration had been restored. Curiously, sodium appetite continued long after internal deficits had been restored and sodium retention had ceased, with the

excess sodium rapidly excreted. Thus, the sodium appetite of rats appears to be characterized by behavioral over-compensation for sodium deficits.

The primary purpose of this study is to examine the role of endogenous aldosterone levels in the over-drinking of salt by sodium deficient rats. Recent findings have suggested that sodium appetite in rats recovered from the acute body fluid disturbances of formalin treatment may have been potentiated by increased aldosterone levels (Jalowiec & Stricker, 1970b). Aldosterone secretion can be augmented in normal rats simply by maintenance on a sodium deficient diet for a few days (Marieb & Mulrow, 1965). Accordingly, some rats were maintained on a sodium deficient diet which enhanced endogenous aldosterone levels, while other rats were maintained with access to a sodium replete diet so that mineralocorticoid activity remained normal. Following this manipulation of endogenous aldosterone levels, acute sodium deficiency was induced by the powerful diuretic and natriuretic, furosemide, and consequent sodium appetite and its relationship to sodium loss was examined.

METHODS

Subjects and pretreatment maintenance:

Male albino rats (Sprague-Dawley), weighing 300-350 gm at the start of the experiment, were housed in individual metabolism cages. The laboratory was continuously illuminated and temperature controlled (24-26 C). All animals were permitted free access to demineralized water and 0.51 M sodium chloride solution, available from graduated

tubes (± 1.0 ml) with stainless steel drinking nozzles, and pelleted Purina Laboratory Diet (Na+ replete; 150.0 mEq/kg).

Procedure:

Furosemide is an anthranillic acid derivative (4-chloro-N-2-furylmethyl-5-sulfamoyl-anthranillic acid), chemically distinct from the organomercurials, thiazides and other heterocyclic compounds used as diuretics. Pure furosemide powder[1] was mixed with a slightly alkaline (pH=7.4) 0.15 M sodium bicarbonate solution since it precipitates as a carboxylic acid, at a pH below 7.0.

Furosemide enters the tubular fluid of nephrons mainly through secretion because its marked protein-binding characteristic prohibits glomerular filtration (Deetjen, 1966). It appears to inhibit sodium reabsorption by interfering with active sodium transport rather than by altering tubular permeability (Dirks & Seely, 1969). Micropuncture studies have demonstrated that this inhibition occurs in the proximal tubule (Brenner, Keimowitz, Wright & Berliner, 1969), the ascending loop of Henle (Morgan, Tadokoro, Martin & Berliner, 1970) and perhaps the distal tubue as well (Dirks & Seely, 1969). The site of action in the loop of Henle is unique among diuretics and accounts for the superior natriuretic and diuretic properties of furosemide.

Preliminary studies were conducted to determine the optimal dose required to produce maximal sodium loss. Forty-six rats were given single intraperitoneal injections of 1.0 ml of either 0, 1.0, 2.5, 5.0, 10.0

1. Generously supplied by Dr. R. A. Malo of Hoechst Pharmaceuticals of Montreal.

or 20.0 mg of furosemide. Following injection, the rats were deprived of food and fluid and urine excretions were collected every half-hour for two hours as described below. This two hour collection period was sufficient since the onset of the diuresis was very fast (3-5 min) and approximately 75% of the total urine volume and sodium loss occurred within the first hour. The rats excreted little urine during the second hour and generally were anuric for the next several hours. In addition, urine sodium concentrations began to decrease while potassium concentrations increased, suggesting the onset of sodium retention mediated by increased aldosterone activity (Simpson and Tait, 1952; Johnson, 1954). The maximal sodium loss was produced by 10.0 mg injections. Smaller doses clearly elicited a diuresis and natriuresis but the losses were less reliable. Doubling the optimal dose did not increase the urine volume or sodium content suggesting that a response "ceiling" had been reached (Fig. 1). Adverse behavioral reactions were never seen following any of the administered doses. In this regard, the drug manufacturer (Hoechst) reports an LD-50 of 680 mg/kg body weight (200-240 mg for the rats in this study) when furosemide is injected intravenously.

Six days prior to furosemide injection, an additional 40 rats, previously maintained on the sodium replete diet (NaR) with water and 0.51 m NaCl solution, were divided randomly into two equal groups. One group remained on the NaR regime while the other was permitted access to a sodium deficient diet (NaD). This pelleted diet was especially prepared by General Biochemicals Co. to contain less than 0.00009 mEq Na^+/kg and normal potassium levels (114.0 mEq/kg). Body weights were recorded to insure that the new diet had not led to anorexia. In

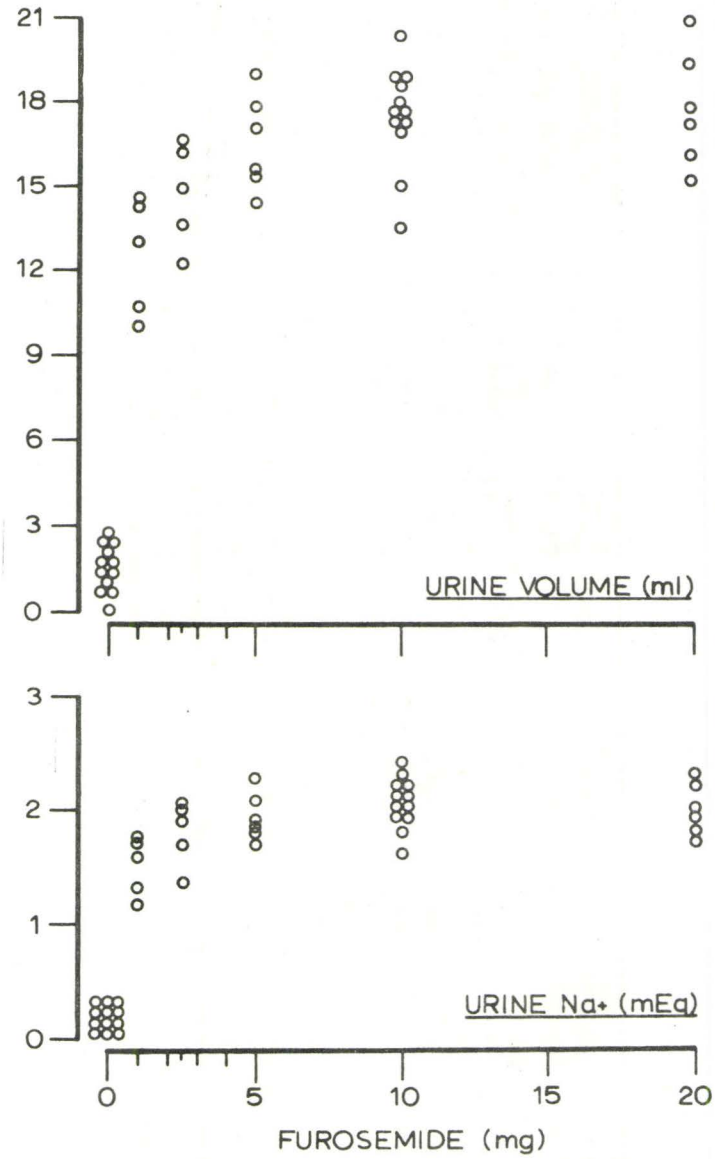

Fig. 1. Urine volume and urine sodium content after injection of various doses of furosemide. Each circle represents a different determination, two hours after injection.

addition, continuous sodium balance was calculated for the NaD rats by analysing daily urine samples for sodium content and subtracting sodium loss from sodium intake (Jalowiec & Stricker, 1970a). Fecal sodium was found to be negligible. Cage interiors were sponged daily to avoid possible accumulation of salts even though cage rinses yielded no measurable amounts of sodium.

On the morning of the seventh day, all animals were weighed and then deprived of food and 0.51 M NaCl solution. Water drinking tubes were refilled and urine collection funnels with feces screens were placed beneath the cages. Ten rats from each dietary program were injected with 10.0 mg of furosemide. The other animals were injected with 1.0 ml of the 0.15 M sodium bicarbonate vehicle. Urine was collected in graduated tubes (±0.1 ml) attached beneath the funnels and water intakes were monitored hourly for 12 hours and then at 24 hours. Urine sodium and potassium concentrations were determined by flame photometry (Instrumentation Laboratories).

The sodium replacement test was begun at the end of the 24th hour after injection when the drinking tube containing 0.51 M NaCl was returned to the cages. Intakes and excretions were recorded subsequently at 5, 10, 15, 20, 30, 60, 120 and 180 min. The salt drinking test and urine collections were then terminated, body weights were recorded, and food was returned to the animals' cages. Final water and salt solution intakes were measured the following morning (21 hr later).

Blood Analyses:

In order to determine the intravascular effects of furosemide injection, an additional 75 rats were used for blood sampling. Some animals were maintained on the NaR regime, injected with furosemide, and either were allowed access to water (n=15) or were water deprived (n=22) for 2, 6, or 24 hours before being bled. Other animals, maintained on the NaD regime, were injected with furosemide and allowed access to water before being bled as above (n=15). NaD rats were also bled 24 hours after furosemide following 15 minutes (n=5) or 2 hours (n=5) of 0.51 M NaCl access. Finally, uninjected control rats were bled after NaR maintenance (n=5) or NaD maintenance (n=8). All rats were anesthetized with ether and blood samples were withdrawn from the abdominal aortas and immediately analysed for hematocrit (microcapillary tubes), plasma sodium and potassium concentrations (flame photometer), plasma protein content (refractometer) and plasma osmolality (Advanced Instruments Osmometer).

RESULTS

Maintenance on a sodium deficient diet clearly resulted in marked sodium retention and high urinary potassium/sodium ratios (see vehicle injected-NaD maintained group, Fig. 2). These indirect indicators of high endogenous aldosterone activity are supported by actual measurements of secretory rates reported by Marieb and Mulrow (1965). Few

animals ingested enough 0.51 M NaCl solution during the maintenance period to prevent a slight negative sodium balance (mean sodium balance of 20 rats = -0.45 ± 0.1 mEq). In most rats virtually all of this loss occurred within the first two days of NaD maintenance. The following four days were characterized by almost total sodium retention and extremely low urine sodium concentrations (less than 2.0 mEq/l). Thus, the rats maintained on the sodium deficient diet began the test on the seventh day with high endogenous aldosterone levels but only minor negative sodium balance. In contrast, NaR maintained rats (vehicle injected) showed normally variable urine sodium concentrations and low urine potassium/sodium ratios (Fig. 2) indicating no sodium retention and low levels of aldosterone (n.b. urine was not collected during NaR maintenance because food particles contaminated samples).

Injection of furosemide resulted in significant increases in water intake and urine excretion in both the NaR and NaD maintained rats when compared to their respective controls (all p's < .001; 2-tailed t test; Fig. 3). The enhanced water intake was apparent within two hours after injection and appeared during the period of maximum diuresis. Water intakes were comparable in both furosemide injected groups during the initial 12 hours, but after 24 hours, NaD maintained rats had ingested significantly more water than NaR maintained rats ($p < .01$). A similar difference in 24 hour intake

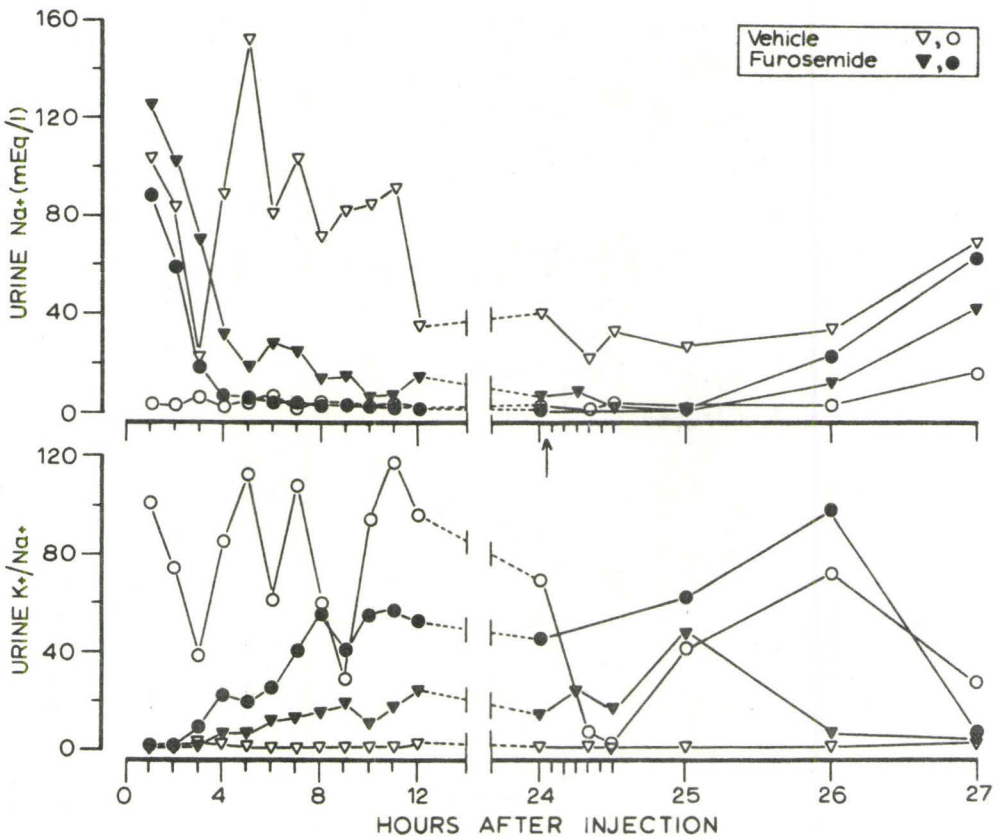

Fig. 2. Urine sodium concentrations and urine potassium/sodium ratios after injection of furosemide or vehicle. Circles represent values from rats maintained on the sodium deficient diet, inverted triangles represent values from rats maintained on the sodium replete diet.

can be seen for the vehicle injected groups ($p < .05$) and thus, can probably be ascribed to an effect of the diet rather than to some aspect of the diuretic. Urine volume losses were similar for both furosemide-treated groups. The diuresis was complete within two hours and was followed by an extended period of approximately normal urine flow (note the similar slopes for cumulative urine loss in diuretic and vehicle-injected rats after two hours; Fig. 3). As a result of the large urine volume loss and the failure of the enhanced water intake to match that extensive loss, "fluid retention" (i.e., volume intake-urine output) was significantly decreased in both furosemide-injected groups (both p's $< .001$, Fig. 4). In contrast, fluid intakes and urine losses were approximately equal in each of the vehicle-injected groups resulting in fluid retention near zero.

Corresponding to the large increases in urine volume after furosemide injection, urine sodium loss was significantly increased during the initial 12 hours (both p's $< .001$; Fig. 5). However, note that sodium loss was significantly greater for the NaR maintained group ($p < .001$) and that this difference was apparent three hours after furosemide injection. A similar difference was seen between the two vehicle injected groups but was not evident until after 24 hours food deprivation (Fig. 5). The vehicle injected rats maintained on the NaD regime lost virtually no sodium during the 24 hour test as might be expected since they began the test with evidence of sodium retention.

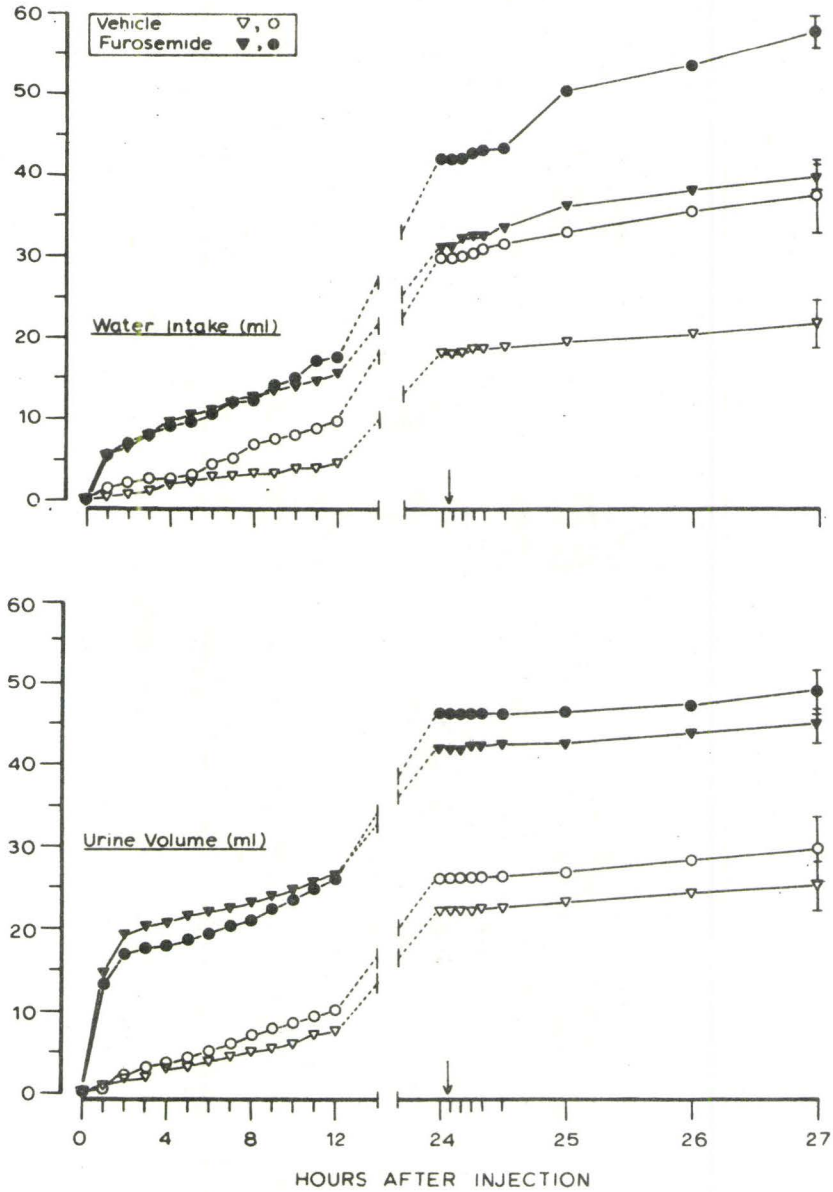

Fig. 3. Water intakes and urine volumes after injection of furosemide or vehicle. Symbols as in Fig. 2.

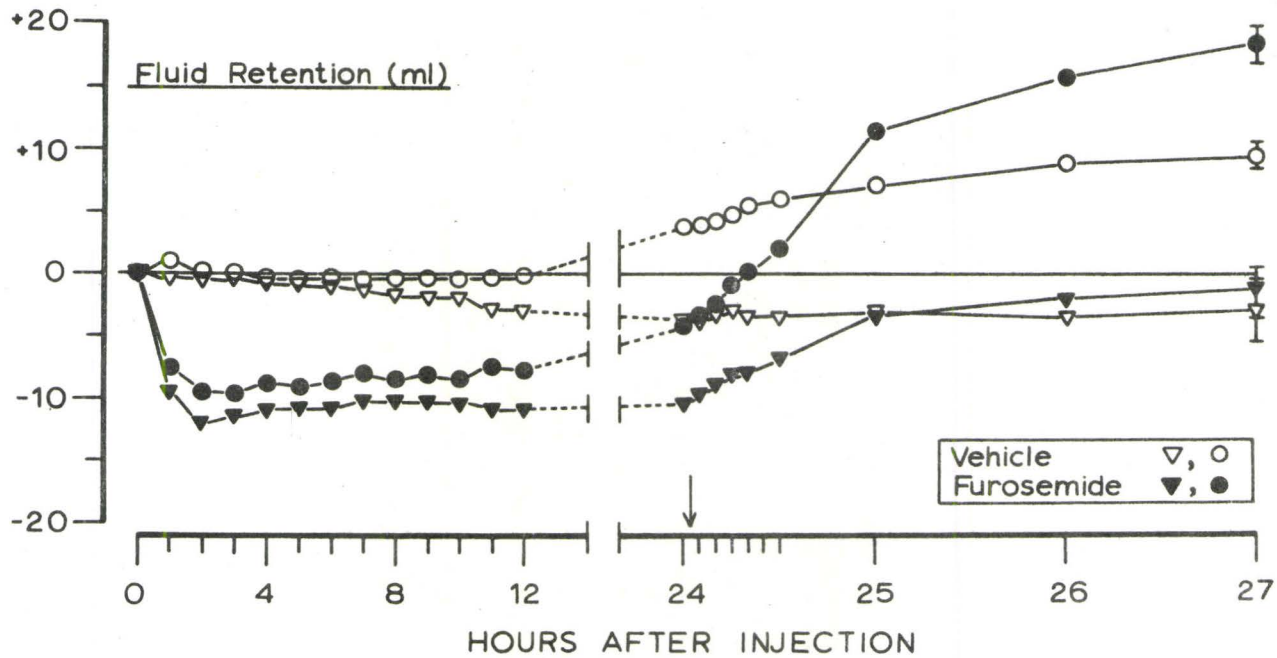

Fig. 4. Fluid retention (fluid intake - urine volume) after injection of furosemide or vehicle. Symbols as in Fig. 2.

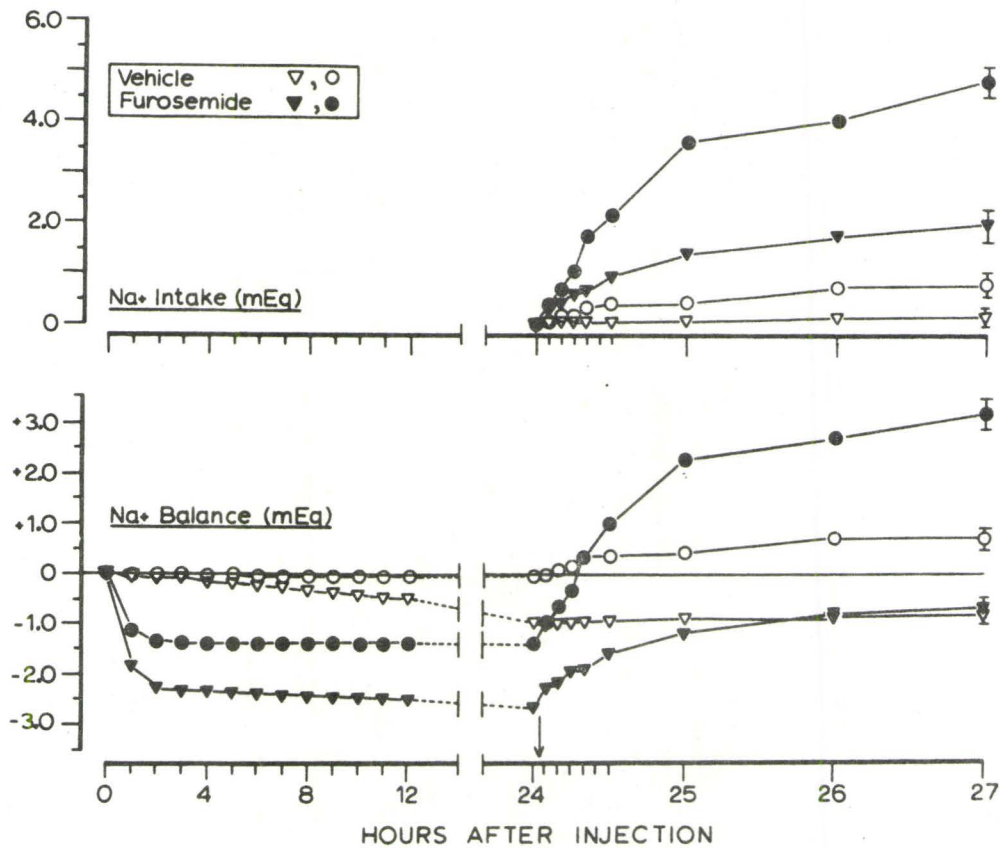

Fig. 5. Sodium intake and sodium balance after injection of furosemide or vehicle. Symbols as in Fig. 2.

However, their NaR maintained counterparts slowly excreted approximately 1.0 mEq Na+ (Fig. 5). These differences in sodium loss were the result of differences in urine sodium concentrations (Fig. 2), since urine volume losses were similar in both furosemide groups and both control groups (Fig. 3). The furosemide-injected animals showed a brief initial period of slightly elevated urine sodium levels which soon decreased to the low levels seen in animals that retain sodium actively (Jalowiec & Stricker, 1970a). Note that for the NaD maintained rats this natriuresis represents an increase from absolutely minimal concentrations followed by a rapid return to those low levels.

Calculation of urinary potassium/sodium ratios to estimate mineralocorticoid activity (Simpson & Tait, 1952; Johnson, 1954) demonstrated that aldosterone activity was most evident in the NaD maintained animals (Fig. 2). In contrast to the NaD maintained controls, the NaR maintained controls had ratios of approximately 1.0 showing no evidence of sodium retention or mineralocorticoid activity. Following the natriuretic period associated with furosemide injection, electrolyte ratios increased to 20 - 60, but the rate and degree of increase were enhanced in the NaD group suggesting higher levels of circulating aldosterone.

Both furosemide-treated groups began to drink immediately when the 0.51 M NaCl solution was returned at the end of the 24th hour after injection (Fig. 5). More rapid salt drinking was evident in the animals previously maintained on the sodium deficient diet. By the end of three hours, both furosemide-injected groups had significantly increased

their salt intakes in comparison to their respective controls (both p's<.001), but the NaD maintained rats ingested substantially more salt than the NaR maintained group ($p<.05$). These three hour sodium intakes represent essentially all of the sodium appetite elicited by furosemide since intakes over the following 21 hours when food was available were small (approximately 1.5 and 0.7 mEq Na^+ for the NaD and NaR maintained groups). There was no evidence of a sodium appetite in either of the vehicle-treated control groups.

The rapid salt intakes of the furosemide-injected rats resulted in the increases in sodium balance observed during the three hour replacement test (Fig. 5). For the rats previously maintained on the NaD regime, sodium intake was grossly in excess (200%) of the sodium deficit established by natriuresis. This behavioral over-compensation was evident within the initial 30 minutes of salt access and was not appreciably reduced during the next 2.5 hours by enhanced sodium excretion. Nevertheless, gradual increases in urine sodium concentration and decreases in electrolyte ratios suggested that sodium retention was waning (Fig. 2). The sodium intake of the other furosemide-injected group (NaR maintained) was more appropriate to their sodium deficit since it restored sodium balance to the level tolerated by their vehicle-treated controls (the significance of the negative level is considered in the following discussion). With regard to the two control groups, sodium balance increased only slightly in the NaD maintained rats and remained essentially unchanged in the NaR maintained group. Water intakes did not increase substantially in either of the vehicle-injected

groups during the three hour salt replacement test but furosemide-injected animals showed some increased water intake following 0.51 M NaCl drinking (Fig. 3).

Blood Analyses:

The animals used to examine the intravascular effects of furosemide injection showed sodium deficits and fluid intakes (when water or salt solution were available) similar to those just described. Analyses of aortic blood samples from non-injected rats demonstrated that differential dietary maintenance had no consistent effect on the blood parameters measured (Fig. 6 & 7). Furosemide injection evoked little change in plasma sodium concentration unless the animals were water deprived (Fig. 6). The significant increases in plasma sodium ($p<.05$) in water-deprived rats were probably due to the dehydrating effects of the extensive loss of hypotonic urine induced by furosemide. Nevertheless, these animals experienced sodium deficits comparable to those previously described. Plasma potassium concentrations were depressed, but not significantly, following furosemide (Fig. 6). However, the diuresis and natriuresis rapidly produced hypovolemia (plasma volume deficit). Both hematocrit and plasma protein concentrations were increased within two hours of treatment ($p<.02$; $p<.05$ respectively) and remained elevated for 24 hours (Fig. 7). The ingestion of water (approximately 30.0 ml in 24 hrs) had little effect on hypovolemia since water alone cannot restore plasma volume (Stricker & Wolf, 1967). In accordance with the absence of substantial alterations in plasma electrolytes, no reliable changes were evident in

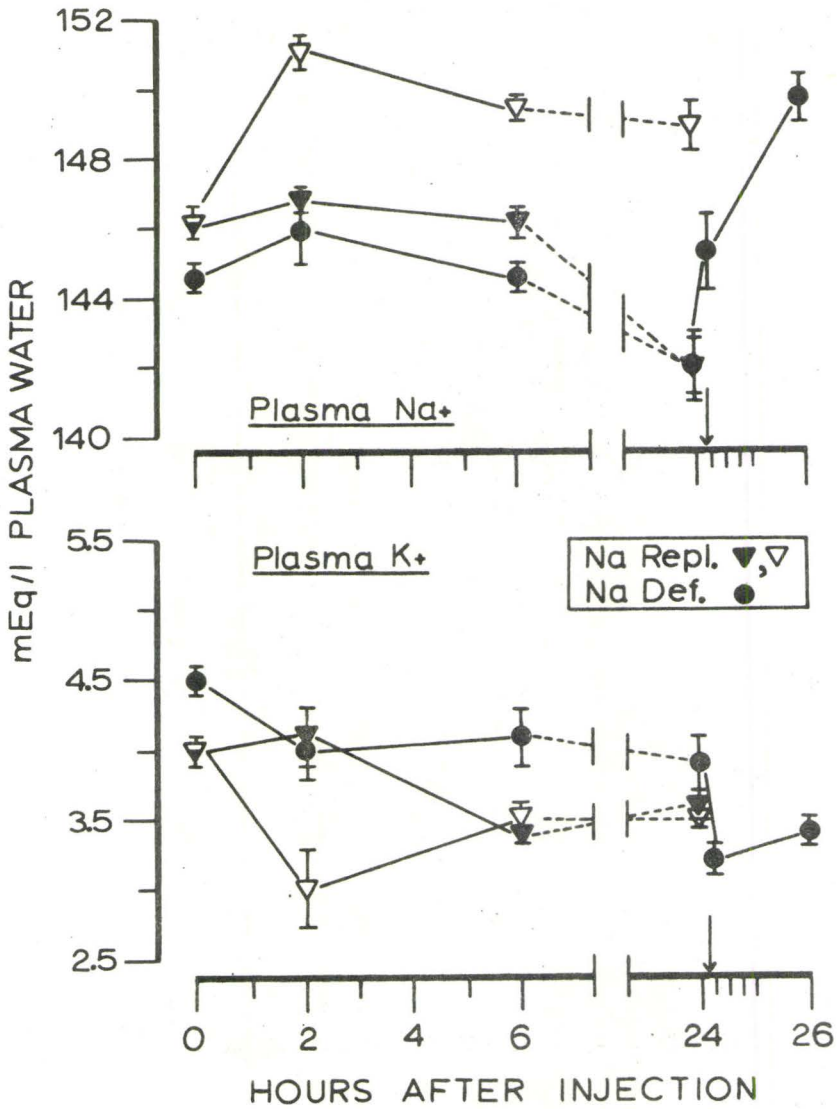

Fig. 6. Plasma sodium and plasma potassium concentrations after furosemide injection in rats maintained on either the sodium replete or sodium deficient diet. Open inverted triangles represent values from water-deprived rats. Access to 0.51 M NaCl indicated by the arrow.

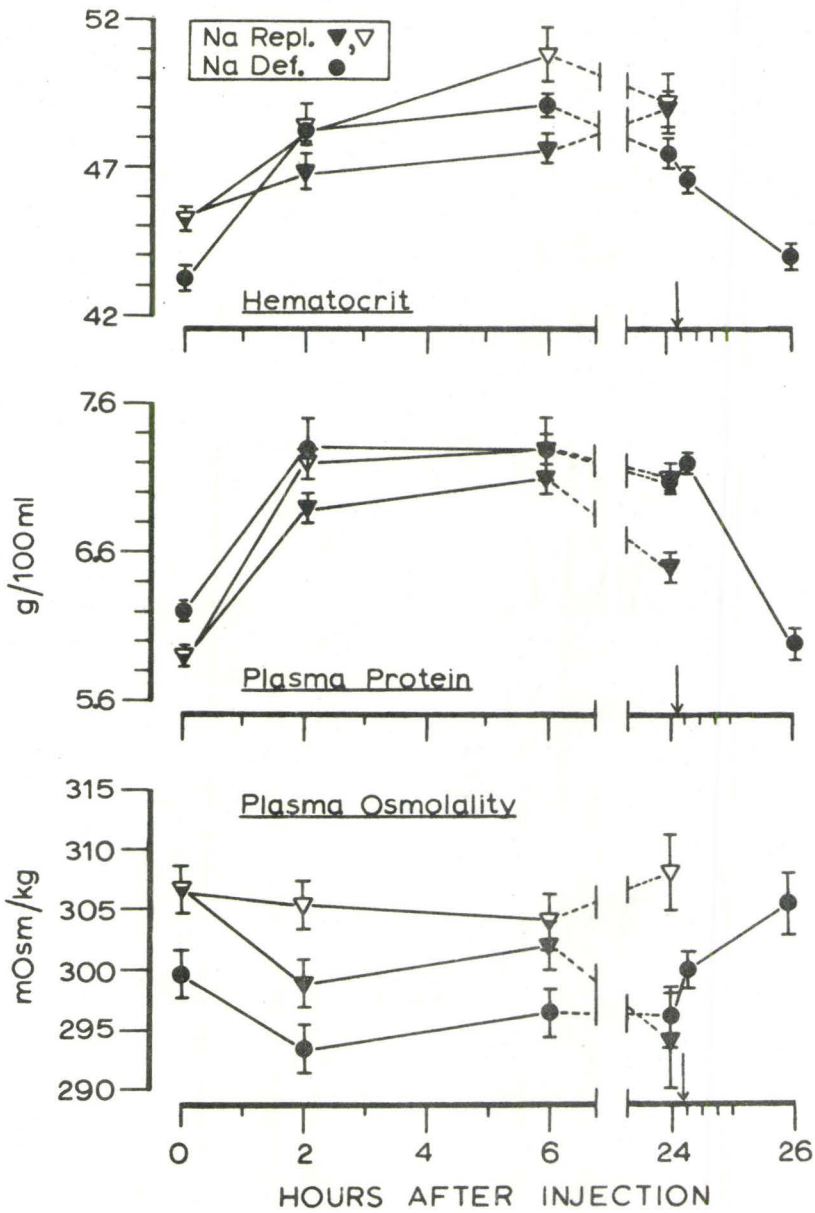

Fig. 7. Hematocrit, plasma protein and plasma osmolality values after furosemide injection in rats maintained on either the sodium replete or sodium deficient diet. Symbols as in Fig. 6.

plasma osmolality (Fig. 7).

Blood samples from furosemide-injected rats permitted access to 0.51 M NaCl (24 hours post-injection) provided information on the intravascular effects of sodium replacement. As can be seen from Fig. 6, plasma sodium concentrations were within the normal range after 15 minutes of NaCl access but were significantly elevated after two hours ($p<.05$). Plasma osmolalities were also increased after two hours of access to concentrated NaCl solution (Fig. 7). These changes may not be the result of absorption of sodium from the gastrointestinal tract, but instead may reflect movement of extracellular water into the gut in response to the hypertonicity of ingested fluid (O'Kelly, Falk & Flint, 1958). Plasma volume was clearly restored after two hours of access to 0.51 M NaCl since plasma protein concentration and hematocrit had returned to normal (Fig. 7).

DISCUSSION

In many respects, the vascular effects of furosemide injection are reminiscent of changes observed following polyethylene glycol (PG) treatment. Subcutaneous PG injection results in uncomplicated hypovolemia by drawing isotonic vascular fluid into an interstitial edema (Stricker, 1966). Furosemide injection also produced hypovolemia but the fluid loss is external and plasma sodium concentration is increased due to the hypotonic diuresis. Both of these treatments can be contrasted to formalin injection which results in hyponatremia (decreased plasma sodium concentration) as well as hypovolemia (Jalowiec & Stricker, 1970a).

All three of these treatments enhance water drinking but, whereas the thirst following PG or formalin injection has been attributed to hypovolemic stimuli (Stricker, 1966; Jalowiec & Stricker, 1970a), the thirst observed after furosemide injection may have been elicited by a complex interaction of hypovolemic and osmotic (cellular dehydration due to hypernatremia) stimuli (Corbit, 1968).

The balance studies demonstrated that furosemide-evoked sodium loss is a powerful stimulus for sodium appetite and thirst as well as for sodium retention once the initial natriuresis is complete. In view of previous suggestions that sodium appetite is not closely related to sodium deficiency (see introduction), furosemide-injected rats were expected to over-compensate for the deficits established by the natriuresis. Surprisingly, behavioral over-compensation was only evident in the furosemide injected group previously maintained on a sodium deficient diet (Fig. 5). The degree of over-drinking in these animals provides a striking contrast to the precise sodium replacement behavior of sheep (Denton, Orchard & Weller, 1969). In addition, note that the over-compensation was evident within the first 30 minutes of salt access. Plasma sodium values from animals bled after 15 minutes of 0.51 M NaCl access indicated that some restoration of extracellular sodium levels might have begun shortly after salt ingestion. However, the rapidity with which over-drinking of salt occurred suggests that previously postulated cues for satiation of sodium appetite, such as oropharyngeal metering (Denton & Sabine, 1961), taste (Nachman & Valentino, 1966), or gut distention and absorption of ingested sodium (Mook, 1963; 1969)

are not effective enough to prevent behavioral over-compensation. In this regard, the present studies increase the plausibility of a reservoir receptor for sodium need whose activity would rapidly signal sodium appetite but would diminish only slowly consequent to restoration of reservoir sodium (Stricker & Wolf, 1969). A rapid and precise satiety mechanism may not be a vital requirement since excessive ingestion of sodium would not be harmful unless complicated by renal failure and the absence of water.

Furosemide injection also enhanced sodium intake in rats maintained on the sodium replete diet, but the ingestion was more gradual and significantly less than that of NaD maintained animals despite a significantly greater sodium deficit during the preceding 24 hours (Fig. 5). If net sodium loss were the only determinant of consequent sodium intake, then NaR maintained animals should have ingested more sodium, not less, than the NaD maintained group. The explanation of this paradox is uncertain, but could involve differences in sodium balance between the NaD and NaR maintained groups at the start of sodium deprivation. NaR maintained rats may have had an excess of body sodium in comparison to NaD maintained animals which were in zero or slightly negative sodium balance. Assuming a daily food intake of 20-25 gm, the rats maintained on the sodium replete diet (150 mEq Na^+/kg) had been ingesting 3.0-3.8 mEq Na^+/day necessitating a substantial daily excretion. This obligatory sodium loss, represented in part by the gradual excretion of approximately 1.0 mEq Na^+ by the vehicle-injected NaR maintained group during sodium deprivation, could

have been accelerated by furosemide injection. Thus, differences in sodium loss between the NaR and NaD maintained groups can probably be accounted for by an obligatory sodium loss required of the NaR group. Accordingly, the negative sodium balance of the vehicle-injected NaR group during the sodium deprivation period probably represents a level comparable to the sodium balance of NaD maintained rats at the start of sodium deprivation. Yet in contrast to the over-compensation noted in the sodium replacement behavior of the NaD maintained rats, the salt intake of NaR maintained rats injected with furosemide was precisely sufficient to re-establish sodium balance at the level tolerated by control rats (Fig. 5).

Acute sodium loss elicited gross behavioral over-compensation for the established deficit in rats maintained on a sodium deficient diet, but resulted in apparently precise replacement behavior in rats maintained on a sodium replete diet. What is the explanation for this difference? Previous reports have demonstrated that maintenance on a sodium deficient diet increases aldosterone secretory rates (Marieb & Mulrow, 1965). In the present study, urine sodium concentrations and urinary electrolyte ratios of vehicle-injected rats showed that NaD maintained rats were actively retaining sodium at the beginning of the food deprivation period while NaR maintained animals showed no evidence of enhanced mineralocorticoid activity (Fig. 2). Thus, the NaD maintained rats had high aldosterone levels even before furosemide injection while the NaR maintained group did not show enhanced urinary potassium/sodium ratios and sodium retention until well after (5 hours)

injection. This evidence taken in conjunction with the results of the sodium replacement test suggests that high endogenous aldosterone levels could be responsible for the potentiation of sodium appetite in the NaD maintained group.

The role of aldosterone as a primary stimulus for sodium appetite is controversial in view of the marked salt appetite of adrenalectomized rats (Richter, 1936) and the natrorexigenic effects of hypovolemia and hyponatremia in adrenalectomized rats (Wolf & Steinbaum, 1965; Wolf & Stricker, 1967). In addition, although dose-response studies have shown that small injections of aldosterone can augment salt intake in normal rats (Wolf & Handal, 1966) some authors consider any exogenous dose to be unphysiological (Denton, Nelson, Orchard & Weller, 1969). At present, it is unlikely that any single stimulus can account for the elicitation of sodium appetite. Instead, it would appear that many stimuli, such as increased aldosterone levels, hypovolemia, hyponatremia, perhaps acting to deplete some sodium reservoir, are sufficient but not necessary for the elicitation of sodium appetite (Stricker & Wolf, 1969). The nature of the interaction between these various stimuli, which usually are all present during acute sodium deficiency, is obscure.

In the present experiment, furosemide treatment had comparable effects on blood volume and plasma sodium whether rats received NaR or NaD maintenance. Therefore, it seems that high endogenous aldosterone levels were probably responsible for the potentiated sodium appetite and consequent over-compensation seen in rats maintained on the sodium

deficient diet. Briefly, acute sodium deficiency accompanied by high levels of aldosterone elicited a greater sodium appetite than either high aldosterone levels alone or sodium deficiency with lower levels of aldosterone. These results suggest that the role of aldosterone in sodium appetite is as a potentiator of the natrorexigenic (sodium appetite stimulating) effects of sodium deficiency (i.e., hypovolemia, reservoir sodium deficits, etc.). Support for this interpretation comes from unpublished data (Jalowiec, 1970) showing an enhanced response to formalin treatment following NaD maintenance and a recent report that sodium appetite occurring after recovery of extracellular sodium and volume may be potentiated by high endogenous aldosterone secretion (Jalowiec & Stricker, 1970b). Thus, it may be appropriate to consider aldosterone in a supportive rather than a primary role in the elicitation of sodium appetite.

The results of this study might also be accounted for by a "dose-response" explanation leaving the primary role of aldosterone intact. Since aldosterone secretion could not be directly measured, it is possible that endogenous levels were not maximized by six days of maintenance on a sodium deficient diet and thus were not sufficient to elicit sodium appetite until after additional stimulation by the effects of acute sodium loss. Accordingly, NaD maintenance may evoke a high but not natrorexigenic level of aldosterone, acute sodium loss in NaR maintained rats may elicit a somewhat higher level capable of increasing salt intake, and finally, acute sodium loss in NaD maintained rats may elicit the highest level thereby evoking the most pronounced sodium appetite.

EXPERIMENT II

SODIUM REPLACEMENT BEHAVIOR IN THE ADRENALECTOMIZED RAT

The results of the previous experiment clearly show that behavioral regulation of sodium balance in the acutely sodium deficient rat is characterized by marked over-compensation for sodium loss. The present study examines behavioral regulation in adrenalectomized rats that are chronically sodium deficient. Unlike the furosemide-treated animal, the adrenalectomized rat must continuously compensate for the continuous loss of sodium in urine due to the lack of endogenous mineralocorticoids. This continued need to replenish body sodium may result in more precise (i.e., more efficient) sodium replacement behavior than was seen in acutely deficient, intact rats. The use of adrenalectomized rats also permits study of sodium replacement behavior over a range of deficits since different degrees of sodium need may be instituted simply by varying periods of sodium deprivation. Perhaps the precision of salt replacement is a function of sodium requirements. In addition, this study will test the hypothesis, based on the preceding experiment, that behavioral over-compensation for sodium loss is due to high endogenous aldosterone levels. If that is the role of aldosterone in mediating sodium appetite, then the sodium appetite of adrenalectomized rats unable to mobilize aldosterone should not be characterized by pronounced over-compensation for sodium deficits.

The relationship between sodium deficiency and sodium intake

has been well documented in sheep and the apparent precision of their salt appetite is independent of functioning adrenals (Denton, Orchard & Weller, 1969). Unfortunately, little is known of the relationship between sodium deficiency and consequent sodium appetite in the adrenalectomized rat except that sodium intake is insufficient to permit continued existence (Richter, 1936). Epstein and Stellar (1955) reported that sodium intake increased as a function of increased sodium deprivation in adrenalectomized rats, but the relationship was not defined because sodium losses were not measured. Thus, the primary purpose of the present experiment is to examine the sodium replacement behavior of adrenalectomized rats and its relationship to varying degrees of measured sodium deficiency.

METHOD

Subjects and pretreatment maintenance:

Male albino (Sprague-Dawley) rats, weighing 300-325 gm at the start of the experiment, were housed in individual metabolism cages. The laboratory was continuously illuminated and temperature controlled (24 - 26 C). All animals were permitted free access to demineralized water and a pelleted sodium deficient diet especially prepared by the General Biochemical Co. to contain less than 0.00009 mEq Na+/kg and normal potassium levels (114.0 mEq/kg). Hypertonic saline (0.51 M NaCl) was also available except during sodium deprivation. Drinking fluids were presented in graduated tubes (±1.0 ml) with stainless steel drinking nozzles.

Procedure:

After five days of maintenance, 20 rats were bilaterally adrenalectomized (ADREX) and an additional 12 animals were sham-operated. The adrenal glands were removed, while the animals were deeply anesthetized with ether, via a small dorsal incision through the skin of the back and two subcutaneous incisions into the abdominal wall just caudal and parallel to the last ribs. Surgery was accomplished within 20 minutes and did not have a noticeable effect on fluid intake or body weight over the following 24 hours. However, five additional days of complete maintenance were permitted for recovery. During these five days rats that had been successfully adrenalectomized showed gradually increasing daily intakes of 0.51 M NaCl solution while intakes of sham-operated animals were unchanged from pre-operative levels (Figs. 8 & 9). Adrenalectomized rats not showing increased daily NaCl intake were discarded and replaced by other rats since autopsies invariably indicated that rats failing to increase their salt intake after adrenalectomy retained accessory adrenal tissue. The success of adrenalectomy of the animals included in this study was confirmed after all testing was completed by mortality within 10 days of total sodium deprivation and by post-mortem macroscopic examination for residual adrenal tissue.

After the recovery period, half of the ADREX animals and half of the sham-operated rats received the following sodium deprivation periods: 3, 8, 24, 48, 72 and 96 hours, each separated by three days of salt access (Fig. 8). The remaining ADREX and sham-operated rats

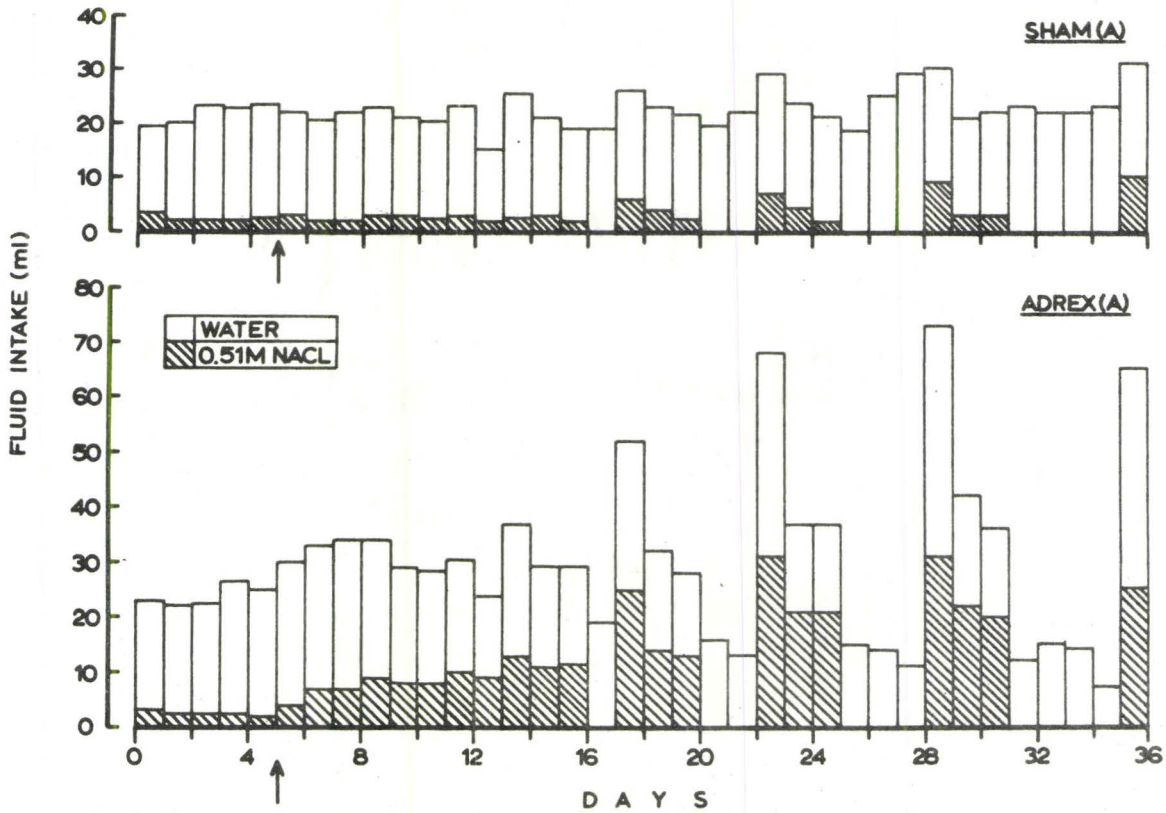

Fig. 8. Daily total fluid intake (water and 0.51 M NaCl) of ADREX and sham-operated rats tested with the ascending series of sodium deprivations. Adrenalectomy and sham operation indicated by the arrow.

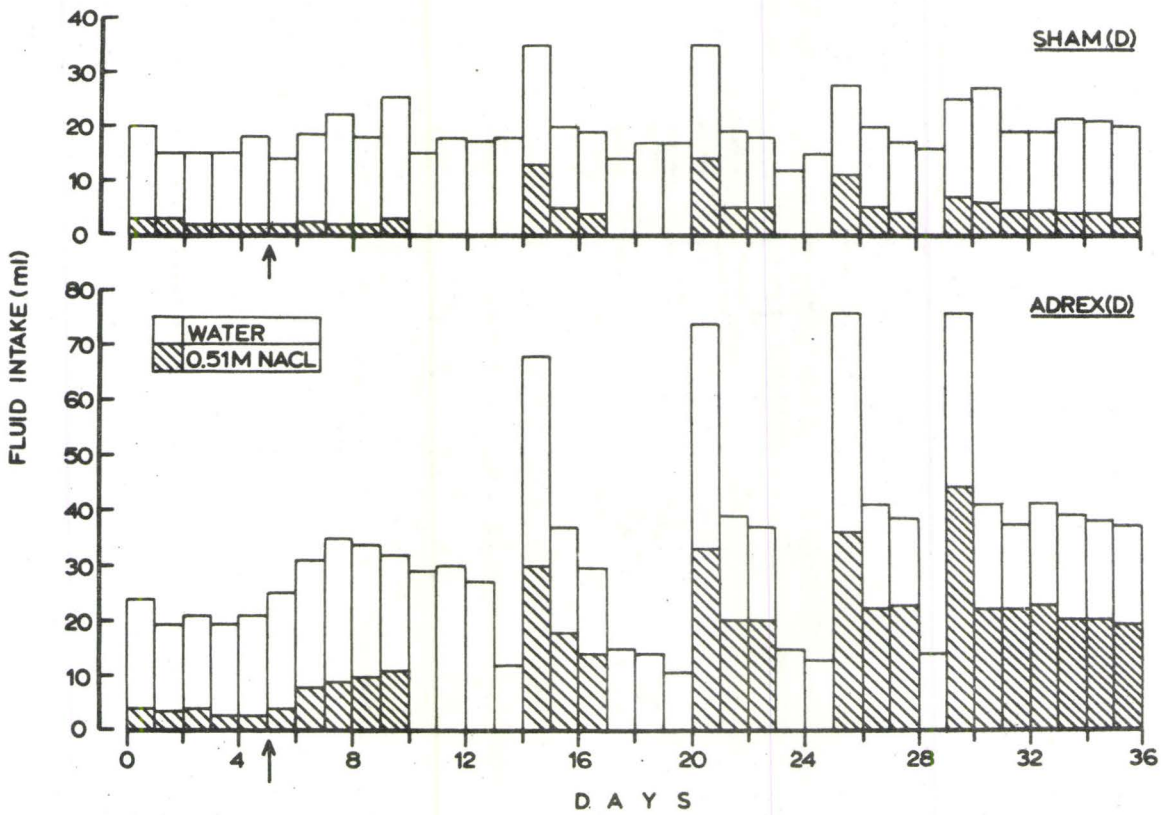

Fig. 9. Daily total fluid intake (water and 0.51 M NaCl) of ADREX and sham-operated rats tested with the descending series of sodium deprivations. Adrenalectomy and sham operation indicated by the arrow.

received the same deprivation periods but in a descending presentation order (Fig. 9). Sodium deprivation was accomplished by removing the 0.51 M NaCl drinking tube (but not the food or water) from the cages and sponging the cage interior with demineralized water. Urine collection funnels with feces screens were placed beneath the cages and urine was collected in graduated tubes (±0.1 ml) attached to the funnels. Total urine output was collected every 24 hours or at the end of the shorter sodium deprivation periods for analysis of sodium content (flame photometer: Instrumentation Laboratories). During the longer sodium deprivation periods, urine collection funnels were replaced every 24 hours to avoid possible accumulation of salt although periodic rinses yielded no measurable amounts of sodium.

The degree of sodium deficiency established during sodium deprivation was calculated simply as the amount of sodium lost in urine. These measures appeared adequate since fecal sodium loss in ADREX rats was found to be small (Fig. 10). In addition, it seems unlikely that ADREX rats had greater sodium requirements than those estimated by urine sodium loss because of self-induced sodium deprivation before the salt solution was removed from their cages since hourly measures of the periodicity of NaCl intake over 24 hours showed that most ADREX rats ingested some sodium every 1-2 hours (Fig. 11). The sodium loss that might occur during these brief intervals would not add appreciably to sodium loss during the scheduled deprivations.

Sodium replacement tests consisted of measuring the intakes and urine excretions during the initial three hours of access to salt solution following each sodium deprivation period, at 5, 10, 15, 20,

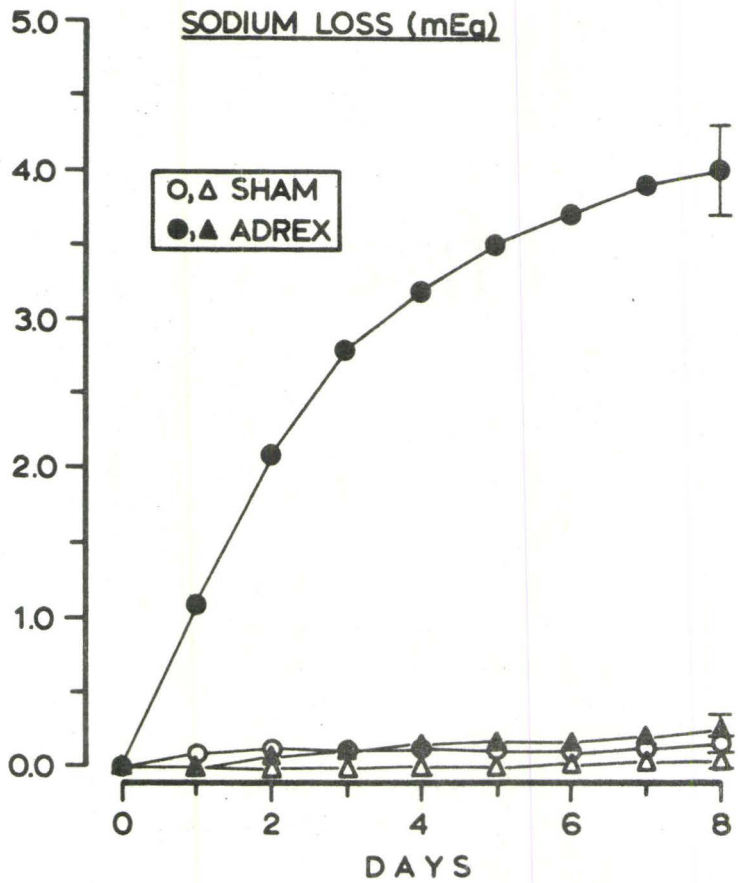

Fig. 10. Daily urine (circles) and fecal (triangles) sodium loss after adrenalectomy (n=12) or sham-operation (n=4). All animals were maintained on the sodium deficient diet without access to 0.51 M NaCl solution.

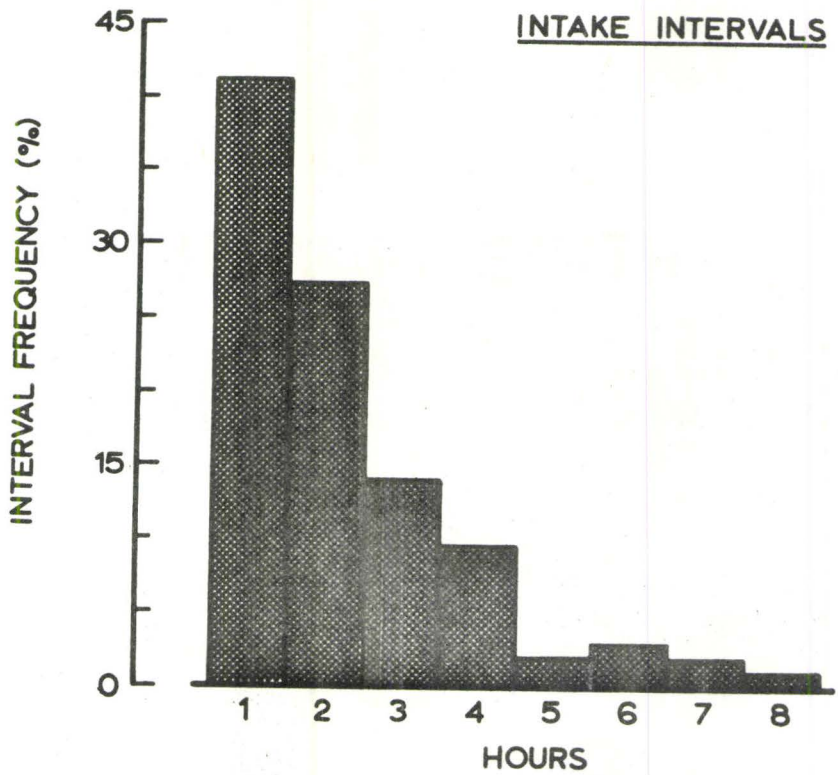

Fig. 11. Frequency of 0.51 M NaCl intakes of nine ADREX rats on the fifth day after surgery. For example, the one hour interval includes the percent of increases in intake that occurred between two consecutive hourly measurements, while the two hour interval includes the percent of increases that occurred between three consecutive hourly measures.

30, 60, 120 and 180 minutes. After these replacement tests, urine collections were discontinued and the animals were left undisturbed, except for daily intake and body weight measures, until the next sodium deprivation period.

Blood Analyses:

Additional ADREX animals were maintained and prepared as described previously, but were used for blood sampling after single sodium deprivation periods (7 groups of 6 rats each). Two groups of ADREX rats were bled after either 15 or 120 minutes of salt access following 24 hours sodium deprivation (n = 5, 5). Finally, groups of sham-operated (n = 8) and unoperated rats (n = 5) maintained on the sodium deficient diet, and ADREX rats (n = 7) maintained on sodium replete Purina Laboratory Diet, were bled for baseline values. All rats were deeply anesthetized with ether and blood samples were withdrawn from the abdominal aortas and immediately analysed for hematocrit (microcapillary tubes), plasma sodium and potassium concentrations (flame photometer), plasma protein concentration (refractometer) and plasma osmolality (Advanced Instruments osmometer).

RESULTS

During the sodium deprivation periods in both the ascending and descending series, adrenalectomized (ADREX) rats excreted urine at higher sodium concentrations than their respective sham-operated controls (Fig. 12) as might be expected because of their inability to

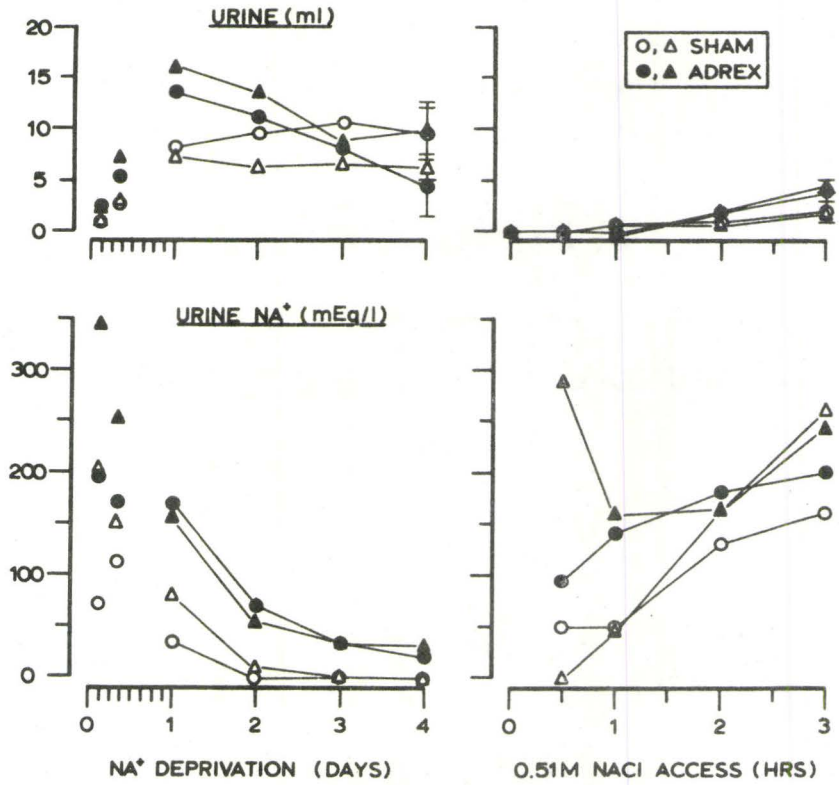

Fig. 12. Urine volume and urine sodium concentrations of ADREX and sham-operated rats during sodium deprivation and salt access. Circles represent values from rats in the ascending series, triangles represent values from rats in the descending series.

retain sodium (Hierholzer, Wiederholt, Holzgreve, Giebisch, Klose & Windhager, 1965). Unfortunately, even such indirect measures of mineralocorticoid activity as urinary potassium/sodium ratios were not available in this experiment since the potassium content of food waste biased urine potassium measures. The decreases in urine sodium concentration seen in ADREX rats during the longer sodium deprivations were similar in degree to those seen in sham-operated animals (Fig. 12), but should not be considered as evidence of active sodium retention. Their urine sodium concentrations never decreased to the low levels seen in sham-operated rats and the evident decreases were likely due to decreases in glomerular filtration (Brenner, Bennett & Berliner, 1968). There were no significant differences in urine volume between ADREX and sham-operated rats during any of the various sodium deprivation periods (Fig. 12). As a result of their generally higher urine sodium concentrations, sodium loss during sodium deprivation was significantly increased in ADREX rats (p's $< .01$ for all but the 3 hour deprivation; two tailed t tests; see initial points on Figs. 14, 16, & 18).

The ingestive behavior of the ADREX and sham-operated rats during the 3 hour sodium replacement tests and its effect on restoring sodium balance are the focal points of this experiment. During the sodium replacement tests following the brief (3 hr and 8 hr) sodium deprivation periods (Fig. 13), ADREX rats from both ascending and descending series ingested significantly more 0.51 M NaCl than their respective controls (both p's $< .001$). However, the ADREX rats from the descending

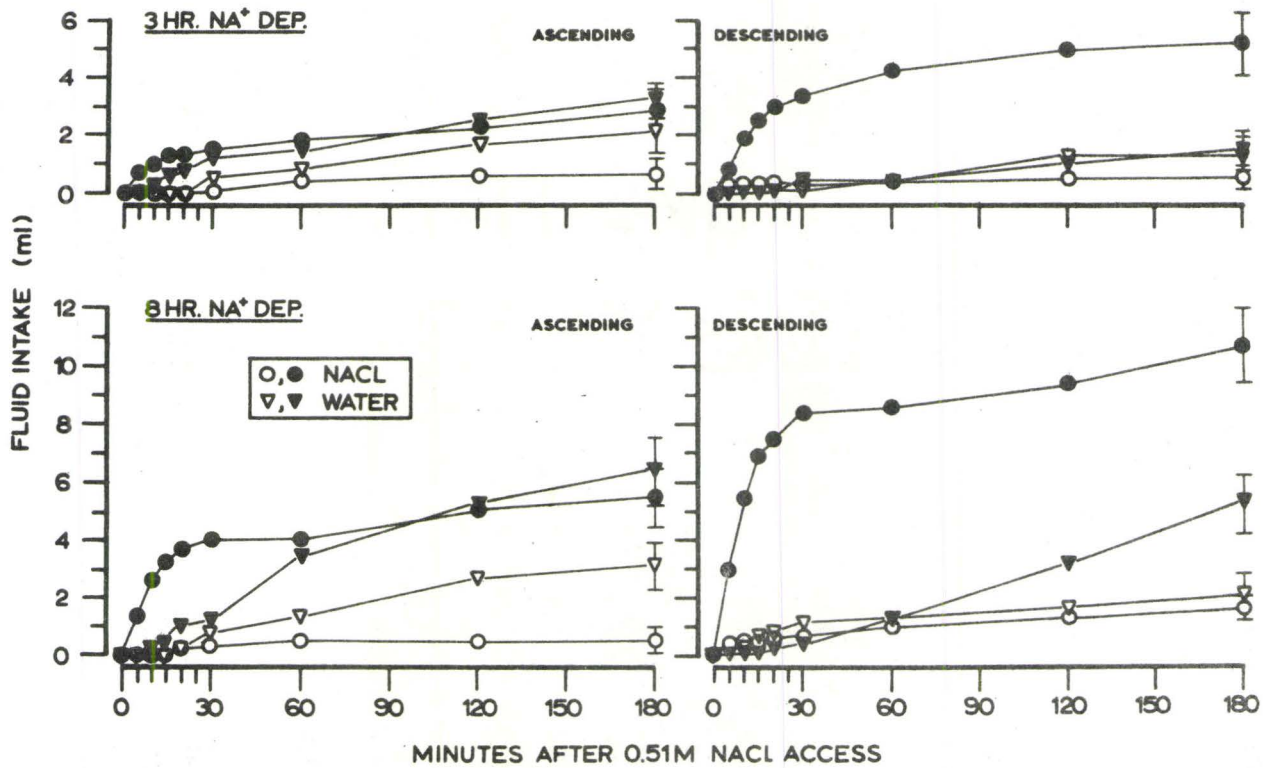

Fig. 13. Water and 0.51 M NaCl intake during the sodium replacement tests following 3 hr and 8 hr sodium deprivation. Open symbols represent values from sham-operated rats, filled symbols represent values from ADREX rats.

series ingested more NaCl solution than ADREX rats from the ascending series ($p < .01$). Note that the animals from the descending series received the 3 hr and 8 hr sodium deprivations at the end of their series, while the rats in the ascending series received these deprivations and replacement tests first (Fig. 8 & 9). Thus, the greater salt intake of the ADREX rats from the descending series may reflect their more extensive experience with sodium deprivation and sodium replacement.

During the sodium replacement tests only minimal amounts of urine were excreted but urine sodium concentrations rapidly increased (Fig. 12) consequent to NaCl ingestion. The effect of salt intake on sodium balance following the brief sodium deprivations is presented in Figure 14. The initial points present the sodium deficits incurred during sodium deprivation which were generally less than 1.0 mEq except for ADREX rats in the descending series. Note that sodium intake by ADREX rats was sufficient to result in gross overcompensation for the deficits. Sham-operated rats from both series lost inconsequential amounts of sodium (less than 0.5 mEq) and, because of small NaCl intakes, sodium balance remained stable.

NaCl solution intake was further enhanced in the ADREX groups during the sodium replacement tests following the longer periods (24 hr and 48 hr) of sodium deprivation (Fig. 15). Salt intake was greater for the rats from the descending series following the 24 hour deprivation ($p < .001$), but not after the 48 hour deprivation. Water intakes were also significantly increased from control levels (all $p < .001$), but water ingestion was delayed until after

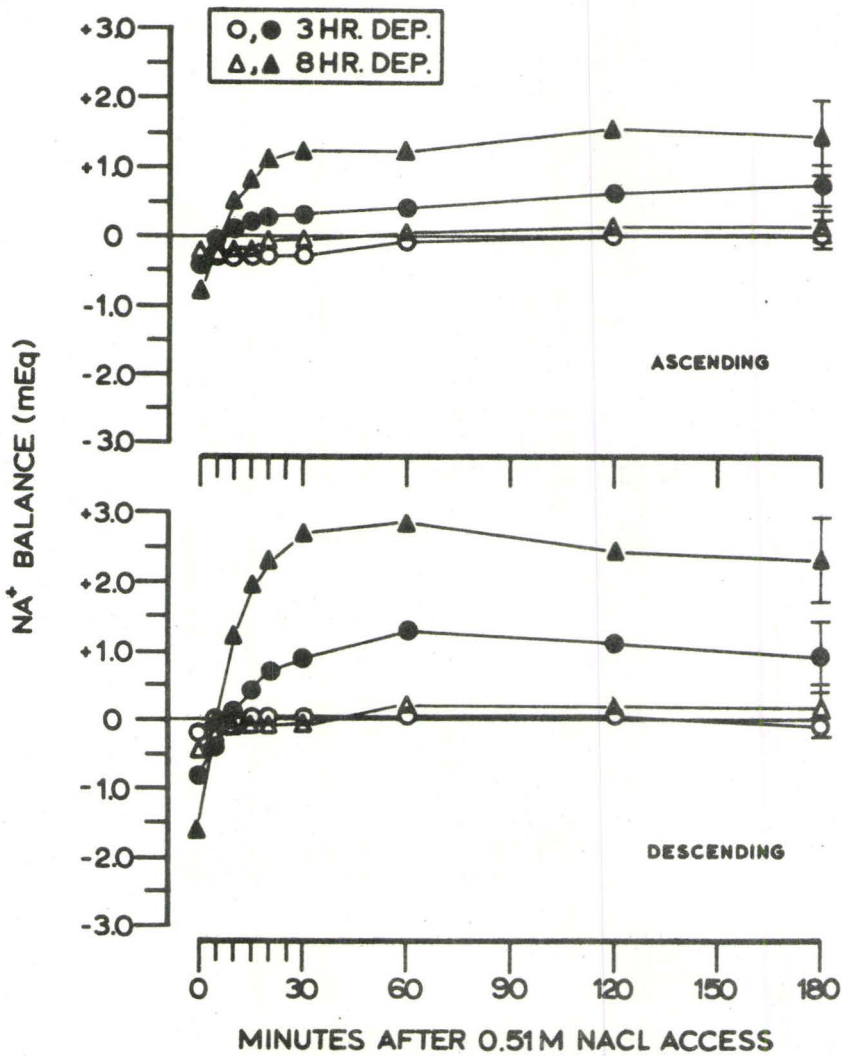

Fig. 14. Sodium balance during the sodium replacement tests following 3 hr and 8 hr sodium deprivation. Open symbols represent values from sham-operated rats, filled symbols represent values from ADREX rats.

substantial drinking of hypertonic salt solution (Fig. 15). Since water was available throughout the deprivation periods, this enhanced intake is probably a consequence of hypertonic NaCl ingestion. It was obvious from this intake pattern that ADREX rats were most interested in drinking salt. In addition, ADREX rats seemed to anticipate salt presentation by sleeping in front of their cages and often licking the cage section where the salt tube was attached. Note that sham-operated rats also showed increased NaCl intake after the 48 hour sodium deprivation in comparison to their drinking after the shorter deprivations (Fig. 15).

Urine sodium loss of ADREX rats deprived of sodium for 24 hr or 48 hr was more extensive than that observed during the shorter deprivation periods (Fig. 16; compare with initial points in Fig. 14), whereas the sodium loss of sham-operated rats was essentially unchanged since urine sodium concentrations had decreased to minimal levels after 24 hours of sodium deprivation (Fig. 12). Sodium deficits were most extensive for ADREX rats from the descending series, perhaps because of their larger NaCl intakes prior to sodium deprivation (Fig. 8 & 9). Intakes of 0.51 M NaCl solution during the sodium replacement tests were more than sufficient to replace sodium loss in ADREX rats, and sodium balance was well above the equilibrium level within 10 minutes of access to saline (Fig. 16). Sodium balance of sham-operated rats was also increased as a result of their enhanced drinking of NaCl despite only minor sodium deficits during sodium deprivation.

Water and NaCl intakes, and the pattern of drinking, after

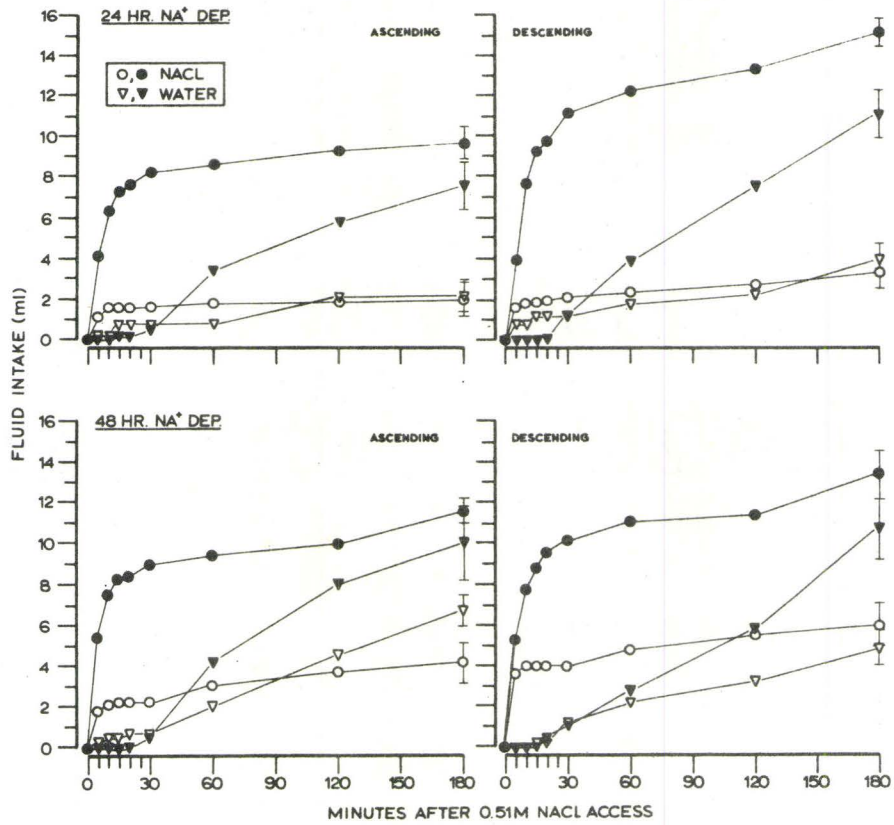

Fig. 15. Water and 0.51 M NaCl intake during the sodium replacement tests following 24 hr and 48 hr sodium deprivation. Symbols as in Fig. 13.

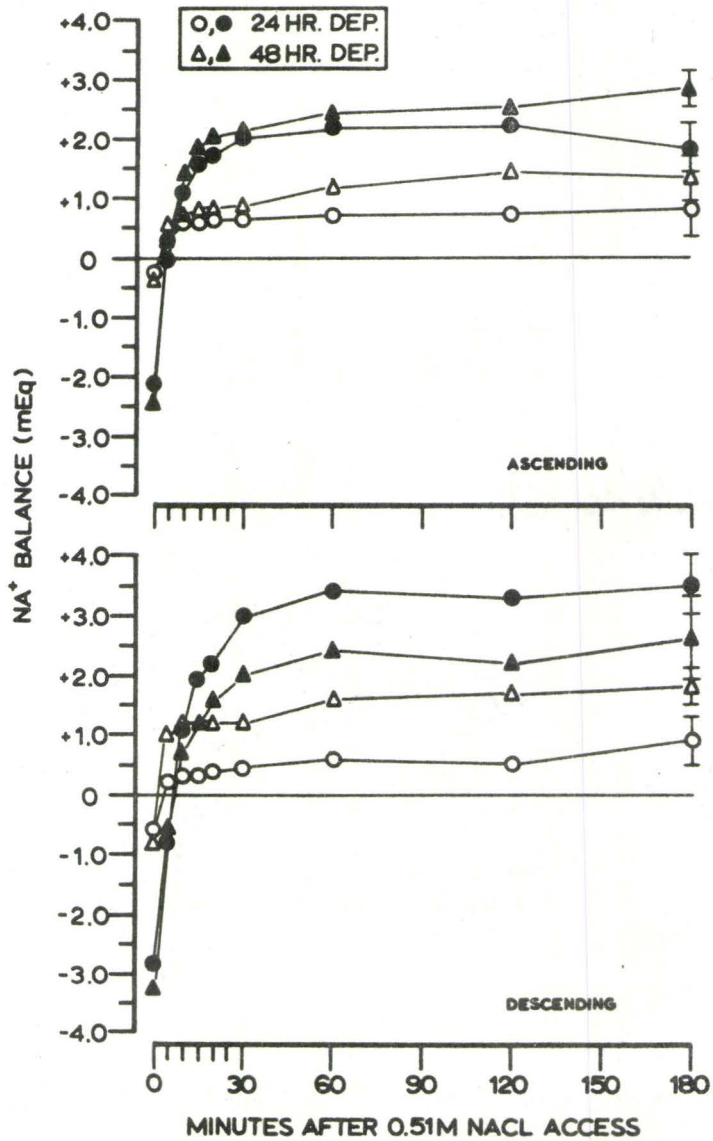

Fig. 16. Sodium balance during the sodium replacement tests following 24 hr and 48 hr sodium deprivation. Symbols as in Fig. 14.

72 hr of sodium deprivation were similar to those following the 24 hr and 48 hr deprivation periods (Fig. 17). However, after the 96 hr sodium deprivation, NaCl intakes of ADREX rats were clearly depressed in comparison to their drinking during the other sodium replacement tests (Fig. 17). This reduced drinking is only one indication of the general malaise occurring in most ADREX rats by the end of the 96 hour deprivation period. At this stage of sodium loss, many ADREX rats evidenced difficulties of motor coordination (stumbling, weaving, righting problems, etc.). In fact, some ADREX rats not included in this study died after three or four days of sodium deprivation. Note that the ADREX rats from the ascending series tended to ingest more NaCl solution after the longest sodium deprivations than ADREX rats from the descending series. The long deprivation periods and sodium replacement tests were the last experienced by the ascending groups, but the first given to the descending series. In conjunction with the opposite of ordering salt intakes seen after the brief deprivation periods, these results suggest that salt drinking was potentiated in ADREX rats after substantial experience with sodium deficiency and salt replacement. Note that sham-operated rats also showed rapid and substantial NaCl intakes after the 72 hr and 96 hr deprivation periods (Fig. 17).

Urine sodium deficits and changes in sodium balance during the replacement tests are presented in Figure 18. Urine sodium losses incurred by ADREX rats during the 72 hr and 96 hr sodium deprivations (initial points in Fig. 18) were the most substantial seen in this

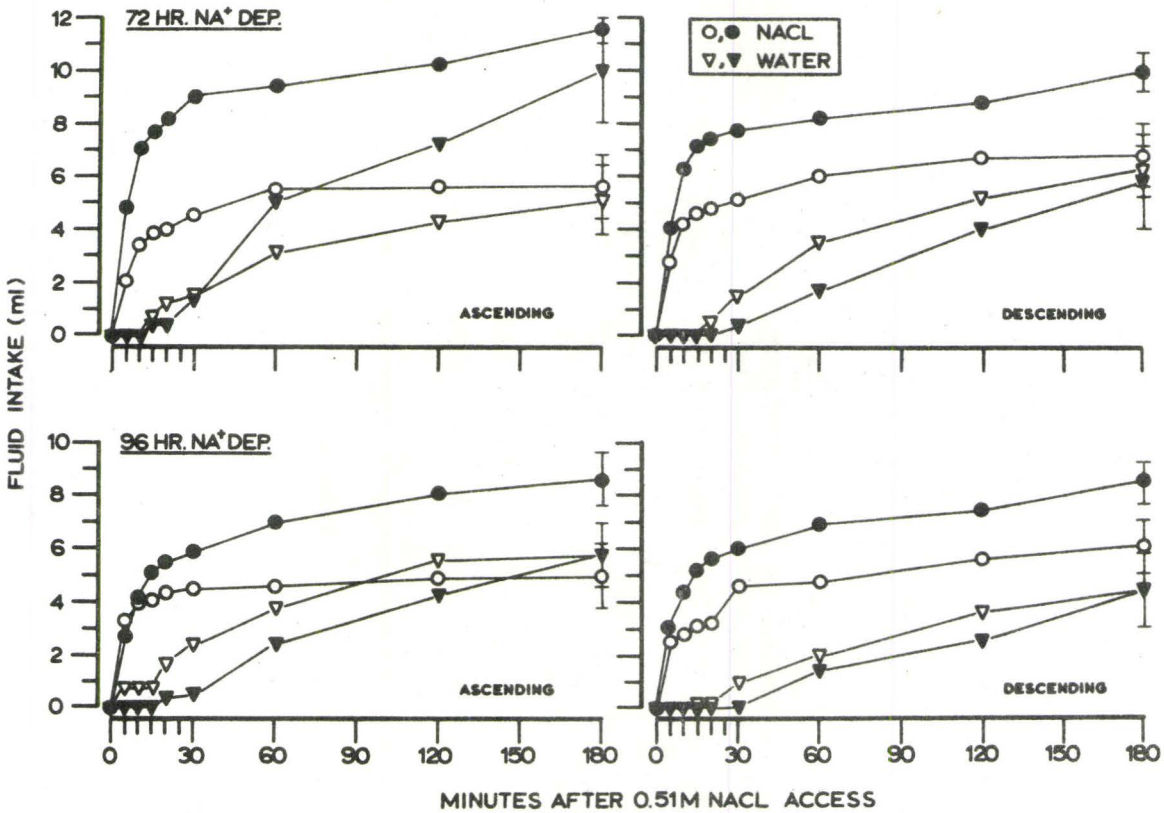

Fig. 17. Water and 0.51 M NaCl intake during the sodium replacement tests following 72 hr and 96 hr sodium deprivation. Symbols as in Fig. 13.

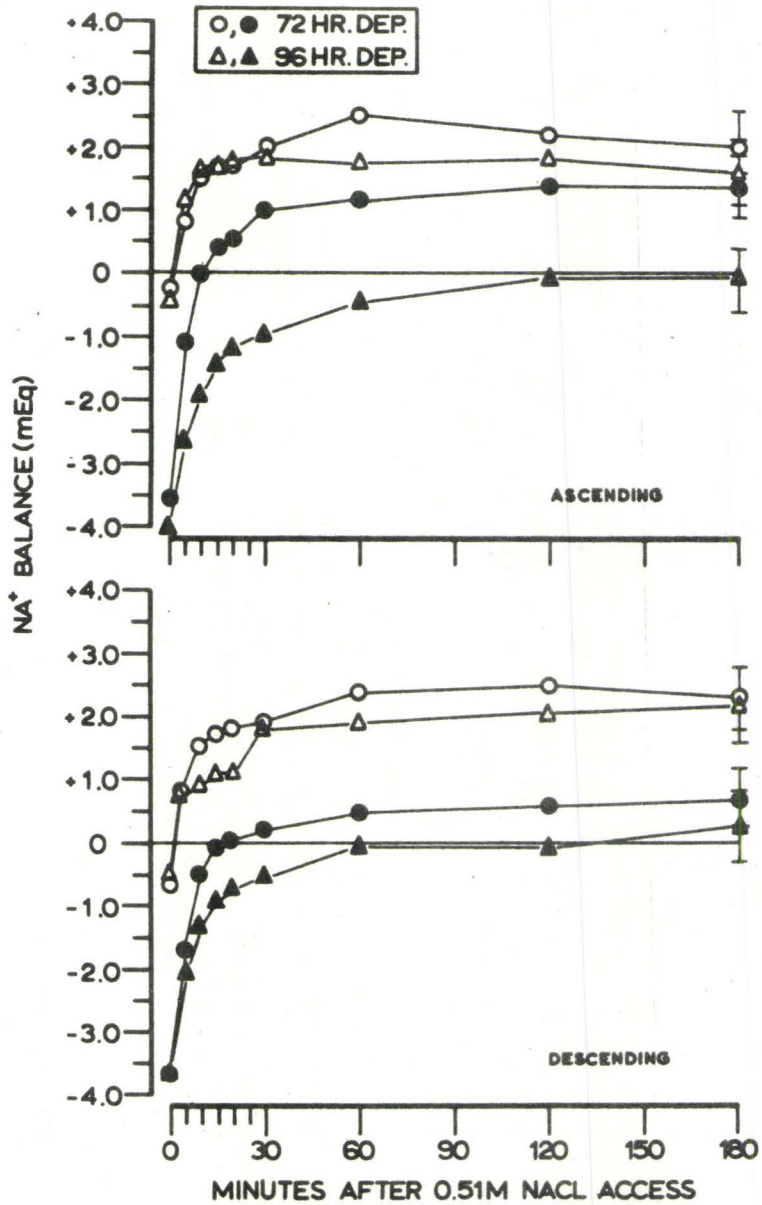

Fig. 18. Sodium balance during the sodium replacement tests following 72 hr and 96 hr sodium deprivation. Symbols as in Fig. 14.

experiment and are similar to losses reported for sodium deprived ADREX rats at death (White & Rolf, 1955). As mentioned previously, animals at these stages of sodium deprivation were obviously ill and their inability to sustain enhanced fluid intake may account for the apparent precision with which they replaced their sodium deficits (Fig. 18). In other words, although sodium intakes of the ADREX rats were precisely adequate to recover urine sodium losses, it is likely that gross behavioral over-compensation for sodium losses would have been seen had these animals been capable of sustained behavior.

The striking intakes of NaCl solution by the sham-operated rats after the 72 hr and 96 hr sodium deprivation periods (Fig. 17) resulted in marked increases in sodium balance despite sodium deficits comparable to those occurring in the shorter sodium deprivation (Fig. 18). It would appear that long periods of sodium deprivation can elicit a sodium appetite even in rats with intact adrenals.

To summarize, sodium deprivation of ADREX rats resulted in substantial sodium deficits with the degree of deficiency directly related to the length of deprivation (Fig. 10). The inability of ADREX rats to exercise physiological control of sodium excretion via mineralocorticoids adequately accounts for these sodium losses. Enhanced NaCl intake was seen after all periods of sodium deprivation except the shortest (3 hr) and sodium intake during the replacement tests was directly related to the degree of sodium deprivation up to 72 hr. However, sodium ingestion was usually markedly in excess of urine sodium

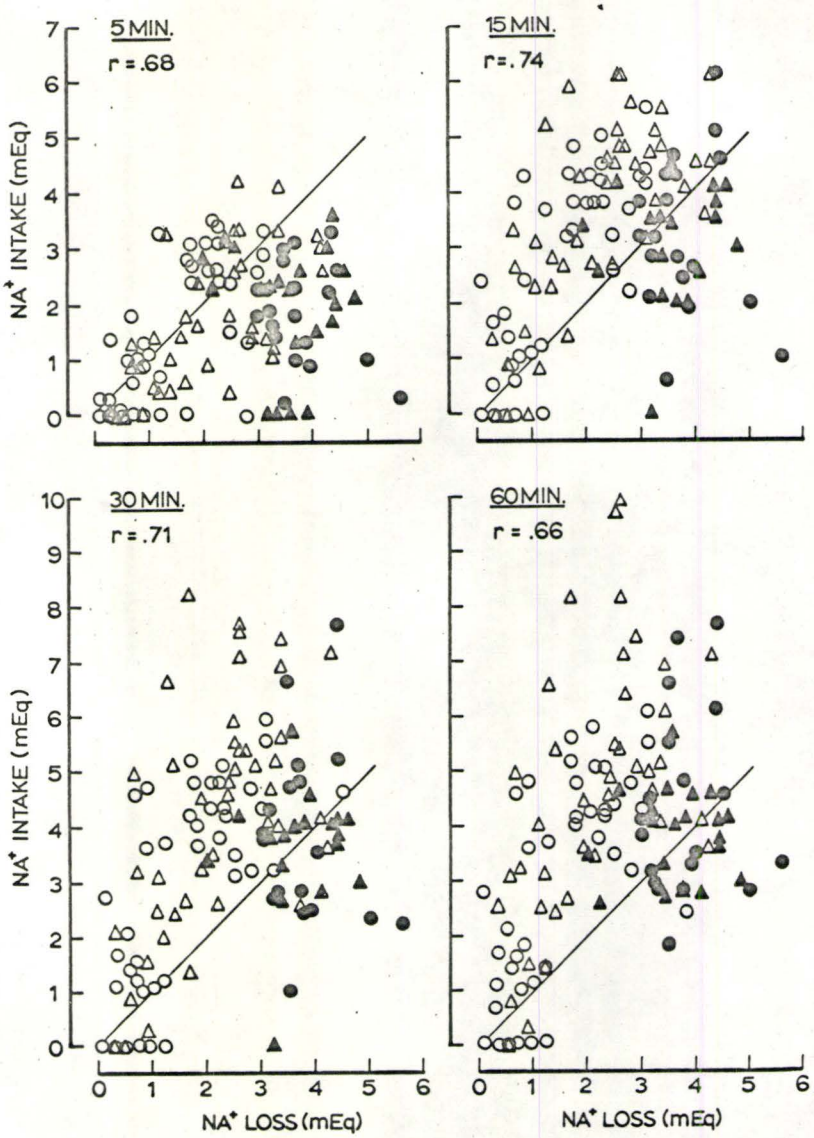

Fig. 19. Correlations between sodium loss during sodium deprivation and consequent sodium intake in ADREX rats after 5, 15, 30 and 60 min of 0.51 M NaCl access. Diagonal lines indicate perfect precision (intake = deficit). Circles represent data from the ascending series, triangles represent data from the descending series and filled symbols the data from the 72 hr and 96 hr tests. Results from the 72 and 96 hr tests were excluded from calculation of the correlations.

losses (Fig. 19). Note that the relatively high correlations (all p's < .001) are not the result of a precise correspondence between loss and intake but instead are due to a direct relationship between sodium loss and salt intake at almost all levels of sodium deficiency despite behavioral over-compensation. In addition, the degree of salt over-drinking seemed partially dependent on the order in which the various sodium deprivations were presented. Generally, the more experience ADREX rats had with sodium deficiency and salt drinking, the more pronounced the sodium intake and consequent excessive increases in sodium balance. Finally, an apparent salt appetite was evident in the ingestive behavior of sham-operated rats after the longer sodium deprivations despite only minor sodium losses that remained constant regardless of the length of sodium deprivation.

Blood Analyses:

ADREX rats maintained on a sodium deficient diet and used for blood sampling showed sodium deficits during deprivation similar to those just described. Plasma sodium concentration was depressed after only 24 hours sodium deprivation ($p < .01$), but did not decrease significantly with longer deprivations (Table 1). Plasma potassium levels increased after 24 hour sodium deprivation and increased still further during the longer deprivations. Adrenalectomy is known to result in decreased potassium secretion into the distal renal tubule in addition to its effects on enhancing urine sodium loss (Hierholzer, et al, 1965) and this accounts for the high plasma potassium concentration. These

TABLE 1

Progressive changes in some blood parameters in adrenalectomized rats maintained on a Na+ deficient diet.

Na+ deprivation (hours)	Plasma Sodium (mEq/1 plasma HOH)	Plasma Potassium (mEq/1 plasma HOH)	Protein (g/100 ml)	Hematocrit (%)	Osmolality (mOsm/kg)
0	143.4 ± 0.6*	5.3 ± 0.2	5.9 ± 0.1	44.3 ± 0.2	296.5 ± 0.9
3	142.6 ± 0.4	5.4 ± 0.1	6.0 ± 0.1	45.7 ± 0.5	289.8 ± 3.9
8	142.0 ± 0.8	5.6 ± 0.2	6.1 ± 0.1	47.0 ± 1.6	294.8 ± 2.0
24	136.7 ± 1.6	6.3 ± 0.2	6.5 ± 0.1	48.8 ± 2.0	286.8 ± 2.5
48	136.1 ± 0.8	7.0 ± 0.2	7.0 ± 0.1	53.8 ± 1.0	306.8 ± 3.3
72	134.4 ± 1.6	7.1 ± 0.3	6.9 ± 0.1	54.5 ± 1.1	301.1 ± 3.2
96	134.9 ± 1.6	7.6 ± 0.3	6.6 ± 0.1	56.8 ± 1.2	314.0 ± 4.8

* mean ± S.E.M.: Six rats bled for each deprivation.

high levels approach those seen in comatose ADREX rats (10.0 - 12.0 mEq/l plasma water) and probably contribute to their general malaise. Sodium deprivation of ADREX rats also resulted in marked decreases in plasma volume as indicated by the significant increases in plasma protein and hematocrit (p's $< .01$; Table 1). Plasma osmolality generally increased during the longer sodium deprivations.

Adrenalectomized rats maintained on either a sodium replete diet or a sodium deficient diet, with continuous access to 0.51 M NaCl and water, showed clear decreases in plasma sodium and increases in plasma potassium concentrations (both p's $< .01$; Table 2). This suggests that adrenalectomy results in chronic difficulty in maintaining plasma electrolyte levels. Plasma protein and hematocrit levels were unchanged by these dietary maintenance schedules (5 days post-operative).

When 24 hour sodium deprived ADREX rats were permitted to drink 0.51 M NaCl for either 15 or 120 minutes, plasma sodium concentration rapidly increased to normal levels (Table 3). Curiously, plasma volume appeared to have decreased at first and then returned to normal, since plasma protein and hematocrit were increased after 15 minutes of NaCl access but normal after 120 minutes. These changes cannot be the result of absorption of sodium from the gastrointestinal tract, since plasma volume should have increased at 15 minutes after acccess, but instead may reflect movement of extracellular water into the gut in response to the hypertonicity of ingested fluid prior to absorption (O'Kelly, Falk & Flint, 1958). However, if this were the case, plasma potassium concentration might be expected to increase as well unless it

TABLE 2

Values for some blood parameters of intact and adrenalectomized rats maintained on Na+ replete or Na+ deficient diets.

Treatment	Plasma Sodium (mEq/l plasma HOH)	Plasma Potassium (mEq/l plasma HOH)	Protein (g/100 ml)	Hematocrit (%)	Osmolality (mOsm/kg)
Intact (regular diet)	146.2 ± 0.2*	4.0 ± 0.1	5.9 ± 0.1	45.2 ± 0.4	306.9 ± 2.3
Sham (deficient diet)	144.6 ± 0.3	4.5 ± 0.1	6.2 ± 0.1	43.2 ± 0.4	299.8 ± 1.7
Adrex (regular diet)	142.7 ± 0.3	5.3 ± 0.2	5.6 ± 0.1	44.4 ± 0.4	293.9 ± 2.9
Adrex (deficient diet)	143.4 ± 0.2	5.3 ± 0.2	5.9 ± 0.1	44.3 ± 0.2	296.5 ± 0.9

*mean ± S.E.M.: n = 5 - 8 in each group.

TABLE 3

Values for some blood parameters of 24 hr Na+ deprived adrenalectomized rats after access to 0.51 M NaCl.

Treatment	Plasma Sodium (mEq/l plasma HOH)	Plasma Potassium (mEq/l plasma HOH)	Protein (g/100 ml)	Hematocrit (%)	Osmolality (mOsm/kg)
Adrex (24 hr Na+ deprived)	136.7 ± 1.6*	6.3 ± 0.2	6.5 ± 0.1	48.8 ± 2.0	286.8 ± 2.5
Adrex (with 15 min NaCl access)	145.9 ± 0.5	5.8 ± 0.2	6.9 ± 0.1	51.6 ± 1.1	293.7 ± 3.6
Adrex (with 120 min NaCl access)	147.0 ± 1.1	5.4 ± 0.4	5.7 ± 0.1	46.4 ± 1.4	296.9 ± 1.5

* mean ± S.E.M.: n = 5 - 8 in each group.

also moved into the gastrointestinal tract (little excretion of urine occurred during the sodium replacement tests). Thus, ingestion of sodium by sodium deficient ADREX rats resulted in rapid restoration of plasma sodium concentration, but true restoration (i.e., post-absorption) may not have occurred for some time as indicated by delayed restoration of plasma volume. Note that there was no internal evidence of the behavioral over-compensation for sodium loss since plasma sodium and plasma volume did not increase above the normal range.

DISCUSSION

The degree of sodium deficiency of ADREX rats depends on the length of sodium deprivation. Such a relationship had been assumed by earlier investigators (Epstein & Stellar, 1955), but never demonstrated. This direct relationship has certain limitations since losses are very minor (less than 1.0 mEq/l Na+) during the short deprivation periods and rapidly become asymptotic (3.0 - 4.0 mEq Na+) after three days of sodium deprivation. Sodium deficits of this magnitude represent 20 to 25 percent of total body sodium in adult rats and have been shown to be fatal to ADREX rats (White & Rolf, 1955). Adrenalectomy results in a number of metabolic disturbances either directly or indirectly related to sodium deficiency, such as general weakness, hypotension, hypovolemia, hyponatremia, hyperkalemia, uremia, as well as an inability to withstand stress (Tepperman, 1968). All of these factors probably contribute to the mortality of ADREX rats deprived of sodium. However,

such rats can remain virtually symptom-free if large quantities of salt are ingested. Thus, survival by ADREX rats depends solely on the increased ingestion of salt.

The increased sodium appetite of ADREX rats was expected but the apparent behavioral over-compensation for sodium deficits was surprising. The results of the first experiment suggested that behavioral over-compensation was due to potentiation of the effects of sodium deficiency by high endogenous mineralocorticoid levels. Accordingly, sodium deficient ADREX rats should not have shown marked over-drinking of salt solution. As noted in the introduction to this experiment, the behavioral over-compensation is at variance with reports of remarkable precision in the behavior of sodium deficient sheep with or without adrenals (Denton, Orchard & Weller, 1969). The significance of this difference is not known, but should at least caution against assumptions of a similar etiology for sodium appetite in the two species.

The behavioral over-compensation for sodium deficits by ADREX rats cannot be attributed to high endogenous mineralocorticoid levels, but instead, may be due to repeated experience with sodium deficiency and sodium replacement. In the first experiment, sodium deficiency was induced rapidly by furosemide injection and the experimental rats were not subjected to repeated testing. Marked over-drinking of salt solution was evident only in rats with high aldosterone levels prior to injection and was attributed to a potentiation effect of mineralocorticoid secretion. On the other hand, the sodium deficiency in ADREX rats

occurred gradually (comparable sodium deficits required one or two days of sodium deprivation) and all ADREX rats had considerable experience with sodium deficiency and salt replacement. This experience could account for the behavioral over-compensation seen in ADREX rats despite the lack of mineralocorticoids. The degree of precision did not improve with experience and over-compensation was evident at almost all levels of sodium deficiency tested. It was not likely a function of the size of the sodium deficit. As already noted, the apparent precision seen after 72 hr or 96 hr sodium deprivation was probably due to depressed responding resulting from illness. It is important to note that the behavioral over-compensation seen in both the furosemide-treated rats of the first experiment and the ADREX rats is not harmful to the animals since the excess sodium can be excreted readily. Thus, the imprecise nature of the behavior is compensated by physiological responses.

In contrast to the lack of sodium appetite in the vehicle-injected rats of the furosemide experiment, long term sodium deprivation (72 hr or 96 hr) was sufficient to elicit a pronounced sodium appetite in sham-operated rats in the second experiment. Sodium deficits were comparably small (less than 0.5 mEq) and both groups of rats began to retain sodium within 24-48 hr of access to the sodium deficient diet and presumably had high levels of mineralocorticoids. The reason for this difference could be that while the rats in the previous experiment had salt solution available during maintenance on the sodium deficient diet (although they did not drink enough to prevent negative sodium

balance and high mineralocorticoid secretion) and were deprived for only 24 hr, sham-operated rats in the second experiment were deprived for 72 hr or 96 hr before the replacement test. This absence of salt solution may have resulted in higher mineralocorticoid levels in the sodium deprived, sham-operated rats. Because of their salt appetite during the sodium replacement test, it might be appropriate to revise the hypothetical role of aldosterone in control of sodium appetite in normal rats. Thus, sodium appetite is elicited when high levels of aldosterone and minor degrees of sodium deficiency occur in the absence of salt, but not when presumably lower levels of secretion and minor sodium deficiency occur in the presence of salt. This appetite would be relatively small in comparison to that elicited by larger sodium deficits accompanied by high aldosterone secretion (sodium replete diet + furosemide) and in comparison to the appetite elicited by equally large sodium deficits but even higher aldosterone levels (sodium deficient diet + furosemide).

GENERAL DISCUSSION

The results of the second experiment and earlier studies with ADREX rats (Richter, 1936; Epstein & Stellar, 1955; Wolf & Steinbaum, 1965, Wolf and Stricker, 1967), demonstrating that sodium appetite can be elicited in the absence of aldosterone, clearly show that even though injection of exogenous mineralocorticoids is sufficient to elicit salt appetite (Wolf, 1965; Wolf & Handal, 1966), their presence is not necessary. Instead, a number of other stimuli known to elicit sodium appetite may have been responsible for the enhanced salt intake of ADREX rats. Plasma sodium concentrations decreased after only 24 hr sodium deprivation. Plasma volume was also decreased as evidenced by increased hematocrit and plasma protein concentration. Similar changes in plasma electrolytes and volume are known to be present during sodium appetite in both normal and ADREX rats (Wolf & Steinbaum, 1965; Wolf & Stricker, 1967; Stricker & Wolf, 1969; Jalowiec & Stricker, 1970a). It should be noted that adrenal insufficiency also enhances renin and angiotensin activity in rats (Gross, Brunner & Ziegler, 1965; Nasjletti & Masson, 1971) although their role, if any, in the elicitation of sodium appetite is unclear (Stricker, 1971b; Fitzsimons & Stricker, 1971).

Hypovolemia can also be postulated as a natrorexigenic stimuli after furosemide injection. Note that plasma sodium concentration did not change substantially after furosemide treatment although values were

at the lower end of the normal range (142.0 mEq/1 plasma water) 24 hr after diuresis and natriuresis. Hyponatremia would appear unnecessary for the elicitation of sodium appetite since isosmotic hypovolemia produced by polyethylene glycol injection enhances sodium intake 24 hr after treatment (Stricker & Wolf, 1966). However, with continuous access to water and NaCl the appetite may be potentiated by hyponatremia which develops slowly following increased renal retention of ingested water (Stricker & Jalowiec, 1970). Sodium appetite appears gradually after PG treatment, whereas it is apparent soon after formalin injection which produces hyponatremia rapidly (Jalowiec & Stricker, 1970a). If drinking fluids are withheld for eight hours after injection, PG treatment does not elicit a salt appetite despite decreased blood volume, but formalin injected rats which are hypovolemic and hyponatremic have a pronounced appetite (Stricker & Wolf, 1966). Although sodium appetite elicited by hypovolemia might be mediated by a receptor system sensitive to blood volume or pressure (Wolf & Stricker, 1967), the critical variable may be the amount or concentration of sodium in a particular component of the mediation system for sodium appetite. Hypovolemia could alter sodium levels at a specific site since depressed plasma volume and consequent water ingestion reduce effective plasma osmolality thereby increasing cell volume (Stricker, 1969; 1971a). This increased cell volume could alter the sodium concentration and perhaps the transmission properties of a receptor site. In addition, minor sodium deficits occurring at a specific site and not necessarily decreasing plasma sodium might elicit sodium appetite in the presence of high mineralocorticoid levels as seen in the sham-operated rats deprived of sodium

for 3 or 4 days. Finally, extensive loss of body sodium occurring as a result of either internal fluid shifts (formalin injection) or through actual external loss of sodium (furosemide injection or adrenalectomy) would also reduce sodium levels at this specific site even though plasma sodium concentration might remain unchanged or slightly increased (furosemide diuresis), thereby eliciting sodium appetite in the presence or absence of aldosterone.

The location of the proposed receptor site is unknown. The "reservoir hypothesis" of Stricker & Wolf (1969) postulates a receptor sensitive to depletion and repletion of a sodium reservoir possibly located in bone. A receptor site might also be located within the central nervous system. Sodium sensitive receptors appear to exist in the periventricular area of the diencephalon (Mouw & Vander, 1970; Dorn & Porter, 1970). Lesions studies in rats have implicated the hypothalamus in control of sodium excretion (Keeler, 1959; Dorn & Rothballer, 1968). In addition, hyperosmotic solutions infused into the third ventricle augment sodium excretion if they contain sodium (Andersson, Dallman & Olsson, 1969; Dorn & Porter, 1970), while iso-osmotic or hypo-osmotic infusions depress sodium excretion (Mouw & Vander, 1970). Lesions in the nearby lateral and medial hypothalamus are known to impair sodium appetite (Novakova & Cort, 1966; Wolf, 1967; Quartermain, Wolf & Keselica, 1969).

Despite the absence of information on just which stimuli are relevant to the control of sodium appetite and how they might interact, it is apparent that sodium ingestion is not easily extinguished or

inhibited once sodium balance has been restored. This does not suggest that sodium balance is poorly regulated but only that the control of sodium appetite is imprecise. Thus, the search for stimuli relevant to the initiation and satiation of sodium appetite should be directed toward parameters of sodium deficiency that are not altered rapidly by salt ingestion. One prime candidate that has not been investigated adequately is the "reservoir receptor" of Stricker and Wolf (1969). The activity of this receptor would rapidly signal sodium deficiency and elicit sodium appetite, but its activity would diminish only slowly consequent to reservoir repletion. This model for the control of sodium appetite is similar to the system recognized for thirst resulting from cellular dehydration (Stricker, 1969). It is a single loop, negative feedback system in which the ingestive behavior initiated by the system reduces the excitatory stimuli thereby eliminating the behavior. Sodium appetite could be mediated by a similar system if the receptor complex were more sensitive to sodium deficiency than to sodium repletion.

An alternative mechanism that would also account for the over-drinking of salt might involve a dual system of excitatory and inhibitory components similar to that postulated in the control of food intake (see Morgane & Jacobs, 1969 for review). The excitatory component might be sensitive to a complex of stimuli accompanying sodium deficiency and the inhibitory component might be relatively less sensitive to the opposite of those stimuli or to different stimuli. A third alternative control system has been suggested (Oatley, 1967; Stricker & Jalowiec, 1970)

in which sodium appetite might be controlled as part of a larger system involved with both intra- and extracellular fluid volumes. This model is based partially on evidence that the development of sodium appetite is mandatory for effective satiation of hypovolemic thirst since water ingestion alone cannot restore intravascular volume and water drinking in inhibited until salt is ingested (Stricker, 1969; Stricker & Jalowiec, 1970).

Unfortunately, present knowledge of the factors involved in the elicitation and satiation of sodium appetite is inadequate to permit a choice between these model systems. The results of the present experiments suggest that any system proposed for control of sodium appetite must consider not only the relevant stimuli capable of eliciting sodium appetite, but also the interactions between those stimuli. Such a system must also account for the pronounced behavioral over-compensation for sodium deficiency seen in both normal and adrenalectomized rats.

SUMMARY

Regulation of body sodium levels is vital for the maintenance of both extracellular and intracellular fluid volume and the integrity of the circulatory system. Adequate regulation requires the interaction of both physiological and behavioral control mechanisms. Physiological mechanisms insure maximal sodium retention during times of need while behavior is of primary importance in the replacement of unavoidable deficits.

Physiological control of sodium balance is achieved through the complex interaction of renal, hormonal and perhaps central mechanisms. Over 90 per cent of the sodium filtered through the kidneys is reabsorbed via mechanisms considered unresponsive to body sodium requirements. Fine control of renal sodium excretion is dependent upon the adrenal hormone, aldosterone, which enhances tubular reabsorption of sodium during sodium deficiency. Animals lacking this hormone invariably die from chronic sodium loss unless salt replacement is instituted. Aldosterone secretion is directly sensitive to sodium requirements and is modulated primarily by the renin-angiotensin system. Renin is a hormone secreted from the kidney and through angiotensin provides renal control of adrenal mineralocorticoids. Thus, elaborate physiological mechanisms exist to prevent sodium deficiency.

Sodium balance regulation also implies behavioral controls since physiological mechanisms alone cannot insure restoration of sodium

deficits. A number of factors that usually accompany sodium deficiency have been implicated in the control of sodium appetite, i.e., they are natrorexigenic. These factors include decreases in plasma sodium concentration, decreases in plasma volume and increases in aldosterone levels. However, no single factor seems necessary for the elicitation of sodium appetite. The experiments reported here examine two major aspects of behavioral regulation of sodium balance; the relationship between sodium deficiency and consequent sodium appetite and the interaction between endogenous aldosterone levels and the other natrorexigenic effects of sodium deficiency in eliciting sodium appetite.

In the first experiment, normal rats maintained on either a sodium deficient diet which augmented endogenous aldosterone levels or a sodium replete diet which had no effect on endogenous aldosterone were injected with the powerful diuretic, furosemide, to induce measurable losses of sodium in urine. Sodium appetite was examined 24 hours after diuresis and natriuresis during a three hour replacement test with 0.51 M NaCl. Rats experiencing high aldosterone levels prior to furosemide injection showed marked behavioral over-compensation for their sodium deficits by drinking at least twice as much sodium as was necessary to recover their urine sodium losses. Sodium balance during the replacement test increased rapidly from -1.5 mEq to approximately +3.0 mEq. In contrast, the salt ingestion of rats maintained on the sodium replete diet prior to furosemide was less marked and was precisely sufficient to re-establish sodium balance at the level tolerated by vehicle injected rats. Blood analyses

demonstrated that furosemide injection resulted in comparable plasma volume deficits regardless of dietary maintenance. Rats maintained on the sodium deficient diet were not significantly sodium deficient prior to furosemide injection because of high aldosterone secretion and consequent sodium retention.

The fact that only rats experiencing high aldosterone secretion before diuresis and natriuresis showed behavioral over-compensation suggested that elevated aldosterone levels determined the amount of salt ingestion. However, vehicle injected rats given the sodium deficient diet regime had high aldosterone levels but no sodium deficit and showed no sodium appetite. Alternatively, aldosterone may have been the critical mediator of sodium appetite, but levels of secretion occurring after sodium deficient diet maintenance may not have been sufficient to elicit salt appetite until after further enhancement by acute sodium loss.

The second experiment tested the proposed role of aldosterone as a potentiator of other natrorexigenic effects of sodium deficiency by examining sodium replacement behavior in sodium deficient adrenalectomized rats incapable of secreting aldosterone. Adrenalectomized and sham operated rats were maintained on a sodium deficient diet with access to 0.51 M NaCl, but then were sodium deprived by removing the salt solution for periods ranging from three hours to four days. The degree of sodium deficiency measured in urine was directly related to the length of sodium deprivation. Blood analyses demonstrated that increasing sodium deprivation of adrenalectomized rats resulted in gradual decreases

in plasma volume and sodium concentration as well as marked increases in plasma potassium concentration. Sodium replacement behavior was examined during the initial three hours of salt solution access following each deprivation period. With the exception of the salt replacement tests after the longest sodium deprivation periods (when the rats were incapable of sustained behavior because of illness), salt intake was largely in excess of sodium deficits. The degree of precision did not improve with repeated testing and was not a function of the degree of sodium deficiency.

The results of this experiment clearly demonstrated that behavioral over-compensation for sodium deficits occurs even in the absence of al-aldosterone. However, the hypothetical role of aldosterone as a potentiator suggested by the first experiment cannot be discounted. Rats in the second experiment were subjected to repeated periods of sodium deprivation with intervening periods of salt access and thereby acquired considerable experience with sodium deficiency and salt drinking. Larger salt intakes were seen in rats having more experience with sodium deficiency and replacement. Thus, the over-compensation of adrenalectomized rats may have been due to experience.

Curiously, sham-operated rats in the second study showed clear evidence of salt appetite following the three and four day sodium deprivation periods despite only minor sodium deficits. These animals had high levels of aldosterone (as indicated by pronounced urine sodium retention), but were not expected to drink salt solution since control rats from the first experiment with high aldosterone levels and little sodium deficiency did not show a salt appetite. Sham-operated

rats in the second experiment may have had higher levels of aldosterone secretion than vehicle injected rats in the first study since they were totally sodium deprived for three or four days whereas rats in the previous study were maintained with 0.51 M NaCl although they did not drink measurable amounts. Accordingly, moderate levels of aldosterone occurring as a result of maintenance on a sodium deficient diet with 0.51 M NaCl available (but not readily ingested) may not elicit a sodium appetite, but more substantial levels occurring during total sodium deprivation will enhance salt intake.

In summary, the results of these two experiments demonstrated the following:

1) High endogenous aldosterone secretion appears to act as a potentiator of the other natrorexigenic effects of sodium deficiency resulting in behavioral over-compensation for sodium deficiency in otherwise intact rats.

2) However, the sodium appetite of rats is generally characterized by over-drinking in response to sodium deficiency even in the absence of aldosterone. Thus, experimental models proposed for the control of salt appetite must include a mechanism capable of dealing with a behavior that is not easily extinguished or inhibited by relief of the initiating deficiency.

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