ABSTRACT

EFFECTS OF LESIONS IN THE POSTERIOR MEDIAL HYPOTHALAMUS ON SALINE SOLUTION PREFERENCE AND BODY WATER REGULATION

By

John William Wright

The maintenance of extracellular fluid volume and tonicity is primarily the responsibility of the antidiuretic hormone and aldosterone systems, which have aided the transition of animals from an aquatic to a terristrial habitat. The more sodium retained in the extracellular fluid the greater is the volume of this fluid space. Therefore the phenomenon of an exaggerated sodium appetite, i.e., saline solution consumption in excess of that needed for normal body maintenance is a paradox not readily explained.

The present series of experiments utilized albino rats housed in unit metabolism cages which allowed for a rather intense monitoring of food and water consumption, urine volume output and urinary constituents. Periodic blood sampling was also employed. The first of five experiments was of a methodological nature and established a recommended delay time of ten days between blood samples or a blood sample and surgery, performed on the same animal. This suggestion is primarily based on the observation that hematocrit and serum sodium values were depressed following blood loss and did not recover to the levels of the initial sample until post-sample days seven and nine, respectively. A second blood sample prior to this delay duration may, therefore, result in a misinterpretation of the effects of an experimental manipulation.

Bilateral electrolytic or radio frequency lesions of the posterior medial hypothalamus were considered in the remaining four experiments and were shown to result in a substantial increase in isotonic saline solution intake that persisted throughout the several post-operative observational weeks in both normal and previously adrenalectomized animals. Considering the intake and excretion of total sodium it was established that a balance existed,i.e., there was an increased output of total sodium but only in response to the heightened sodium intake. The isotonic saline polydipsia did not appear to be due to an inability of the kidney to conserve water for the administration of exogenous pitressin tannate resulted in a decreased fluid intake and increased urinary specific gravity and electrolyte concentrations (sodium and potassium were measured).

Water deprivation differentially affected the experimental and sham lesioned groups. With the initiation of water deprivation all animals evidenced slight and inconsistent decreases in food intake and urine volume output. There were urinary constituent differences between the control and experimental groups. In general the experimentally lesioned animals revealed an inhibited ability to concentrate their urinary electrolytes to a degree equivelant with their corresponding control groups. Urinary specific gravity also reflected this deficit. It is not known whether these differences were directly due to the experimental brain lesions or possibly reflected kidney function alterations due to the polyuria accompanying the heightened isotonic saline solution intake, an indirect effect of the lesions.

These data are interpretted to support a neural model proposed for the control of body water regulatory processes. Experimentally induced damage to medially located brain structures have been noted in the literature to often result in increased fluid intake while lateral brain structure damage has often led to an adipsic and/or aphagic condition. Bilateral posterior medial hypothalamic lesions represent damage near the brain midline and isotonic saline polydipsia results. Bilateral lesions in what the author has chosen to call the posterior lateral hypothalamus, the area just lateral to the region ablated in this investigation, have yielded adipsia and aphagia.

There are neural pathways that link the medial structures and comparable neural systems that connect the lateral brain structures. Fiber tracts appear to arise from the ventromedial, anteriomedial and posteriomedial hypothalamic nuclei and contribute to the periventricular system at least a portion of which flows into the stria terminalis which courses around the anterior commissure terminating primarily in the central nucleus and medial portion of the basal nucleus of the amygdala. There is also a branching from the stria terminalis in the form of the stria medularis which establishes connections with the medial habenular nucleus. On the other hand, fibers originating from the posterior and anterior lateral hypothalamus pass through the medial forebrain bundle forming the ventral amygdalofugal pathway which appears to terminate in and about the lateral amygdaloid nucleus. Reciprocating fibers have been identified between the posterior lateral hypothalamus and the lateral septal nucleus and between the lateral septal nucleus and the lateral habenular nucleus.

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By

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DEDICATION

To my Family, Donna, Tim and Shonna Ann

For they alone realize the true cost that these pages represent

In effort expended over the last several years.

And they alone remember the Hellos and Good-byes

Separated by much too little time.

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INTRODUCTION

The existence of mechanisms for maintaining body fluid constancy has been appreciated for many years (Peters, 1935; Starling, 1896). The development of such homeostasis has depended upon two major mechanisms aiding in the transition of animals from an aquatic to a terrestrial habitat. Both of these systems are primarily concerned with the extracellular fluid (ECF) volume, for water is thought to freely diffuse across the majority of cell membranes equating extracellular and intracellular concentrations.[1]

Antidiuretic Hormone

The first fluid regulation mechanism, concerned with body water conservation, involves the antidiuretic hormone (ADH) which is sensitive to changes in the effective osmolality of the extracellular fluid (Sims and Solomon, 1963). It was Verney (1947) who first pointed out the relationship between the ADH effect and possible osmoreceptors in the forebrain. Verney infused equal osmolarity hypertonic solutions of NaCl, glucose and urea into the carotid artery of dogs during the apex of water diuresis. The order of effectiveness of these solutions in reducing the diuresis was sodium, glucose then urea. Since this order is the reverse of their ability to penetrate cell membranes from the extracellular fluid he proposed that osmotic removal of water from "osmoreceptor" cells located in the supraoptic nucleus of the hypothalamus initiated neural impulses from that nucleus for the release of

[1]There is some debate on this issue with the suggestion that intracellular concentration may be slightly hypertonic to that of the extracellular fluid (Conway and Geoghehan, 1955; Robinson, 1954).

ADH from the posterior pituitary. Thus Verney envisioned a negative feedback system, i.e., a rise in blood osmotic pressure results in cellular dehydration causing an increased release of ADH which in turn facilitates water reabsorption by the kidney. This added reabsorption of water, together with excretion of ions decreases plasma osmotic pressure and ADH output decreases.

Histological evidence (Jewell, 1953) coupled with the results of intracranial vascular ligations of the cerebral vascular bed (Jewell and Verney, 1957) and recording studies that revealed "osmopotentials" (changes in firing rate in preoptic and supraoptic areas resulting from hypertonic saline injections (Brooks, Ushiyama and Lange, 1962; Cross and Green, 1959; Nakayama, 1955; Sawyer and Gernandt, 1956; Von Euler, 1953) have identified the anterior region of the hypothalamus with ADH secretion.

Antidiuretic hormone is synthesized in the supraoptic nucleus. It then migrates down the supraoptic-hypophyseal tract to the pars nervosa of the pituitary gland where the ADH is stored until release is signalled (Leveque and Sharrer, 1953; Sawyer and Mills, 1966). Cort (1963b) has suggested that ADH may be secreted directly from the hypothalamus, however this has not been substantiated (Raisman, 1966). With the advent of new ADH analysis techniques (Gilmore and Vane, 1970; Heller and Stule, 1959) this possibility may be explored further.

The method by which ADH acts upon the kidney nephron is not clear. It may be that the characteristics of the vasopressin chemical bond alters cell membrane water permeability (Koepoed-Johnsen and Ussing, 1954) thus allowing more water to pass through the membrane. Water reabsorption is also aided by the osmotic gradient created by the

"counter current multiplier system" in the papilla of the loop of Henle (Wirz, 1956; Gottschalk, 1960). ADH does not seem to act on the proximal tubule or upon the descending or ascending loop of Henle. The action is specific to the distal tubule and collecting duct (Pitts, 1968).

Under normal conditions the adequate stimulus for ADH release is hyperosmolarity, or cortical influence upon the hypothalamus resulting from fear or pain (Moran and Zimmermann, 1967). A decrease in ADH secretion accompanies mechanical stretch of the left atrium and stretch of carotid sinus receptors (Pitts, 1968). The control of water excretion by ADH in addition to regulating osmotic pressure of the ECF, has some affect on the volume of the ECF. Infusion of isotonic saline causes changes in ECF volume which results in a water diuresis (Strauss et al., 1951) suggesting that ADH may be controlled by ECF volume changes alone (Cort, 1955; Leaf and Mamby, 1952).

Aldosterone

Primary input into the second fluid regulation mechanism, the aldosterone system, comes from stretch receptors in the carotid sinus, right atrium and juxtaglomerular apparatus. With a decrease in blood volume the afferent glomerulus becomes passively constricted and there is a reduction in ECF volume. An enzyme, renin, released from the kidney into the blood, acts on the glycoprotein of the plasma resulting in the reduction of a decapeptide to angiotensin I which is converted via a plasma enzyme to an octapeptide, angiotensin II. Angiotensin II functions as 1) a vasoconstrictor within the blood and 2) acts on its target organ, the cortex of the adrenal gland, stimulating secretion of aldosterone into the blood resulting in a greater reabsorption of sodium

by the nephron. This appears to be accomplished by a change in the protein synthesis, i.e., aldosterone stimulates sodium transport by influencing a nuclear receptor to increase the DNA directed synthesis of RNA wich codes a particular protein. This protein may function in one of three ways: 1) to control entry of sodium into the mucosal epithelium from the urine side; 2) to act as the ion carrier of the sodium pump; or 3) to regulate the production of ATP enzymatically, and thus control the energy supply on which transport depends (Crabbi and DeWeer, 1964; Edleman et al., 1963; Fimognari et al., 1967; Forte and Landon, 1968; Porter et al., 1964; Sharp and Leaf, 1966). Because protein synthesis has a minimum time lag of one or more hours after the introduction of aldosterone, changes in sodium reabsorption of the kidney are delayed for that time period.

If the reabsorption of sodium is considered along the nephron, the proximal tubule accounts for the reabsorption of some 75% of the sodium. Since approximately 99% of the body sodium is reabsorbed, the descending limb of Henle accounts for some of the remaining 24% by accepting sodium from the ascending limb via the "counter current multiplier mechanism" (Gottschalk, 1960). Reabsorption by the distal tubule and collecting duct, as governed by aldosterone level, accounts for the remaining sodium reabsorption, and obviously, the more sodium retained in the ECF the greater is ECF volume (Pitts, 1968).

Sodium Appetite

Because animals are capable of closely monitoring their body fluid concentrations in a homeostatic manner, the phenomenon of exaggerated sodium appetite (saline consumption in excess of that needed for normal body maintenance) is a paradox not readily explained. Rats offered a

choice between hypotonic saline solutions and water evidence a heightened ingestion of the saline solution (Bare, 1949; Nelson, 1947; O'Kelly, 1954; Stellar and McCleary, 1952). Young and Chaplin (1949) simultaneously offered normal rats eight concentrations of saline: 0.1, 0.2, 0.4, 0.7, 1.5, 3.0, 6.0 and 12.0 per cent. These animals drank more from the 0.7% than from all other solutions. Bare (1949) found that as he increased the sodium chloride concentration, fluid consumption increased while tap water intake slightly decreased. Maximum solution intake occurred with a 0.9% saline solution and fell off with concentrations above this level. At saline concentrations above 2.5% consumption was almost entirely of tap water.

The intake method has also been employed in such preference tests, typically assuming the form of the presentation of one test fluid for one hour. The animals are under a water deprivation schedule until fluid presentation. With this approach Weiner and Stellar (1951) placed maximum saline solution consumption at a concentration of 0.8%. Stellar, Hyman and Samet (1954) also indicated maximum preference at between 0.7 and 0.8% using similar intake methods.

In general investigations utilizing two drinking tubes have established a preference for salt solutions in the normal rat beginning at concentrations of 0.05 to 0.06 per cent. Maximum differential consumption appears to take place with concentrations of 0.7 to 0.9 per cent. At a saline solution of approximately 1.5% the normal rat drinks equal quantities of tap water and the solution.

Young and Falk (1956) and Falk and Titlebaum (1963) have criticised earlier saline preference work for the implicit assumption that "the more an animal drinks of a fluid the better he likes it". They have

argued that a brief-exposure preference test relieves the experimenter from subscribing to this definition of preference. Under these conditions an animal is released from a start box and is allowed to approach the two test fluids (either distilled water and a saline solution or two saline solutions; the saline solutions tested in the Young and Falk study were 0.38, 0.75, 1.5, 3.0 and 6.0%). Once substantial contact was made with one of the two fluids the cups containg the fluids were withdrawn out of reach. Under these experimental conditions the preferred saline concentration of non-thirsty rats was between 0.75 and 1.5 per cent. Thirsty rats often chose water over the saline solution and took the weaker of two salt solutions.

Isotonic saline preference may be relatively independent of bodily sodium need in that the preference is not significantly affected by parenteral saline loading or by changed sodium food content (Fregly, et al., 1965). Also rats given prolonged free choice between water and isotonic saline ingest saline to such excess that they may reveal signs of chronic sodium overloading (Nelson, 1947).

This preference exhibited by the normal rat for saline solutions is of some interest for such solutions would appear to be less effective in reducing thirst than pure water. Mook (1963) has argued for the relative ineffectiveness of such solutions in relieving thirst on two bases: 1) "isotonic saline solutions are absorbed less rapidly than water from the rat intestine (O'Kelly, Falk and Flint, 1958)" 2)"sodium chloride is confined primarily to the extracellular space after absorption (Gamble, 1954)". Mook suggests that osmotic pressure equilibration holds the solutes of the ECF at isotonicity thus ingestion

of an isotonic solution will only add to the ECF volume with no water gain by the cells. A number of seemingly adequate theories have been postulated in an effort to explain this apparant discrepency between intake and need.

The Diluted-Water hypothesis by Deutsch and Jones (1959; 1960) argues that the Central Nervous System has receptors sensitive to the taste of water. Saline solutions, to some degree, mask the "water signal" resulting in the imbibition of more saline before a given level of satisfaction is reached. They cite neurophysiological work by Zotterman (1956) and Zotterman and Diamant (1959) as support for their hypothesis. These investigators found that nerve fibers carrying water and salt signals fire at high spontaneous rates and are markedly inhibited when water is placed on the tongue, but inhibited to a lesser extent when hypotonic saline is applied. Thus hypotonic saline acts as a "diluted" water yielding a weaker water signal and consumption of a greater volume to reach satiation.

Deutsch (1953) has reported that a choice between equal quantities of water and saline in a T maze situation should result in the selection of water which has a greater water signal. His results confirm this prediction. These basic findings utilizing a T maze have been replicated by Chiang and Wilson (1963) and by Brookshire (1967a) who also found that water reward resulted in faster running by water deprived rats than did a saline solution reward. However, if rats were raised from weaning on hypotonic saline they preferred the saline rather than water in the T maze situation (Brookshire, 1967b).

Fisher (1965) held rats on a slight water deprivation schedule and reported that his animals would lick from a spout containing tap water

in order to obtain access to a second tube containing 0.58% saline. In Weiner and Deutsch's 1967 paper they criticized the work reported by Fisher (1965) and Falk and Titlebaum (1963) in that animals used in these experiments may have been predisposed to saline solution due to inadvertent salt deficiency.

Recently Myer and Hemmel (1969) confirmed the dilute water hypothesis in a lever pressing experiment. Rats kept at 80% body weight were placed on a 1-min. variable interval reinforcement schedule for one hour per day. A 0.025 ml. water or saline solution (0.10-2.0M) was offered as reinforcement. With higher concentrations of saline, response rates decreased; at no time did a saline solution prove more reinforcing than water.

A second explanation emphasizes gastrointestinal diultion of hypotonic saline rather than oral receptor influences in explaining saline solution preference. Mook (1963) prepared each of his rats with both an esophageal fistula and a gastric fistula. In this way he could allow the animal to drink a particular fluid (water or saline) which flowed out the esophageal fistula and introduce the same quantity of that, or a different fluid into the stomich. Utilising this preparation Mook found that when isotonic saline reaches the stomach, water intake is elevated above normal ingestion levels. It would appear that isotonic saline is less effective in hydrating the animal when compared with water and as a result the animal must drink more of the isotonic saline solution. It is interesting to note that if the consequences of drinking water or of drinking isotonic saline are the same in terms of what is placed into the stomach, the voluntary intake of these two fluids is nearly equal.

With regard to this postingestional osmotic effect and mouth receptors Mook states: "This osmotic mechanism must influence intake in interaction with mouth factors; for when hypertonic saline enters the stomach water drinking is enhanced and the drinking of concentrated solutions is depressed, even though the postingestional events are precisely the same in both cases".

Hatton (1965) placed rats on a 23½ hour water deprivation schedule and once accustomed to this he stomach loaded the animals with tap water 2% BW, tap water 4% BW, 2%, 4% or 6% saline to 2% BW. He then measured urine output for a 3 hour period following such loading. The curve of urine output beginning with a sham stomach load group and progressing through the saline loads, i.e., 2%, 4% and 6% NaCl, was negatively accelerated with a greater urine output as saline solution concentration increased. Upon reaching criterion performance (10 min. without a response) for two extinction periods under a lever pressing design for a water reward, Hatton allowed animals water for a one hour period. The animals showed differential intakes of water that were dependent on the type of load earlier received. a 4% BW tap water load group consumed a mean of 10 cc, 2% BW tap water load group 16 cc, sham load group 21 cc, 2% NaCl load group 20 cc, 4% NaCl group 23 cc and the 6% NaCl group approximately 31 cc. If these intakes are accepted as a measure of the degree of dehydration suffered by the animals it appears that in general as the concentration of saline solution increases water intake and therefore level of dehydration also increases.

A third explanation takes a more general form and has been referred to as the "Sodium Reservoir" hypothesis by Stricker and Wolf (1967; Wolf and Stricker, 1967). Sodium appetite is thought to be

elicited by hyponatremia, hypovolemia or increases in mineralcorticoid production. Bone sodium is the only reservoir that Stricker and Wolf have thus far identified (Bergstrom, 1955). The hypothesized reservoir is thought to release sodium as a response to hypovolemia, hyponatremia or increased mineralocroticoid levels. Sodium appetite thus appears in an animal when sodium loss from a reservoir reaches some threshold value (Stricker and Wolf, 1969). Stricker and his colleagues have concerned themselves with manipulating this sodium reservoir by the use of several techniques. The injection of 5 ml of 10% polyethylene glycol (subcutaneous) results in a decreased blood volume with an unaltered serum sodium level approximately 8 hours later. About the same length of time following the injection of 2.5 ml formalin both serum sodium and blood volume are decreased. And following a 5% body weight stomach load of distilled water blood volume is unchanged but the serum sodium level drops (Stricker and Wolf, 1966).

In a recent study (Jalowiec and Stricker, 1970) rats were injected with formalin and then offered either water, 0.07M, 0.15M or 0.51M saline solutions to drink. Body fluid balance was restored most quickly by those animals given isotonic saline and less quickly by hypotonic and hypertonic solutions even though the solutions were ingested at comparable rates.

Animals given subcutaneous injections of polyethylene glycol lose isosmotic intravascular fluid (Stricker, 1966). The animal's response to such treatment is similar to that following formalin injection. Fluid consumption increases and a preference for hypotonic saline over water occurs with some ingestion of hypertonic saline

solutions (Stricker, 1966; Smith and Stricker, 1969). This hypothesis suggests that the ultimate responsibility for a sodium appetite lies with the sodium loss sufferred by some body reservoir which then affects body function (Wolf and Stricker, 1967; Stricker and Wolf, 1969).

Novin (1962) developed a method of measuring brain electrical conductivity in rats. With this technique he has measured the impedance of the brains of thirsty rats (22 hour water deprivation schedule) given one of the following saline solutions: 0.0, 0.1, 0.2, 0.4, 0.8 per cent (Novin,et al., 1966). It was found that measurable changes in conductivity as a result of fluid intake occur within five minutes after the initiation of drinking. In Novin's words, "As the concentration of saline decreases, the change in conductivity increases". A saline solution of 0.8% results in very little impedance change. If it is accepted that changes in brain impedance parallel deviations in systemic electrolyte concentrations or effective osmolarity then Novin would suggest that if effective osmotic pressure must reach a threshold change for the termination of thirst to occur the realization of this threshold would require correspondingly larger volumes of solution ingested as the salinity concentration increased.

The brain lesion technique has thus far offered limited insight into the identification of the underlying neural components of this saline preference in rats. Bilateral damage inflicted upon the ventromedial hypothalamus (VMH) of the rat results in a substantial increase in a 1% sodium chloride solution intake (Kawamura et al., 1970). Bilateral lesions just below the VMH which appear to damage the arcuate nucleus yield a heightened 2% saline intake (Covian and Antunes-Rodrigues, 1963). Bilateral electrolytic lesions destroying the VMH, dorsomedial

hypothalamus (DMH) or fornix are followed by a diabetes insipidic syndrome with an unaltered 2% NaCl ingestion pattern (Antunes-Rodrigues and Covian, 1965). A more recent paper (Wolf and Quartermain, 1967) describes anterior lateral hypothalamic (LHA) lesioned animals that were maintained through adipsia and aphagia by intragastric feeding until normal food and water ingestion was again self-initiated. Such animals, once recovered, exhibited a preference for weak saline and an aversion for hypertonic saline undistinguishable from a normal animal's preference. When adrenalectomized, however, these lesioned animals failed to satisfactorily regulate their saline intake and fell into a sodium deficiency.

Wolf (1967) has shown that normal regulation of saline and water ingestion continues after the elimination of the lateral septal, lateral preoptic or ventral tegmental regions. Lesions in the thalamic gustatory nucleus and in the reticular formation just caudal to the above nucleus decrease intake of a 0.5M (2.9%) saline solution instigated by a 2.5 ml injection of a sodium depleting 1.5% formalin solution (Wolf and Steinbaum, 1965; Wolf, 1968). Large septal lesions involving the anterior and posterior areas and extending from the medial to the lateral portions caused substantial increases in 1.5% saline intake (Vilar et al., 1967) and 0.87% saline intake (Donovick et al., 1969) under a self selection regime involving saline solutions and water.

Support for a neural circuit linking hypothalamus, septal and amygdaloid areas to bodily sodium balance is enhanced by the discovery that amygdaloid lesions result in altered saline intakes by rats. Bilateral damage to the corticomedial complex of the amygdala was

followed by increased 1.5% NaCl intake; similar damage to the lateral nucleus brought decreases in 1.5% saline intake (Gentil et al., 1968).

The posterior region of the hypothalamus has also been suggested as an area involved in the regulation of saline ingestion. Cort (1963a) has claimed that this area is the site of production of a neuroendocrine hormone, a "Substance X", that serves as an anti-natriuretic agent at the kidney level. Bilateral destruction of the posterior hypothalamic area appears to reflect an increase in 0.9% NaCl intake with an accompanying urinary sodium loss of substantial magnitude. Electrolytic lesions of the posterior hypothalamus of rats and cats have been immediately (30 minute latency) followed by salt loss in the urine (Cort and Keeler, 1954; Cort and Lichardus, 1963d; Cort, 1955; Lichardus and Jonec, 1961). There is no evidence of adrenal damage and steriod therapy does not restore salt balance (Lichardus et al., 1965) but availability of saline solutions (0.9 and 3.0%) for drinking, restores salt balance within about five days in rats (Cort, 1963a).

Rats given a 2% saline solution to drink for ten days revealed significantly decreased cell nucleus size in the posterior hypothalamic nucleus as compared with the ventromedial, dorsomedial and arcuate nuclei. The supraoptic and paraventricular nuclei increased nucleus size (Lichardus, Mitro and Cort, 1965).

Cort's present position may be summarized as follows: In that salt loading results in decreased cell nucleus size in the posterior hypothalamic nucleus, a neuroendocrine function may exist at this locus. The carotid occlusion work (Cort and Lichardus, 1963a; 1963c; Cort, 1965, p. 145) suggests that during natriuretic "volume" reflexes a natriuretic

substance is released from the diencephalon. This substance is probably not a steroid or a catecholamine. The rapidity of natriuretic onset and termination given the carotid occlusion stimulus indicates a peptide. Vasopressin and angiotensin have been eliminated (Cort et al., 1965; Cort and Lichardus, 1963b). The substance may have a chemical structure similar to oxytocin.

A question of major significance concerning Cort's work may be approached as follows: A natriuretic hormonal agent is secreted from a brain site during thoracic vascular bed expansion and is not present, i.e., a blood sample does not cause natriuresis in a recipient cat, after bilateral posterior hypothalamic lesioning. Bilateral posterior hypothalamic lesioning results in natriuresis and diuresis in rats, cats, dogs and humans with sudden onset and an extreme negative sodium balance. My question: How is it that bilateral posterior hypothalamic lesioning during carotid occlusion prevents natriuresis and diuresis and in a normal animal causes acute natriuresis and diuresis? It must be pointed out that the most recent of a series of attempts to replicate evidence of a blood-born natriuretic factor has offered an alternative explanation to this phenomenon. In cross-circulation experiments utilizing rats, Bonjour and Peters (1970) expanded the extracellular space of a donor animal by the slow infusion of isotonic saline (0.02 ml/min.). The donor animals demonstrated diuresis and natriuresis due to hemodilution (plasma protein dilution) and/or expansion of the extracellular space. The recipient animals which experienced hemodilution via cross circulation but no expansion of the extracellular space, did not evidence any diuresis or natriuresis. Prevention of expansion of the extracellular space in the recipient animal was accomplished by placing the animal

on a sensitive balance (sensitivity 20 mg.) and adjusting the rate of blood flow in drops per minute so that the recipient animal was not allowed to gain weight in excess of the intravenous infusion which it received, i.e., 0.075 g/min. The cross circulation was achieved by cannulation of a femoral artery to a femoral vein. A blood-born mediator is thus ruled out under these conditions.

An alternative explanation for Cort's findings concerns the stimulation of intrarenal mechanisms by extracellular space expansion. Thus the urinary patterns reported by Cort (Cort et al., 1968; Cort and Lichardus, 1968) and co-workers (Lichardus and Pearce, 1966) may be the result of extracellular fluid volume changes per se rather than a "Substance X".

Lesion Location and Proposed Experiments

For purposes of future reference the posterior medial area of the hypothalamus is that region dorsal and caudal to the dorsomedial nucleus in the post-tuberal region (Haymaker et al., 1969) and medial to the fornix and mammillothalamic tract.

The present set of experiments are an attempt to more systematically ascertain the role of the area posterior hypothalami in body water regulation, with special emphasis on saline preference and ingestion.

Experiment 1 is methodologically concerned with sampling techniques, i.e., what time duration must pass following a 1½ to 2 cc heart puncture blood sample before the body fluids return to pre-sample levels as determined by available indices.

Experiment 2 focuses attention upon the application of bilateral lesions in the area posterior medial hypothalami. Comparisons are made

with reference to the results obtained from electrolytic DC lesions and Radio Frequency (RF) lesions. The electrolytic lesion is referred to as an irritative focus in that metal ions may slough off the electrode with the passage of current and become deposited at the lesion site (Dahl and Ursin, 1969). The radio frequency technique has been said to be non-irritative for it relies upon high intensity heat which results in coagulation of tissue at the lesion point (Rolls, 1970).

Experiment 3 is a preference experiment utilizing three groups of animals. Within each group the animals are equally divided into an experimentally lesioned segment with bilateral lesions of the area posterior medial hypothalami, and a sham lesioned group with identical surgical preparation as the experimental group but with a sterile dura puncture rather than lesions. Group 1 received tap water for its fluid intake. Group 2 was provided both tap water and 0.87% saline to drink. Group 3 consisted of previously adrenalectomized rats and was allowed both 0.87 and 2.0% saline. These same three groups of animals were utilized through the subsequent experiments.

Experiment 4 addresses the possibility of neural damage to the antidiuretic hormone system. Subcutaneous injections of pitressin tannate and peanut oil were administered the experimental lesion and control groups in a counter-balanced design. Cort (1963b) has indicated that ADH may be secreted directly from the posterior hypothalamus in addition to its release by the pars nervosa. This experiment, therefore tests whether the heightened isotonic saline ingestion exhibited by experimentally lesioned animals can be reduced with exogenous pitressin.

Experiment 5 is concerned with the first day of a 23½ hour water deprivation schedule. Such a schedule represents a rather stressful condition in that body water and electrolyte saving mechanisms are required to function well in order to satisfactorily maintain ECF volume and tonicity. Attention is given to comparisons between the experimental and control groups' responses to this regimen.

EXPERIMENT 1

Repeated Blood Samples

Since it is necessary to use repeated blood samples in this set of studies an appreciation of the effects of a 1½ to 2 cc heart puncture blood sample is advised. The literature offers no indication of urine and blood electrolyte changes following such a blood sample. Hatton and Thornton (1968) used ten days between heart puncture blood samples with good success. The following experiment was therefore designed to determine whether the rat's body water balance recovers in less than ten days following a blood sample as measured by available indices of body water volume and constituencies.

Method

Subjects

Thirty male albino rats of the Holtzman strain, approximately 120 days of age were adapted to living in metabolism cages under constant light for ten days before the initiation of experimental observations. The animals were maintained on *ad libitum* tap water and powdered Wayne Breeder Blox.

Metabolism Cages

The metabolism cages were of the Acme Metal Products design. The living dimensions were 26.5 x 20.5 cm, and 16.5 cm high. Each cage was supported by an accompanying base (also Acme Metal Products) that was 50 cm tall. There were 30 unit metabolism cages of this type

arranged on three tables, 10 per table, the surfaces of which were elevated 94 cm above the floor. All cages were kept in the same room which was maintained at approximately 23-25 C with a relative humidity of 20-50%.

Procedure

Following ten days of adaptation to the metabolism cages the animals were placed into five groups of six animals each. Experimental observations then began with eight pre-sample days prior to the first blood sample. The groups were randomly assigned to one of five treatment conditions. These conditions were: first blood sample taken on day 0, second blood sample on day 1, this comprised the group henceforth referred to as Inter-Sample Interval (ISI) - 1. The next group was designated ISI - 3 with the first blood sample taken on day 0 the second sample on day 3. Other groups were treated in similar fashion with five days between blood samples, i.e., group ISI - 5, seven days between samples group ISI = 7 and nine days between the two samples group ISI - 9.

The first blood sample was taken from the animals in a random order on day 0. The second sample taken from an animal was determined by the group to which it had been previously assigned. The second sample for a given animal was taken within five minutes of the time of day the first sample had been taken. The ISI - 3 group, for example, represents animals that were sampled at a given time on day 0 and then sampledagain 72 hours later within five minutes of that given time for each animal. The samples were taken under light ether anesthesia between 0800 and 1330 hours on all sample days. The serum samples were taken via left ventricle heart puncture and were immediately

centrifuged at 3300 rpm for 3 minutes. Two hematocrit samples per animal were prepared and centrifuged in standard heparinized capillary tubes (1.5-2.0 mm I.D., length 75 mm, Scientific Products) at 10,000 rpm for 4 minutes, and subsequently read. A small portion of the retained serum was immediately analyzed for protein in a refractometer (American Optical Company). The remaining serum was divided between two analysis methods. A 0.025 ml serum sample was taken for sodium and potassium concentration determination by flame photometer (instrumentation Laboratories, Model 140). The remaining serum was quickly frozen for later determination of osmolality by freezing point osmometer.

Throughout the ten day adaptation period, the eight day pre-sample period and the nine days of blood samples, urine samples were taken at the same times twice daily, 0800-0830 and 2000-2030 hours. Food and water consumption and urine volume output were recorded at the same time as the urine collection. Body weight was recorded at the 0800-0830 reading time. Sodium and potassium concentrations were determined for each urine sample by flame photometry and total solids readings (specific gravity) were obtained by the use of a refractometer.

Results

The data for water and food intake, urine output and electrolyte concentrations have been grouped across two day intervals for the eight days preceding the first blood sample and nine or eleven days after the first sample depending upon the group being considered. When a blood sample was taken the next reading period values are given following the sample. This is the only departure from the presentation of data grouped across two days. The means and standard errors (S.E.) of the means are plotted across days for each of the five groups. Points are

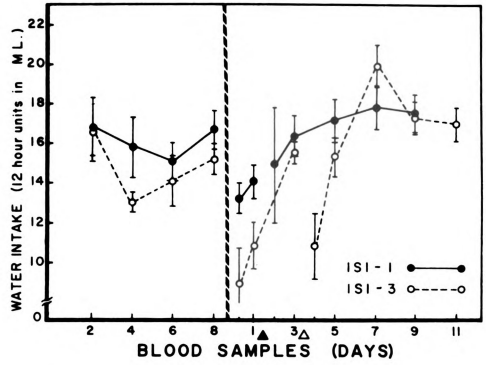
reliably different from one another at a minimum of the 0.05 probability level where S.E. bars do not overlap.

Water intakes for the five groups are shown in Figures 1, 2 and 3. The points represent mean values of tap water intake per 12 hour reading periods with reference to a given sample group. The eight days prior to the first sample represents a base rate period and in the case of water intake the five groups are significantly different from one another ($F=5.28$, df 4/15, $p<0.01$). Groups ISI-3 and ISI-9 deviated from the other three groups. The next reading period after the first blood sample for all groups revealed significant drops in water intake, as compared with the base rate periods for the ISI-1 group which dropped an average of 3.2 ml and the ISI-3 group which dropped 6.2 ml. The other three groups (ISI-5, -7, -9) were not different from the base rate water intakes. The effects of the second blood sample upon water intake were that groups ISI-3, -5 and -9 significantly decreased water intake as compared with the grouped reading periods prior to the sample, with declines of 4.87, 2.88 and 5.33 ml, respectively. Utilising the above criteria, four out of the five groups revealed decreased water intake following the first and/or the second blood sample. The exception, group ISI-7 also revealed a significant decrease in water intake following the initial blood sample if post-sample day 1 is used as a reference value to compare with the base rate periods rather than the first reading period after the sample.

Comparisons between groups concerning food intake disclose tendencies not unlike those evidenced for water intake. Figures 4, 5 and 6 represent food intakes for the five groups for the 12 hour reading

Figure 1. Mean (± S.E.) water consumption in 12 hour units for the intersample interval (ISI) - 1 day and - 3 day groups during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 2. Mean (± S.E.) water consumption in 12 hour units for the intersample interval (ISI) - 5 day - 7 day groups during the eight days prior to blood sampling and eleven days following the initial sample.


WATER INTAKE (12 hour units in ML.)
22
20
18
16
14
12
10
0
ISI - 1
ISI - 3
2 4 6 8 1▲ 3△ 5 7 9 11
BLOOD SAMPLES (DAYS)

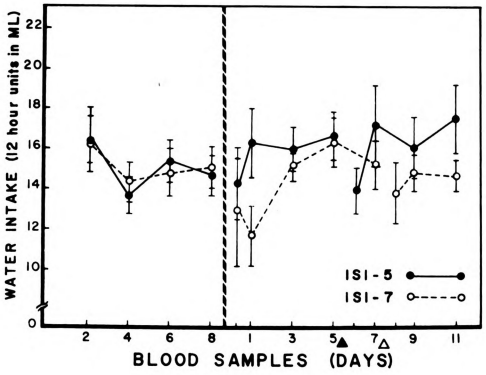
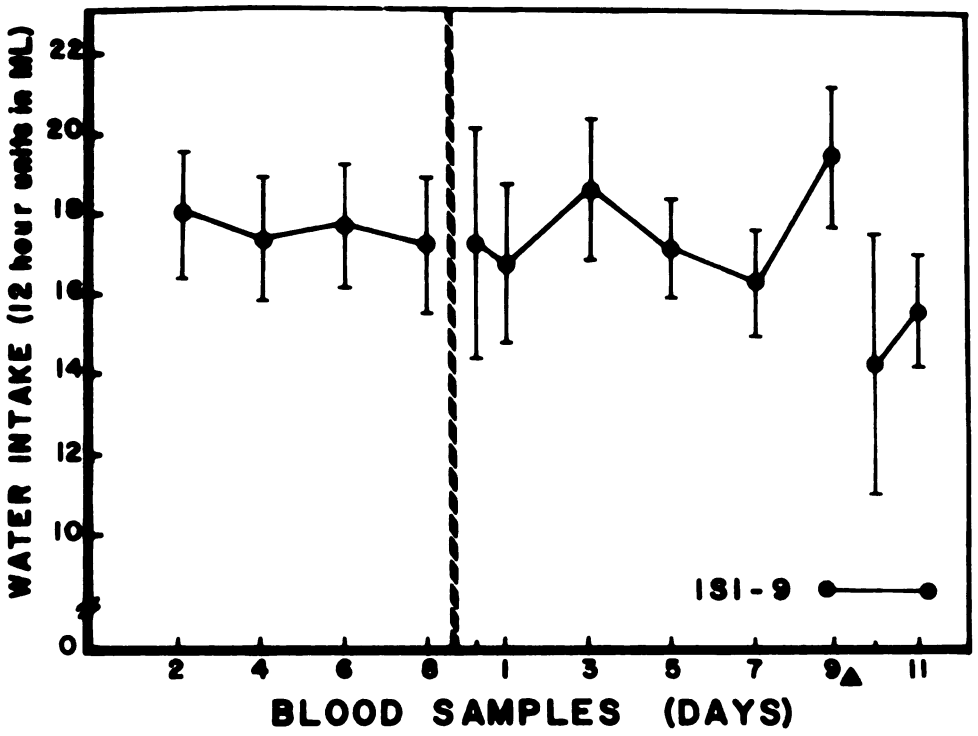
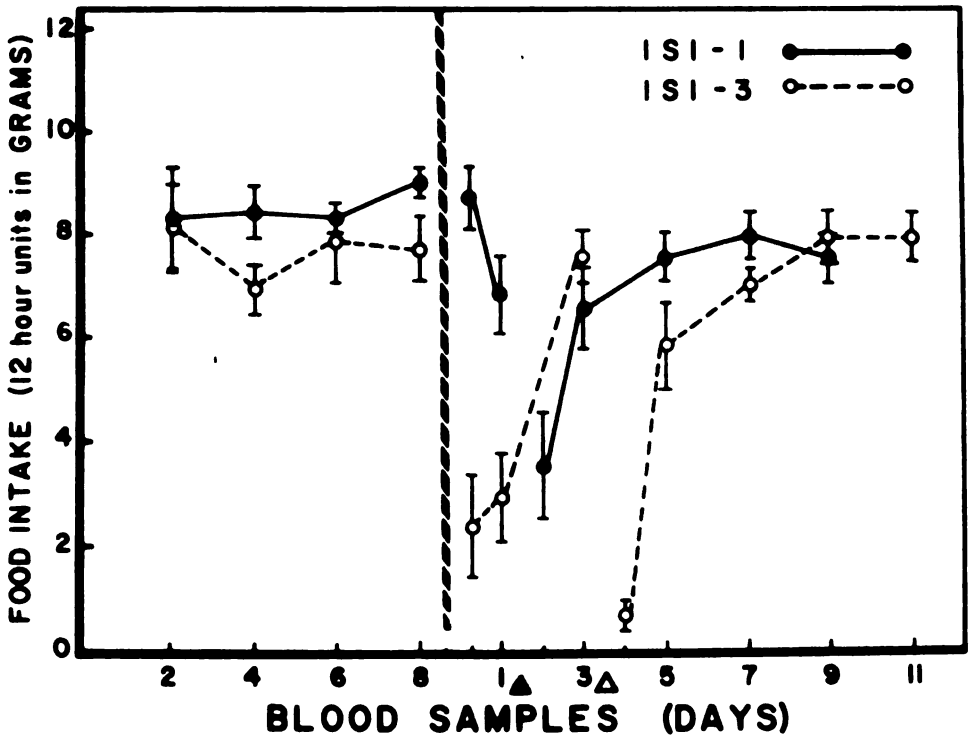

WATER INTAKE (12 hour units in ML.)
22
20
18
16
14
12
10
0
ISI - 5
ISI - 7
2 4 6 8 1 3 5▲ 7△ 9 11
BLOOD SAMPLES (DAYS)

Figure 3. Mean (± S.E.) water consumption in 12 hour units for the intersample interval (ISI)-9 day group during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 4. Mean (± S.E.) food consumption in 12 hour units for the intersample interval (ISI)-1 day and -3 day groups during the eight days prior to blood sampling and eleven days following the initial sample.


WATER INTAKE (12 hour units in ML)
0
10
12
14
16
18
20
22
ISI-9
2
4
6
8
1
3
5
7
9
11
BLOOD SAMPLES (DAYS)


FOOD INTAKE (12 hour units in GRAMS)
0
2
4
6
8
10
12
ISI-1
ISI-3
2
4
6
8
1
3
5
7
9
11
BLOOD SAMPLES (DAYS)

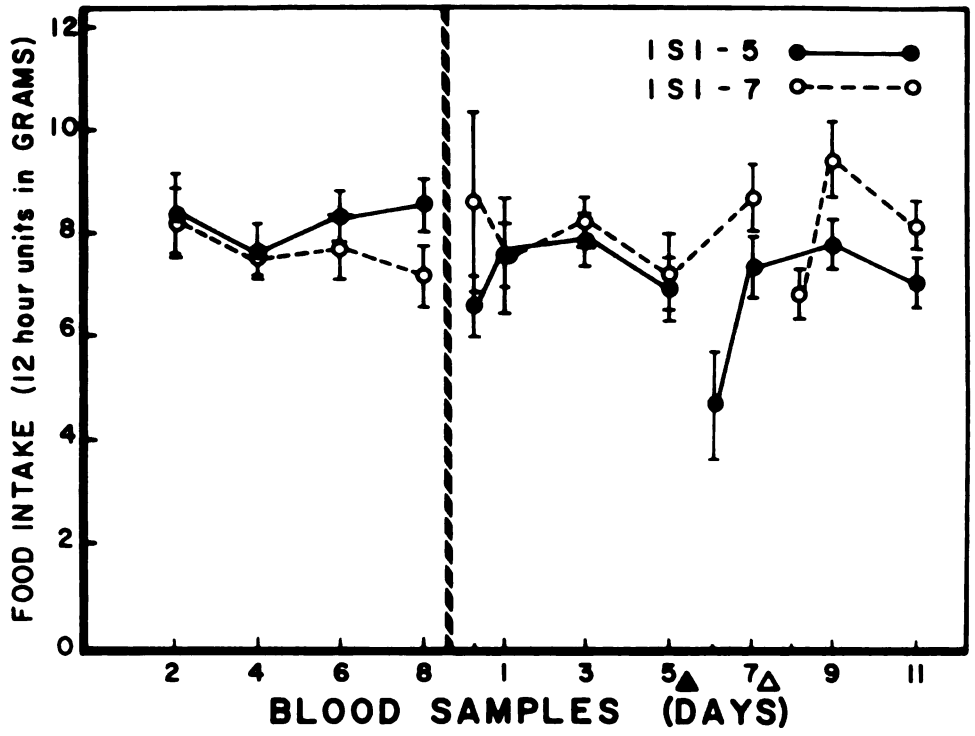
periods, again grouped over two day periods. The eight day base rate period showed the groups to be non-significantly different from one another ($F=2.57$, df 4/15, $p>0.05$). Food intakes dropped significantly for groups ISI-3 and -5 after the initial blood sample, i.e., 6.87 and 2.18 grams. There were significant decreases in food intake as compared with the grouped reading period prior to the sample after the second blood sample for all five groups, ISI-1, -3, -5, -7 and -9 with average drops in food intake of 3.58, 6.87, 2.18, 1.79 and 1.92 grams, respectively.

The mean urine output for each group across days is provided in Figures 7, 8 and 9. There were base rate differences between the groups for the eight days preceding the first blood sample ($F=6.73$, df 4/15, $p<0.01$). Groups ISI-1 and -7 demonstrated a significantly altered urine outuput after the first blood sample as compared with the base rate data. Group ISI-1 dropped 2.66 ml while group ISI-7 increased its mean urine output by 2.79 ml, however the ISI-7 animals registered base line urine outputs that were very low (Figure 8) below a mean of 8 ml per 12 hour reading period. The first reading period urine output value after the initial blood sample was 9.83 for the ISI-7 group which is not different from the last base rate urine output mean for all five groups combined, 8.96 ml. Group ISI-3 showed a significant increase in urine output after the second blood sample, up 2.88 ml, while group ISI-7 decreased significantly by 3.38 ml. The other three groups revealed no significant change.

Turning to urine sodium concentration and specific gravity, the findings are presented in like manner as the previous volumetric intake-output data. The eight day base rate period revealed no differences between the five groups in terms of urine sodium concentration

Figure 5. Mean (± S.E.) food consumption in 12 hour units for the intersample interval (ISI)-5 day and -7 day groups during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 6. Mean (± S.E.) food consumption in 12 hour units for the intersample interval (ISI)-9 day group during the eight days prior to blood sampling and eleven days following the initial sample.


ISI-5
ISI-7
FOOD INTAKE (12 hour units in GRAMS)
0
2
4
6
8
10
12
2
4
6
8
1
3
5▲
7△
9
11
BLOOD SAMPLES (DAYS)

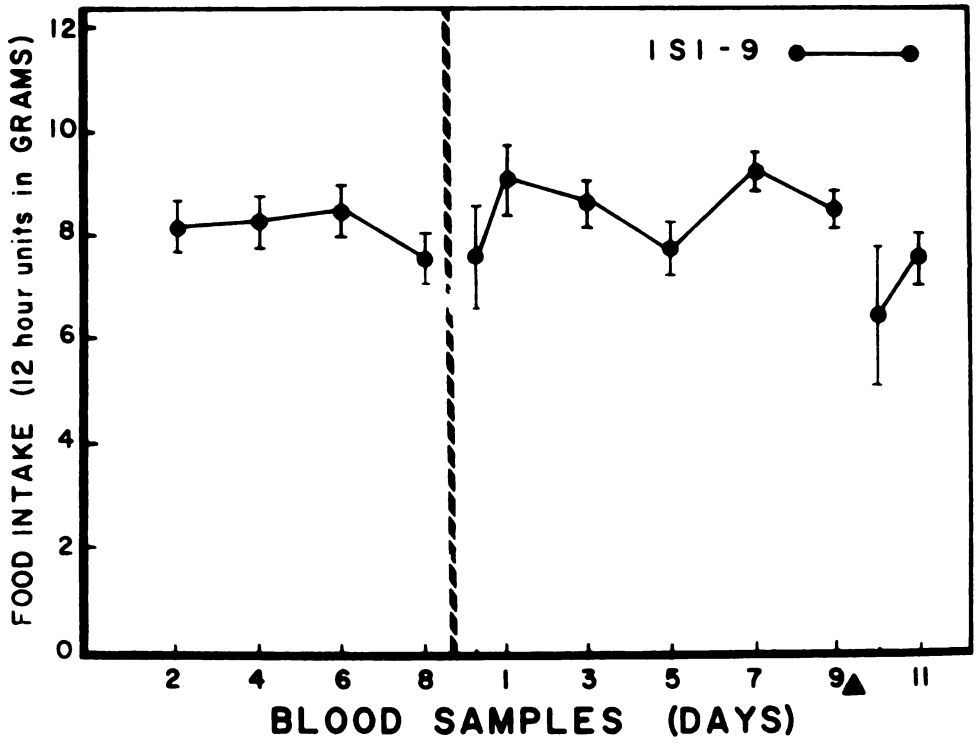
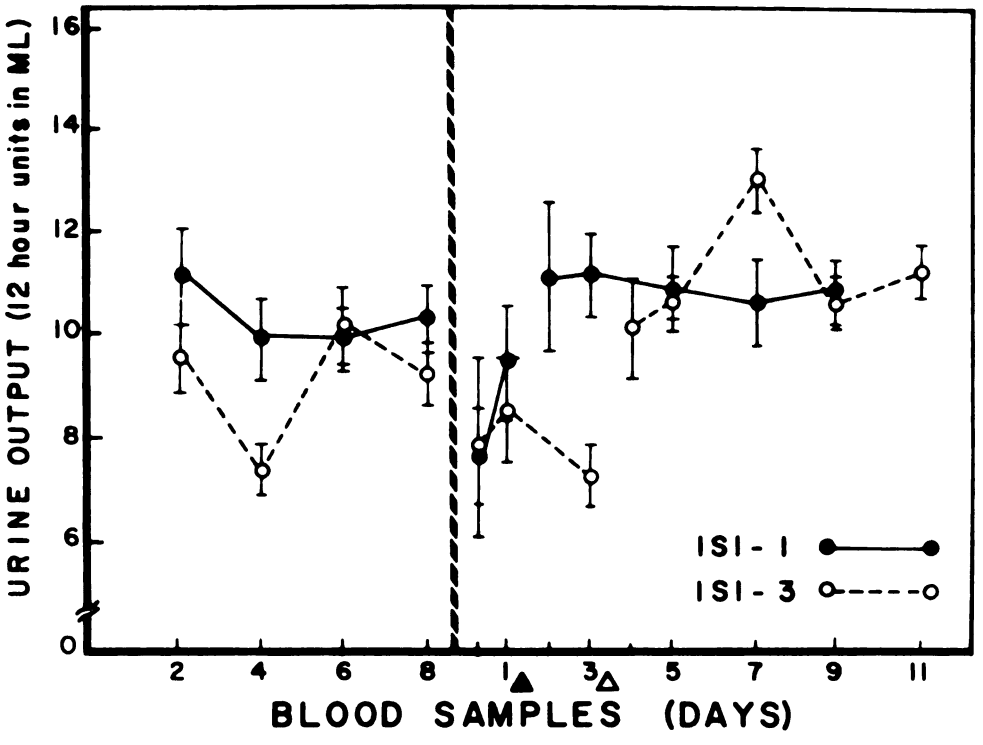
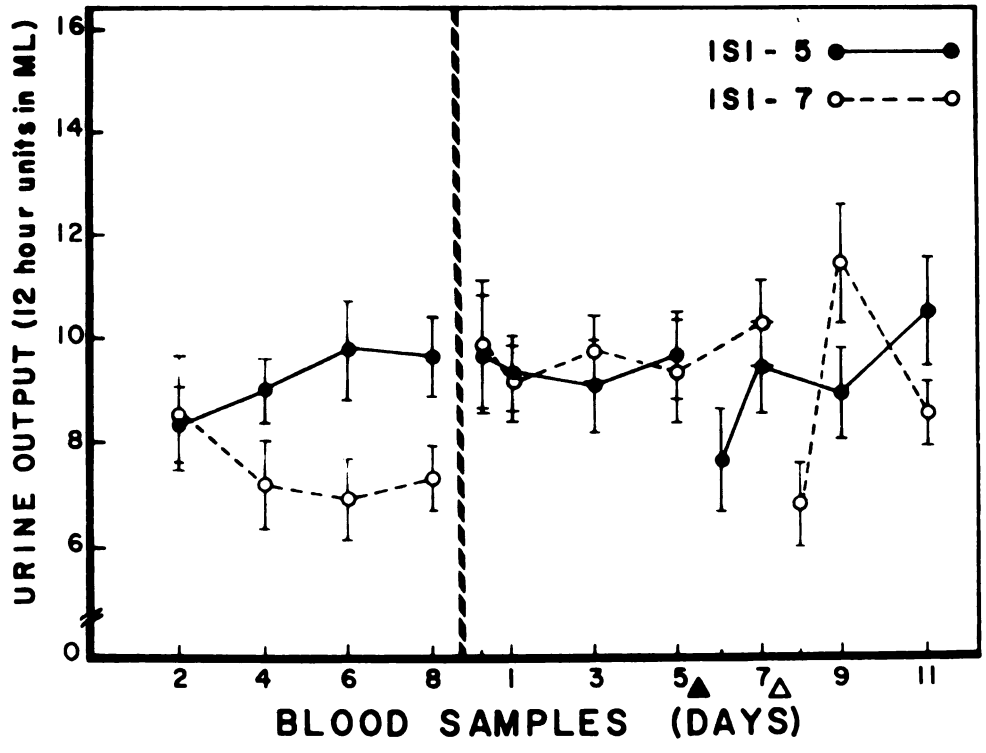

ISI-9
FOOD INTAKE (12 hour units in GRAMS)
0
2
4
6
8
10
12
2
4
6
8
1
3
5
7
9▲
11
BLOOD SAMPLES (DAYS)

Figure 7. Mean (± S.E.) urine volume output in 12 hour units for the intersample interval (ISI)-1 day and -3 day groups during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 8. Mean (± S.E.) urine volume output in 12 hour units for the intersample interval (ISI)-5 day and -7 day groups during the eight days prior to blood sampling and eleven days following the initial sample.


URINE OUTPUT (12 hour units in ML)
16
14
12
10
8
6
0
ISI - 1
ISI - 3
2
4
6
8
1▲
3△
5
7
9
11
BLOOD SAMPLES (DAYS)


URINE OUTPUT (12 hour units in ML)
16
14
12
10
8
6
0
ISI - 5
ISI - 7
2
4
6
8
1
3
5▲
7△
9
11
BLOOD SAMPLES (DAYS)

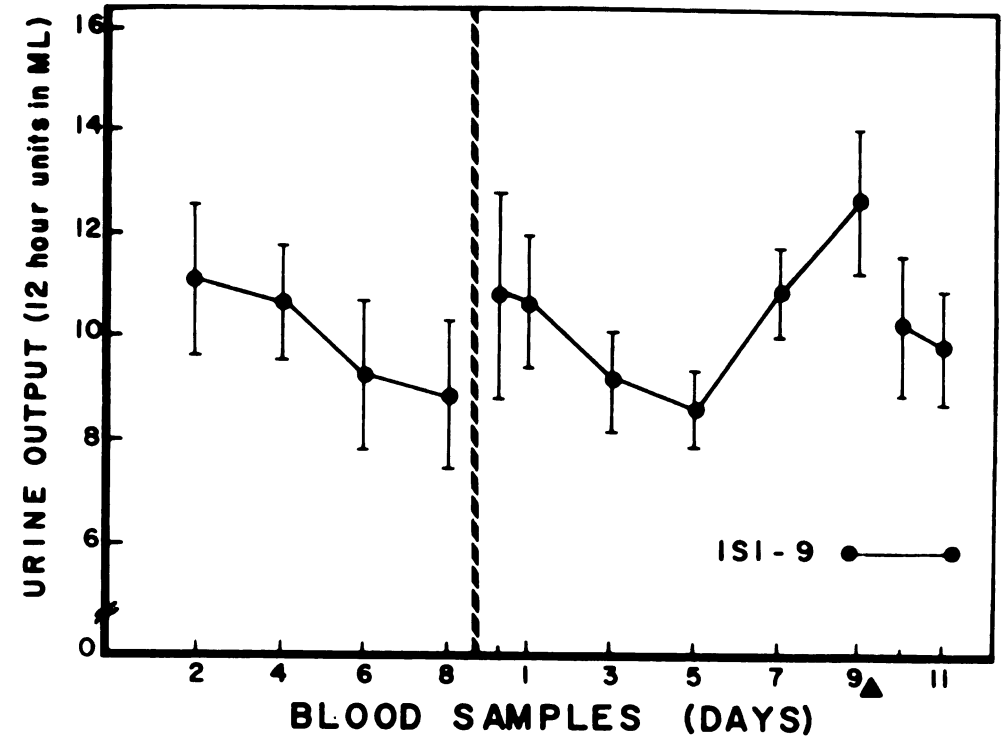
(Figures 10, 11 and 12; F=2.06, df 4/15, p>0.10). All five groups showed tendencies to decreased urine sodium concentration following the first and second blood samples. With regard to the next reading period after the first blood sample, groups ISI-3, -5, -7 and -9 were significantly different from the last pre-sample day means, with decreases of 85.5, 40.6, 61.9 and 93.5 mEq/L. respectively. The same groups registered significant drops again in urine sodium concentration after the second blood sample, 61.5, 74.2, 60.2 and 31.4 respectively.

Urine specific gravities aregiven for the five groups in Figures 13, 14 and 15. In general the total solids changes are more conservative than urine sodium alterations, however, the total solids changes are in the same direction as those recorded for sodium. The eight day base rate period yielded non-significant differences between the groups (F=1.89, df 4/15, p>0.10). Groups ISI-1, -3, -7 and -9 revealed significant decreases in urine specific gravity following the first blood sample. Decreases of 0.00236, 0.00189, 0.00480 and 0.00320 respectively, were registered. Following the second blood sample urine specific gravity fell significantly in groups ISI-3 and -5 by 0.00249 and 0.00285 respectively.

The blood samples were analyzed for sodium, potassium and protein concentrations and a hematocrit value was determined for each sample. The blood data are presented for each group beginning with the ISI-1 day group through the ISI-9 day group. The open bars labelled "a" represent the corresponding mean concentrations and S.E. for the first blood sample taken from the animals of a given group. The closed bars labelled "b" are means and S.E. for the second blood sample. To the

Figure 9. Mean (± S.E.) urine volume excretion in 12 hour units for the intersample interval (ISI)-9 day group during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 10. Mean (± S.E.) urine sodium concentration for the intersample interval (ISI)-1 day and -3 day groups during the eight days prior to blood sampling and eleven days following the initial sample.


URINE OUTPUT (12 hour units in ML)
16
14
12
10
8
6
0
ISI-9
2
4
6
8
1
3
5
7
9▲
11
BLOOD SAMPLES (DAYS)

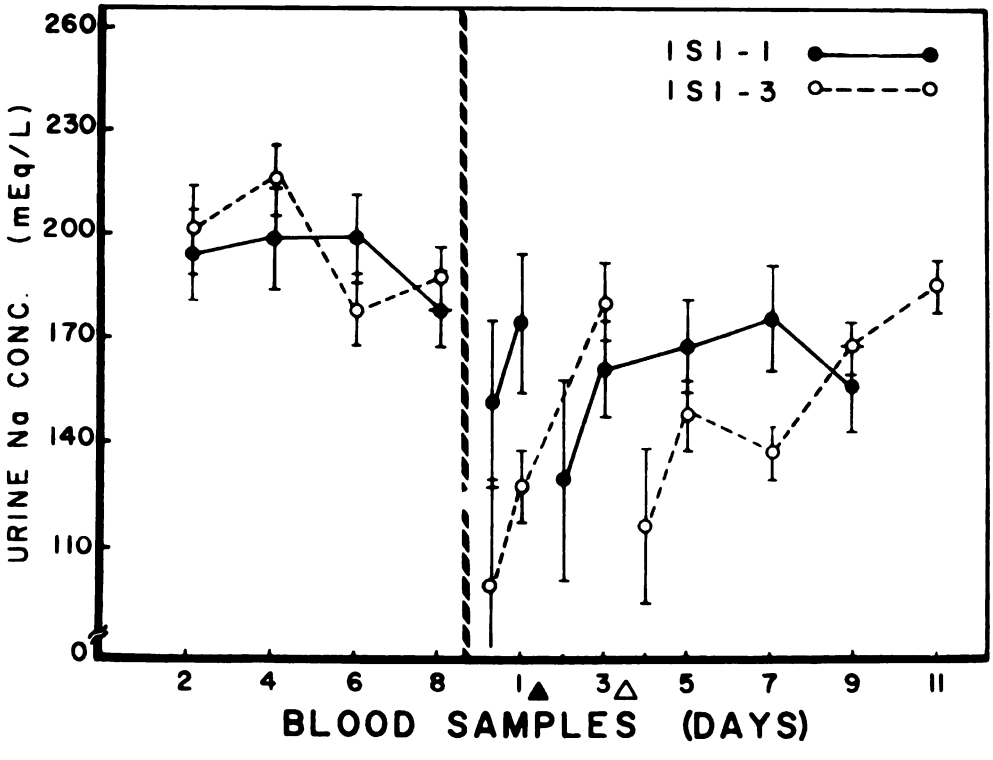
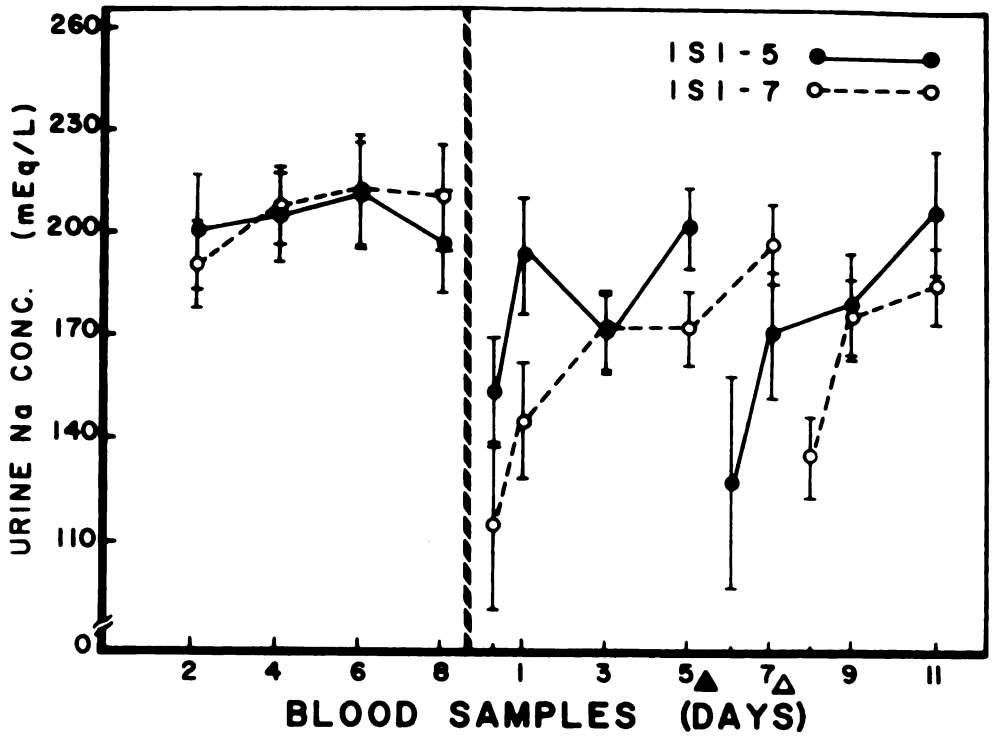
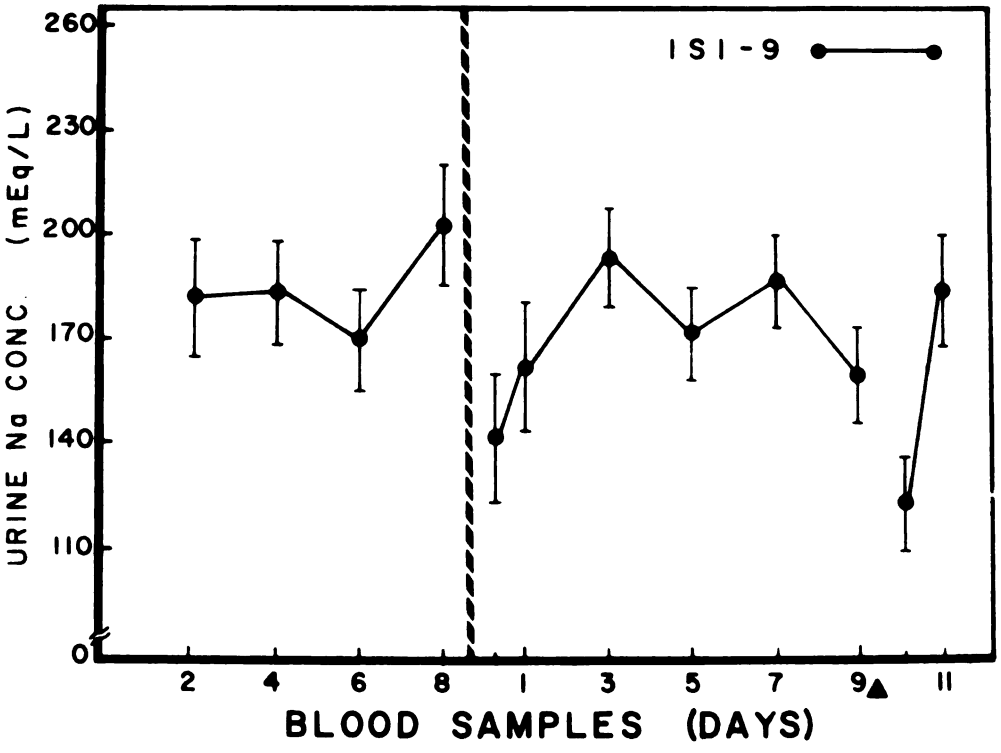

URINE Na CONC. (mEq/L)
260
230
200
170
140
110
0
ISI-1
ISI-3
2
4
6
8
1▲
3△
5
7
9
11
BLOOD SAMPLES (DAYS)

Figure 11. Mean (± S.E.) urine sodium concentration for the intersample interval (ISI)-5 day and -7 day groups during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 12. Mean (± S.E.) urine sodium concentration for the intersample interval (ISI)-9 day group during the eight days prior to blood sampling and eleven days following the initial sample.

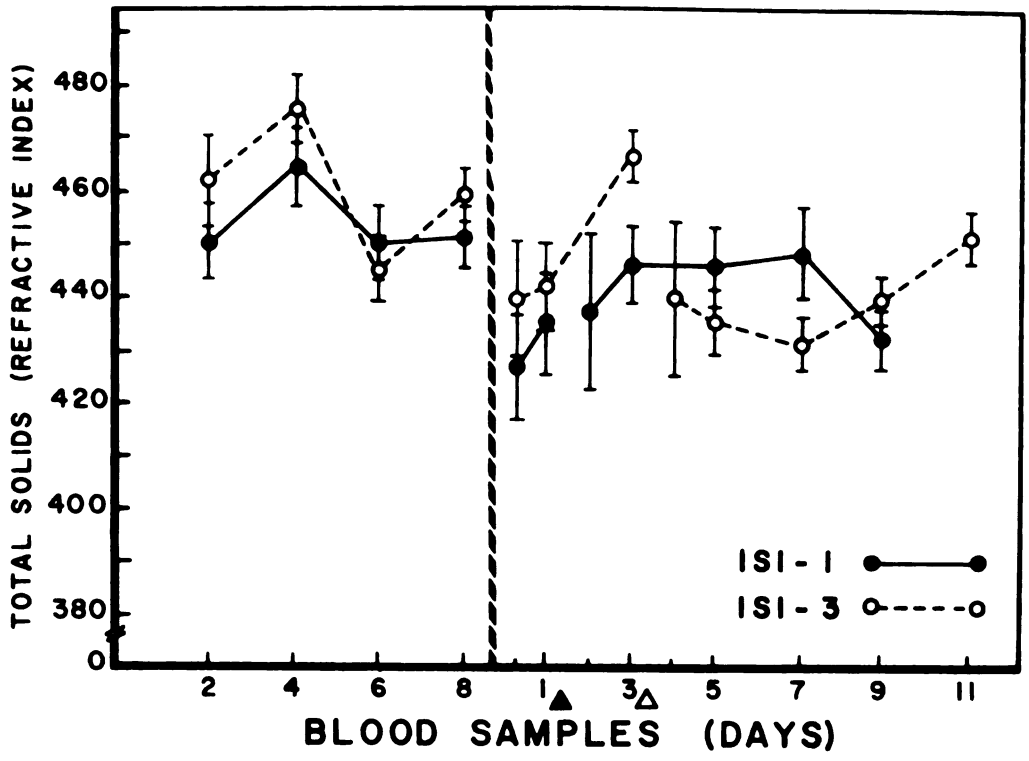

260
230
200
170
140
110
0
URINE Na CONC. (mEq/L)
ISI-5
ISI-7
2
4
6
8
1
3
5▲
7△
9
11
BLOOD SAMPLES (DAYS)

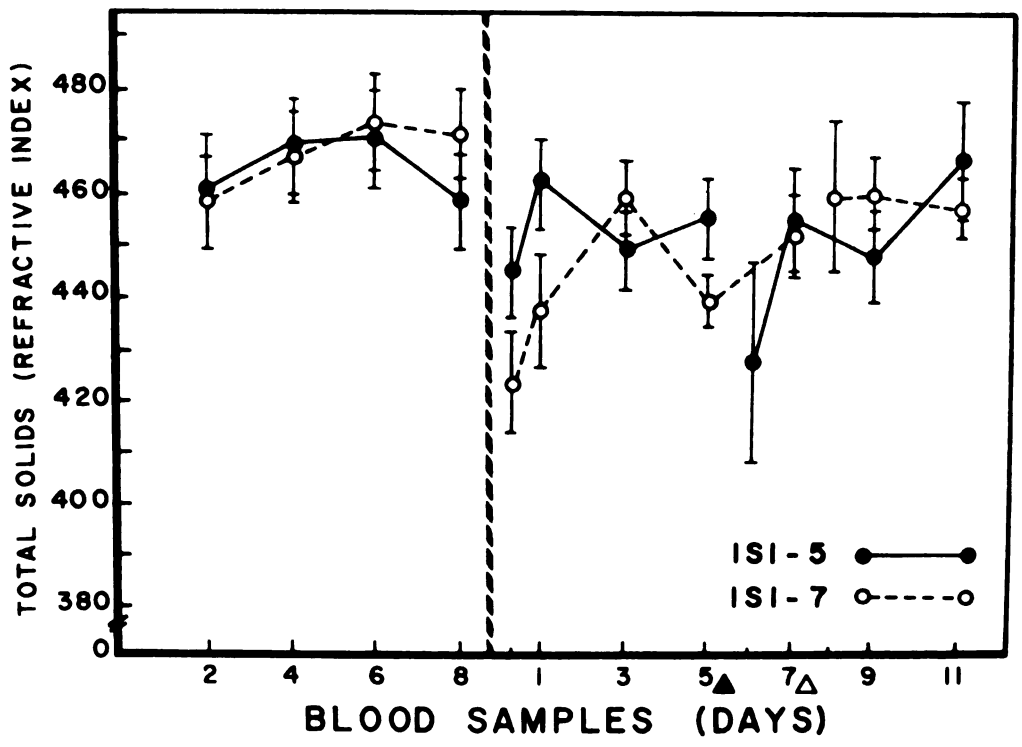

260
230
200
170
140
110
0
URINE Na CONC. (mEq/L)
ISI-9
2
4
6
8
1
3
5
7
9▲
11
BLOOD SAMPLES (DAYS)

Only the last three numbers from the refractive index are presented on the ordinate of Figures 13, 14 and 15. Complete read outs would take the form 1.3___.

Figure 13. Mean (± S.E.) urine specific gravity for the intersample interval (ISI)-1 day and -3 day groups during the eight days prior to blood sampling and eleven days following the initial sample.

Figure 14. Mean (± S.E.) urine specific gravity for the intersample interval (ISI)-5 day and -7 day groups during the eight days prior to blood sampling and eleven day following the initial sample.


TOTAL SOLIDS (REFRACTIVE INDEX)
480
460
440
420
400
380
0
2
4
6
8
1▲
3△
5
7
9
11
BLOOD SAMPLES (DAYS)
ISI - 1
ISI - 3


TOTAL SOLIDS (REFRACTIVE INDEX)
480
460
440
420
400
380
0
2
4
6
8
1
3
5▲
7△
9
11
BLOOD SAMPLES (DAYS)
ISI - 5
ISI - 7

far left of each figure labelled "MEAN" are provided a composite mean and S.E. for all the groups combined for a given characteristic of the first blood sample.

Figure 16 presents the serum sodium concentration in mEq/L. for each group on the first and second blood samples taken from members of each group. The mean of all the groups combined was 147.47 mEq/L. sodium. With respect to first blood sample means, groups ISI-1 and -9 were significantly different. Comparing second sample means, group ISI-7 was significantly different from the other four groups. Comparing within groups, first blood sample versus the second blood sample, ISI-1 and -7 were significantly different.

The mean potassium concentration (Figure 17) of all groups combined for the first blood sample was 4.72 mEq/L. Group ISI-7 was significantly different from the other groups in terms of mean potassium concentration for the first blood sample. Groups ISI-5 and -9 were significantly different from one another and from the other three groups in terms of mean potassium concentration for the second blood sample. Within group comparisons suggested significant differences between the first and second blood samples for groups ISI-1 and -9.

The mean protein (figure 18) value of all groups combined for the first blood sample was 6.42 grams/100ml. Groups ISI-7 and -9 differed significantly in mean protein concentration on the first blood sample. Concerning the second blood sample group ISI-1 significantly differed from the other four groups. The ISI-1 animals were the only ones to show significantly different means for the first and second blood samples.

The mean hematocrit value (Figure 19) for all animals combined was 45.02% red blood cell volume (RBC) in proportion to plasma fluid volume.

Figure 15. Mean (± S.E.) urine specific gravity for the intersample interval (ISI)-9 day group during the eight days prior to blood sampling and eleven days following the initial sample.

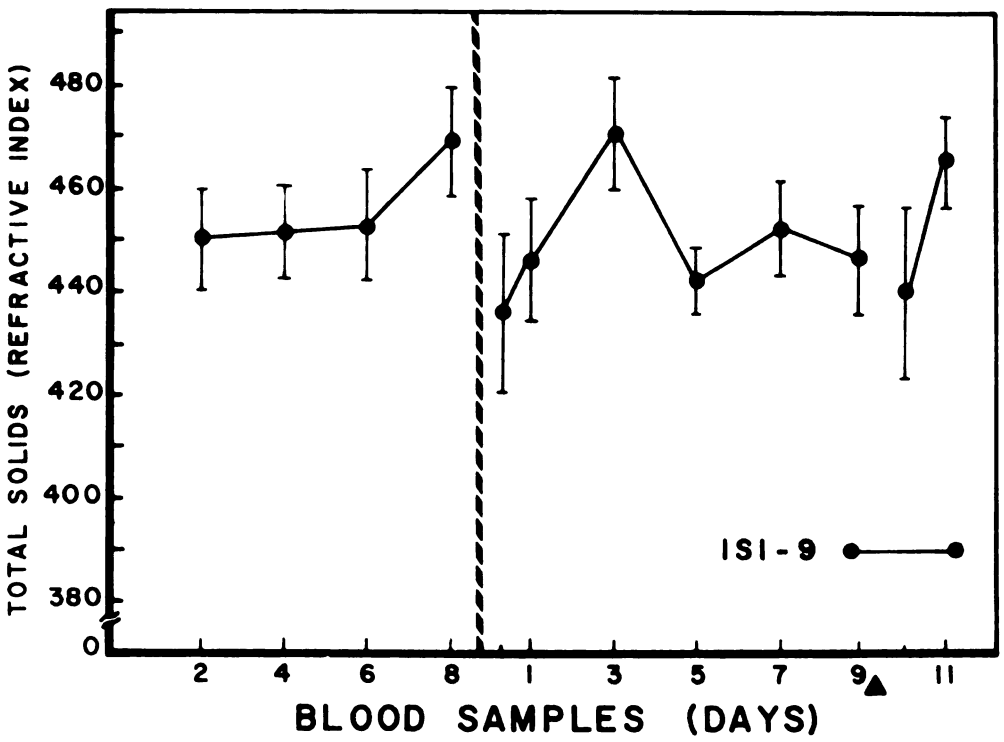
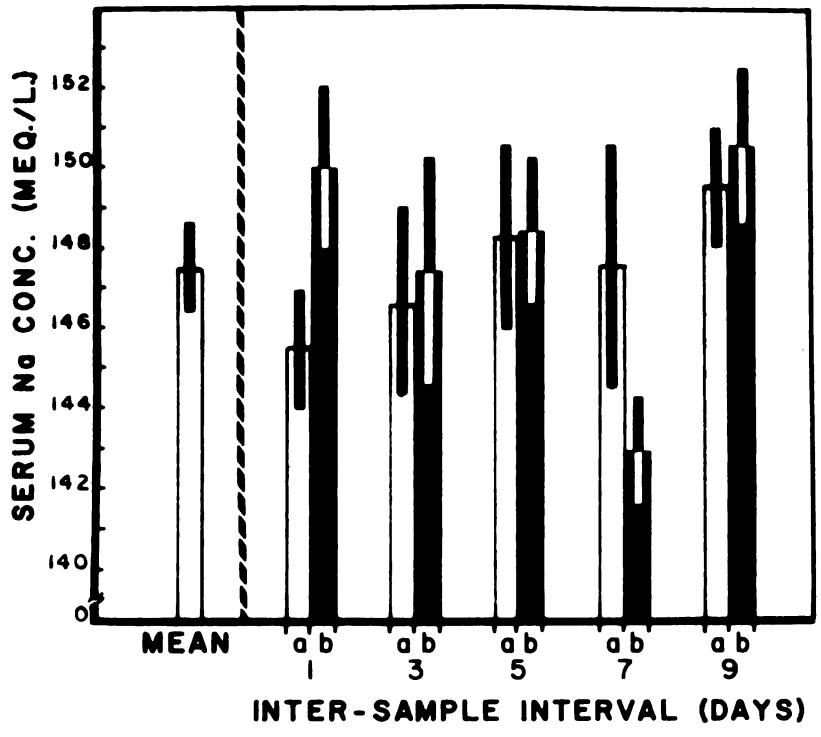

TOTAL SOLIDS (REFRACTIVE INDEX)
480
460
440
420
400
380
0
2
4
6
8
1
3
5
7
9
11
BLOOD SAMPLES (DAYS)
ISI-9

Figure 16. The mean (± S.E.) serum sodium concentration for each ISI group on its first (a) and second (b) blood samples. The mean serum sodium concentration across all ISI groups for the first blood sample (a) is also provided, labelled MEAN.

Figure 17. The mean (± S.E.) serum potassium concentration for each ISI group on its first (a) and second (b) blood samples. The mean serum potassium concentration across all ISI groups for the first blood sample (a) is also provided, labelled MEAN.


SERUM Na CONC. (MEQ./L.)
152
150
148
146
144
142
140
0
MEAN
a b
1
a b
3
a b
5
a b
7
a b
9
INTER-SAMPLE INTERVAL (DAYS)

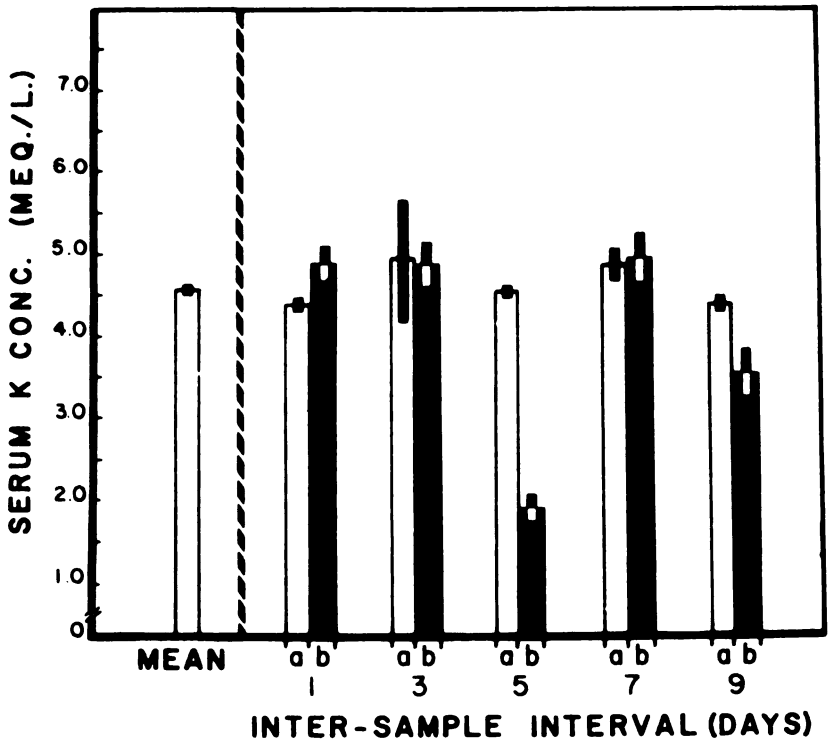
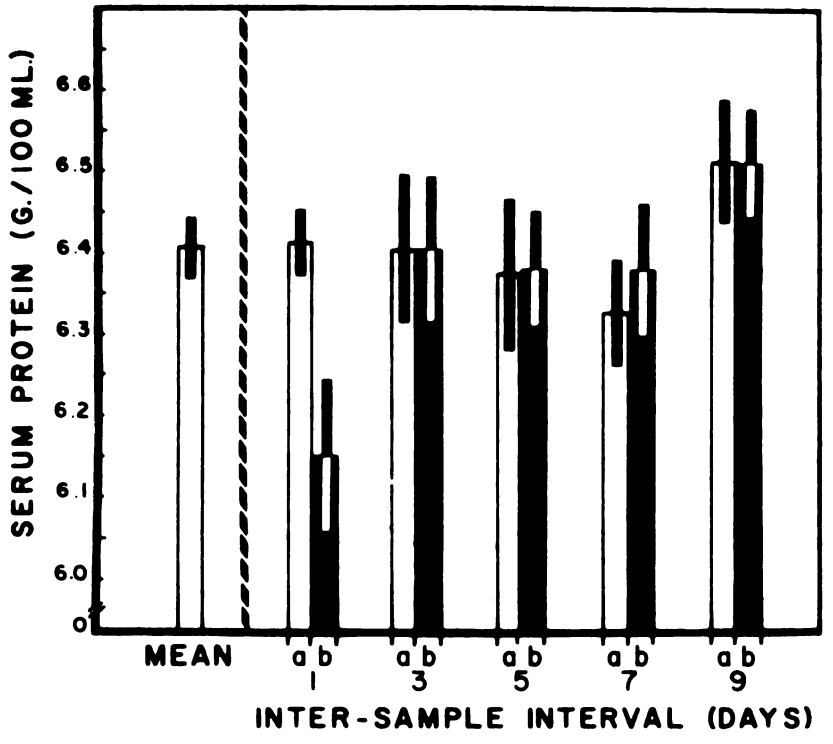
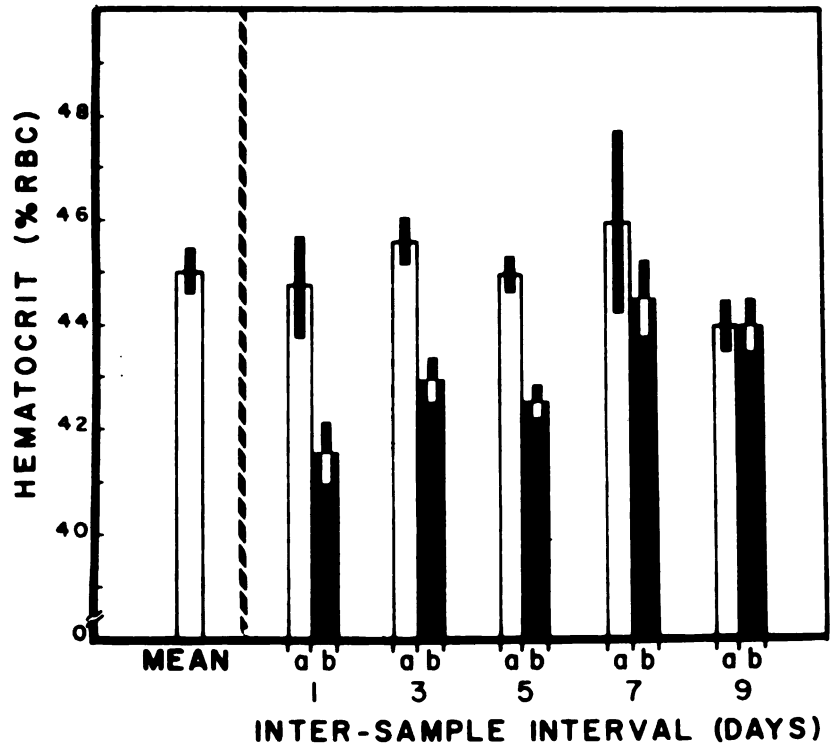

SERUM K CONC. (MEQ./L.)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
MEAN
a b
1
a b
3
a b
5
a b
7
a b
9
INTER-SAMPLE INTERVAL (DAYS)

Figure 18. The mean (± S.E.) serum protein concentration for each ISI group on its first (a) and second (b) blood samples. The mean serum protein concentration across all ISI groups for the first blood sample (a) is also provided, labelled MEAN.

Figure 19. The mean (± S.E.) hematocrit for each ISI group on its first (a) and second (b) blood samples. The mean hematocrit across all ISI groups for the first blood sample (a) is also provided, labelled MEAN.


SERUM PROTEIN (G./100 ML.)
6.6
6.5
6.4
6.3
6.2
6.1
6.0
0
MEAN
a b
1
a b
3
a b
5
a b
7
a b
9
INTER-SAMPLE INTERVAL (DAYS)


HEMATOCRIT (% RBC)
48
46
44
42
40
0
MEAN
a b
1
a b
3
a b
5
a b
7
a b
9
INTER-SAMPLE INTERVAL (DAYS)

Group ISI-9 was significantly different from ISI-3 and -5 in terms of mean hematocrit values for the first blood sample. There were significant differences between group ISI-1's second blood sample mean and the other groups' second blood sample mean, this also held for groups ISI-3 and -5 compared with the other groups, and ISI-7 and -9 versus the other groups. Within group comparisons between the means of the first and second blood samples showed groups ISI-1, -3 and -5 to be significantly different.

Discussion

Experiment 1 was intended to answer the question: How long must the investigator wait between heart puncture blood samples in rats before the body fluids return to pre-sample levels as indicated by available indices? Although the results may appear somewhat inconclusive on lesser questions there are ample indications from the data to suggest a preferred time delay between blood samples. In general the intake-output indices reveal drops in water and food intake following blood samples. Although such drops may or may not occur after the first sample, if they do not occur after the first sample they do occur after the second sample. Once a drop is evidenced a recovery time of from one to four days is generally required before the pre-sample level is reattained. This time period appears to vary with the degree of depression of food and water intake following the blood sample. The greater the magnitude of the depression the longer the recovery time required.

The consistent drops in urine sodium concentration after the blood samples are in large part due to the decrease in food intake and its interaction with other effects of the blood sample *per se*,

such as the two to five minutes under ether anesthesia. Where food intake was most severely depressed, i.e., group ISI-3, urinary sodium concentration was also maximally affected. A similar food deprivation effect upon urinary sodium concentration has been previously documented for normal rats (O'Kelly and Wright, 1971). The base rate level of urine sodium concentration was somewhat higher in the prior study (averaging about 240-250 mEq/L.) than in the present study during which these rats demonstrated mean values of approximately 180-200 mEq/L. Also in the earlier experiment the sodium concentration decrease under 24 hour food deprivation fell to around 40 mEq/L. by the end of the deprivation period while the present decreases, even in the maximally affected group (ISI-3) was to a mean of about 90 mEq/L. during the 12 hours following the sample. The primary difference between the two studies in addition to blood samples in the present investigation is that Experiment 1 animals were free to increase food intake at any time following the blood sample, for the food was never removed as it was in the former study. Close inspection of the food intake data of the Experiment 1 animals further suggests that none of the groups completely stopped eating during the first 12 hours after the blood sample and they fully recovered to their former food ingestion levels by one to three days after the blood sample was taken.

The data of most conern appear to be the blood sample measures proper. The serum sodium concentration values indicate recovery by nine days following the first sample. Serum protein offers a different picture with recovery by three days after the first sample while hematocrit readings, which will be weighted heavily suggest seven days for the red blood cell per cent volume to return to a value not different from the first blood sample values.

From this experiment it is recommended that a minimum of seven days be allowed to pass between blood samples. To insure against confounding effects introduced by sampling too soon the method to be followed in subsequent experiments of this series will be to allow at least ten days between blood samples or between surgery and blood samples.

EXPERIMENT 2

Posterior Medial Hypothalamic Lesions

This experiment was an attempt to replicate previous findings concerning bilateral lesions of the area posterior hypothalami. Cort (1963a) has indicated urinary "salt wasting" following such lesions in rats with death occurring within approximately ten days if a saline solution is not provided for purposes of body sodium replacement. This study closely inspected saline and water intake pre- and post-operatively and resulting alterations in blood osmolality, protein, sodium and potassium concentrations as well as urine total solids, sodium and potassium concentrations.

Subjects

Eighteen male albino rats of the Holtzman strain approximately 120 days of age were adapted to metabolism cages under constant light (the same cages as described in Experiment 1) for a pre-lesion period of 20 days. The animals were maintained on ad libitum tap water, 0.87% saline solution and powdered Wayne Breeder Blox. The tap water and saline drinking cylinders were switched daily on the cages in a random sequence to avoid the establishment of position preference by the animals.

Procedure

The animals were stratified into three groups of 6 animals each. The first group received bilateral electrolytic lesions to the nucleus mediodorsalis thalami. The other two groups received bilateral damage to the area posterior hypothalami. In one group the damage was inflicted by the electrolytic lesion method; the other group received radio frequency lesions. During the 20 days of adaptation and subsequent 22 post-lesion days urine samples were taken from each animal once a day between 0800-0830 hours. At this time body weight, water, saline and food consumption and volume of urine excretion were also recorded. Urine samples were prepared for determination of sodium and potassium concentration by flame photometry and total solids readings were also obtained.

On pre-operative day 11 a blood sample was taken from each animal between 0830 and 1230 hours. The blood samples were treated in the same way as those described in Experiment 1. A second blood sample was taken from each animal on post-operative day 11 and a third sample on post-operative day 22.

Surgery was performed on all animals after the 0800-0830 readings of pre-operative day 20. The surgery lasted from approximately 0900 to 2000 hours. The lesions were applied in a randomized order of the eighteen animals and with a random order applied to the lesioning of the hemispheres, i.e., right-left or left-right. The electrode consisted of a 0.28 mm diameter stainless steel shaft entirely insulated except for a 0.5 mm uninsulated tip. The anode was grounded anally with the radio frequency and electrolytic lesion techniques.

Following the conclusion of this experiment the animals were sacrificed by ether overdose, perfused with 10% formalin and the brains retained in 10% formalin for a minimum of two weeks prior to histology. Both the frozen and celloidin brain histological procedures were employed. Two brains from each of the three groups were celloidin embedded and subsequently sectioned at 25μ and stained with thionin (Nissl method) for cell bodies and hematoxylin (Weil and Heidenhein methods) for myelinated fibers. Approximately four days preceding frozen sectioning the other four brains from each group were placed in a sucrose-formalin solution. They were sectioned at 40μ and stained with cresyl violet acetate, a cell nissl body stain and by the Fink-Heimer method (Hjorth-Simonsen, 1970) for degenerating axons.

Results

The body metabolism data have been grouped across two day intervals. The last ten days of the twenty day pre-operative basal rate period is presented along with the entire twenty day post-operative period. The means and standard errors of the means are plotted across days for each of the three groups. Once again the points are reliably different from one another at a minimum of the 0.05 probability level where standard error bars do not overlap.

Tap water and 0.87% NaCl intakes are provided for each individual group in Figures 20, 21 and 22. There were no differences for any of the groups between tap water versus saline intake during the pre-operative days 10-20. After the experimental lesions the thalamic, posterior hypothalamic radio frequency (PH-RF) and posterior hypothalamic electrolytic (PH-IRR) groups all showed significant differences between

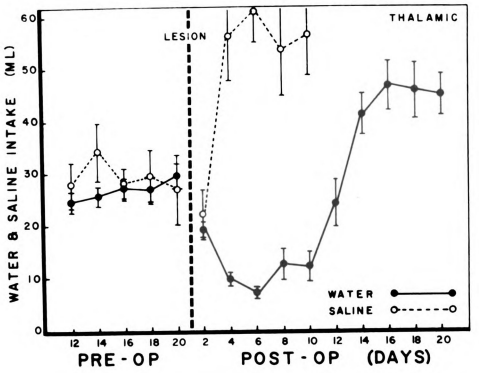
water and saline intakes ($F=25.97$, 21.83, 11.15 respectively, df 1/8, $p<0.01$) with the saline consumption rising a mean of 24.4 ml to 52.0 ml by post-operative day 10 and the tap water intake dropping a mean of 12.9 ml to 12.3 ml by post-operative day 10. On post-operative day 10 following the compilation of the daily intake-output records and urine sample collection, the saline cylinders were removed from the metabolism cages of all groups. Tap water consumption was then noted to rapidly rise a mean of 30.8 ml to 43.1 ml by post-operative day 20.

Comparisons between groups concerning food intake (Figure 23) revealed no differences for the pre-operative period presented ($F=0.95$, df 2/12, $p>0.10$). There was a substantial drop in food intake following the surgery for all groups, approximately 9 grams reduction comparing pre-operative days 19 and 20 grouped with post-operative days 1 and 2 grouped. Subsequently there was a food recovery period that resulted in the reestablishment of a food intake similar to pre-lesion levels by about post-operative day 8. Removal of the saline appeared to have minimal affects upon food intake.

The groups were significantly different concerning urine output (Figure 24) registered during the pre-operative period presented ($F=17.98$, df 2/12, $p<0.01$). The major contribution to this deviation came from the PH-IRR group which evidenced urine output significantly below those of the other two groups. The post-operative urine outputs for the groups were non-significantly different ($F=1.56$, df 2/27, $p>0.10$). However, the mean urine output for all groups on post-operative day 10, the last day on both saline and water was significantly higher ($\bar{X}=38.11$) than the mean for all groups on the last day of tap water only ($\bar{X}=20.44$) post-operatively day 20 ($t=8.51$, df=34, $p<0.01$).

Figure 20. Mean (± S.E.) tap water and isotonic saline solution consumption exhibited by the control lesioned group for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 21. Mean (± S.E.) tap water and isotonic saline solution consumption exhibited by the posterior medial hypothalamic radio frequency lesioned group for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


THALAMIC
LESION
WATER & SALINE INTAKE (ML)
0
10
20
30
40
50
60
WATER
SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

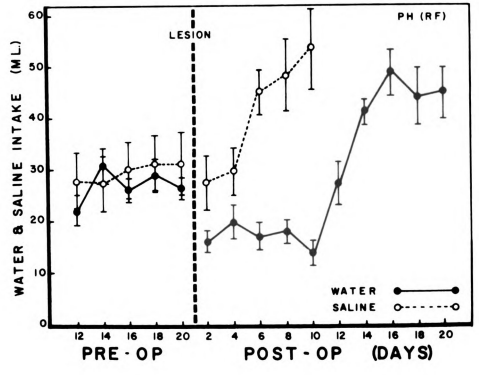
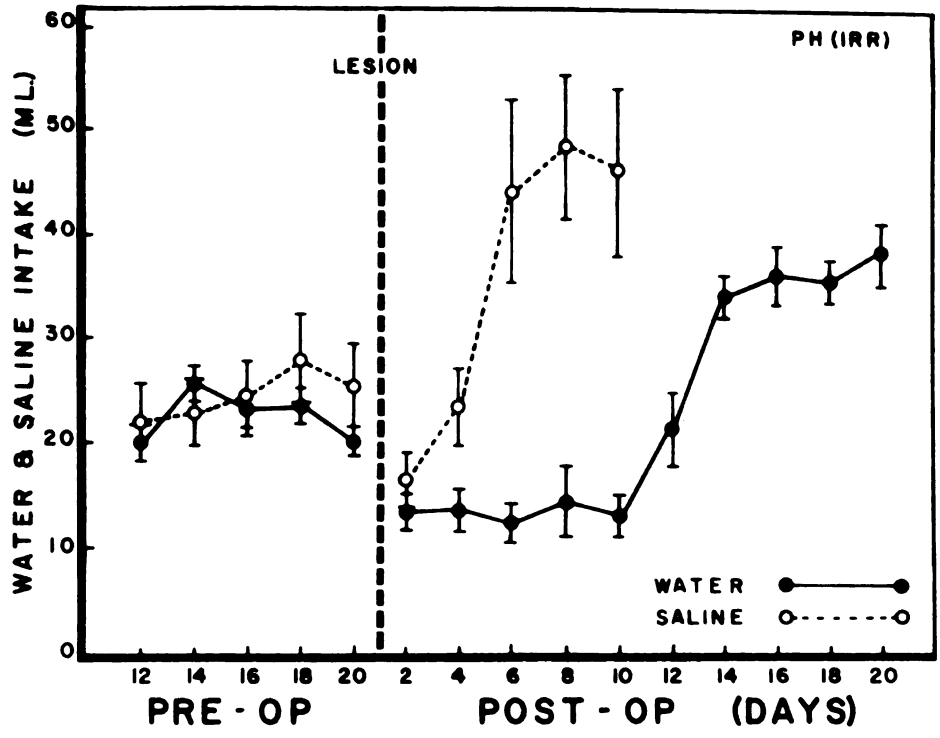
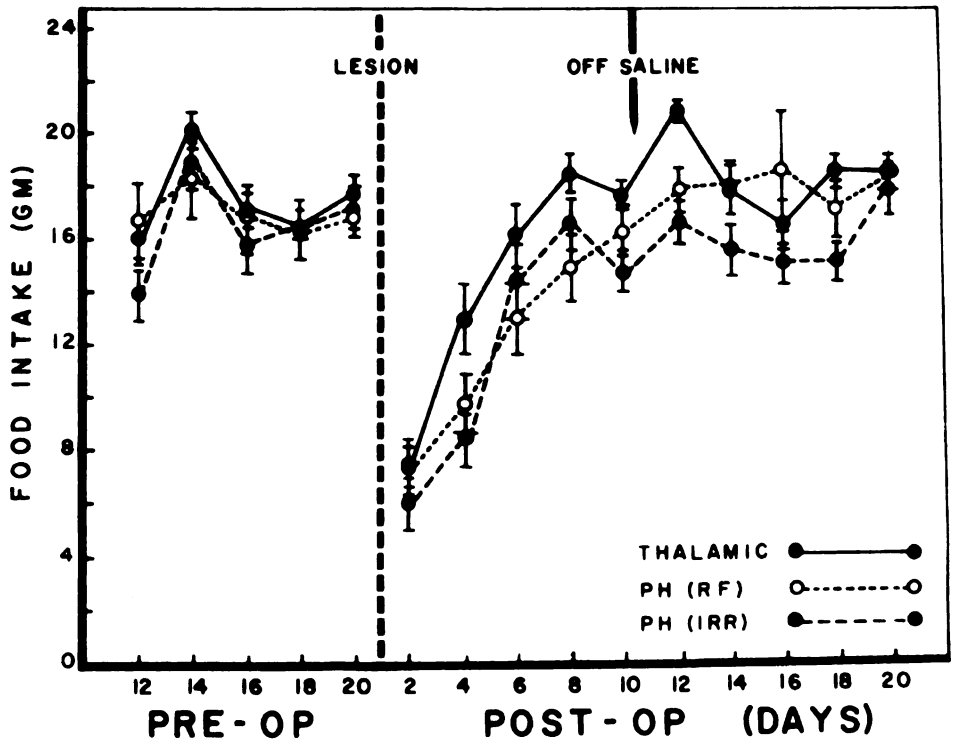

PH (RF)
LESION
WATER & SALINE INTAKE (ML)
0
10
20
30
40
50
60
WATER
SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 22. Mean (± S.E.) tap water and isotonic saline solution consumption exhibited by the posterior medial hypothalamic electrolytically lesioned group for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 23. Mean (± S.E.) food intake in 24 hour units for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH (IRR)
LESION
WATER & SALINE INTAKE (ML.)
0
10
20
30
40
50
60
WATER
SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)


LESION
OFF SALINE
FOOD INTAKE (GM)
0
4
8
12
16
20
24
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

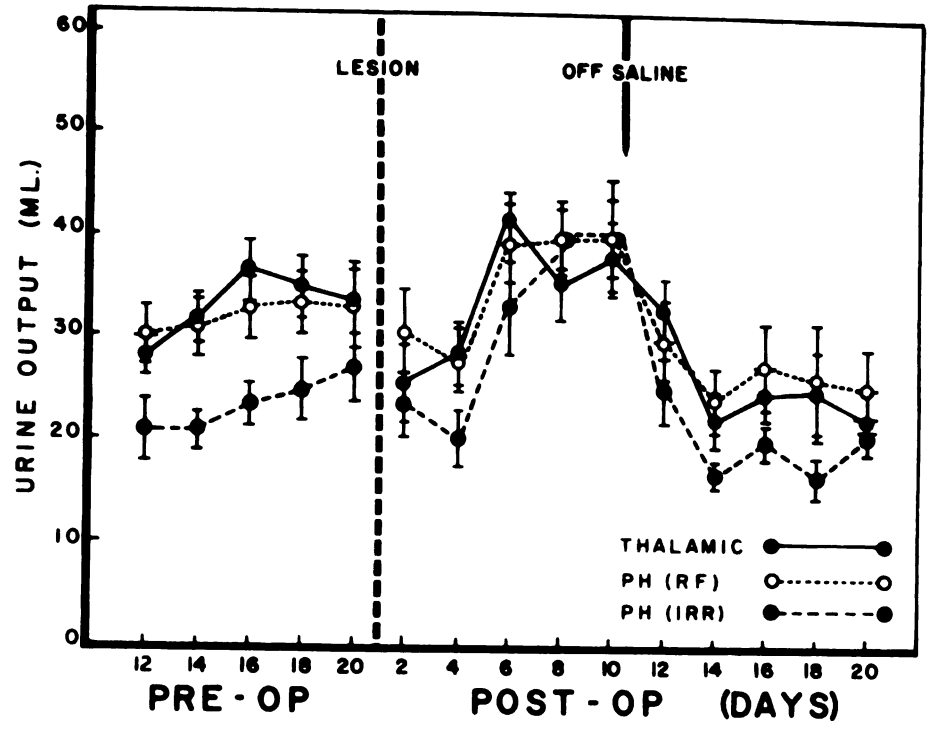
Comparisons between groups concerning urine sodium concentration (Figure 25) suggest significant differences during the ten day pre-operative period ($F=10.42$, df 2/12, $p<0.01$), however, the post-operative twenty day period revealed non-significant differences between the groups ($F=0.59$, df 2/27, $p>0.10$). There was a significant difference between the mean sodium concentration for the last day on saline and tap water, post-operative day 10 and the last day on tap water only, post-operative day 20 (t for related measures, two tailed test=3.49, $p<0.01$) with a mean drop from 184.35 to 133.12 mEq/L.

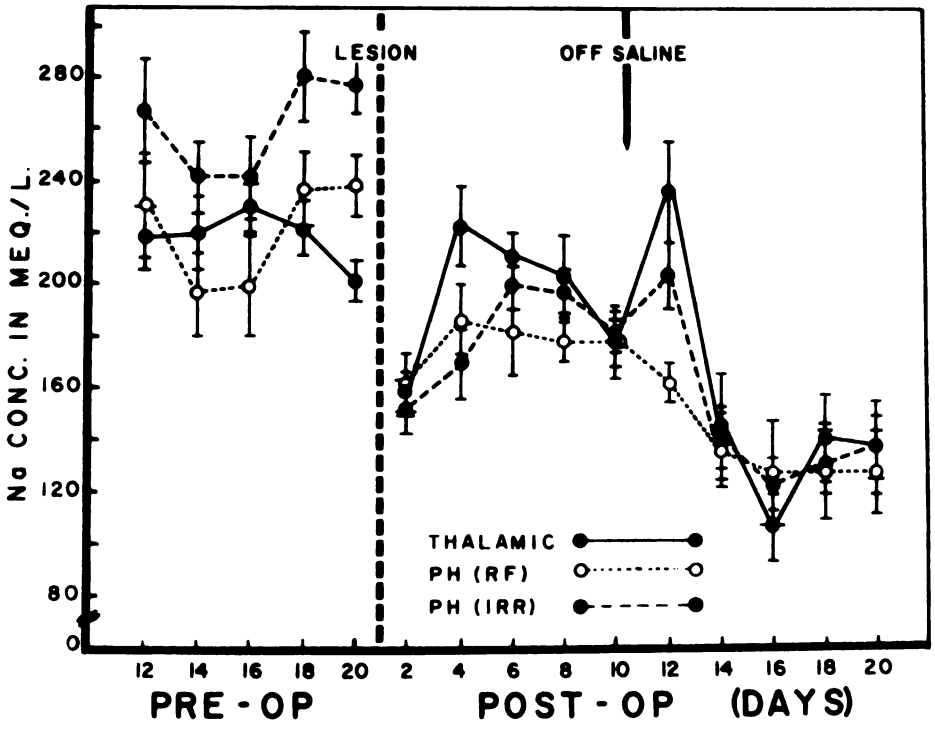
With respect to total urinary sodium (Figure 26), originally computed as a urine concentration multiplied times urine volume over unit time, both the ten day pre-operative and twenty day post-operative periods revealed non-significant differences between groups ($F=2.40$, 0.18, df 2/12, 2/27 respectively, $p>0.10$). There was a general trend difference, however, over time with total sodium decreasing after the lesion, followed by recovery and then a substantial drop in total sodium output when the saline was removed. This pattern is not too unlike that followed by sodium concentration (Figure 25) with the exception that total urinary sodium output recovered to a greater degree. This increased recovery on the part of total sodium appears to be primarily due to the increased urine output between post-operative days 8 and 12. Accompanying the withdrawal of the saline, post-operative day 10, came a decrease in urine output and urine sodium concentration that resulted in a dramatic decrease in total urinary sodium output registered during post-operative days 12 to 20.

It is of some interest that urinary potassium concentration (Figure 27) contrasts with this post-operative pattern evidenced by

Figure 24. Mean (± S.E.) urine volume output in 24 hour units for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 25. Mean (± S.E.) urine sodium concentration for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


LESION
OFF SALINE
URINE OUTPUT (ML.)
60
50
40
30
20
10
0
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)


LESION
OFF SALINE
Na CONC. IN MEQ./L.
280
240
200
160
120
80
0
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

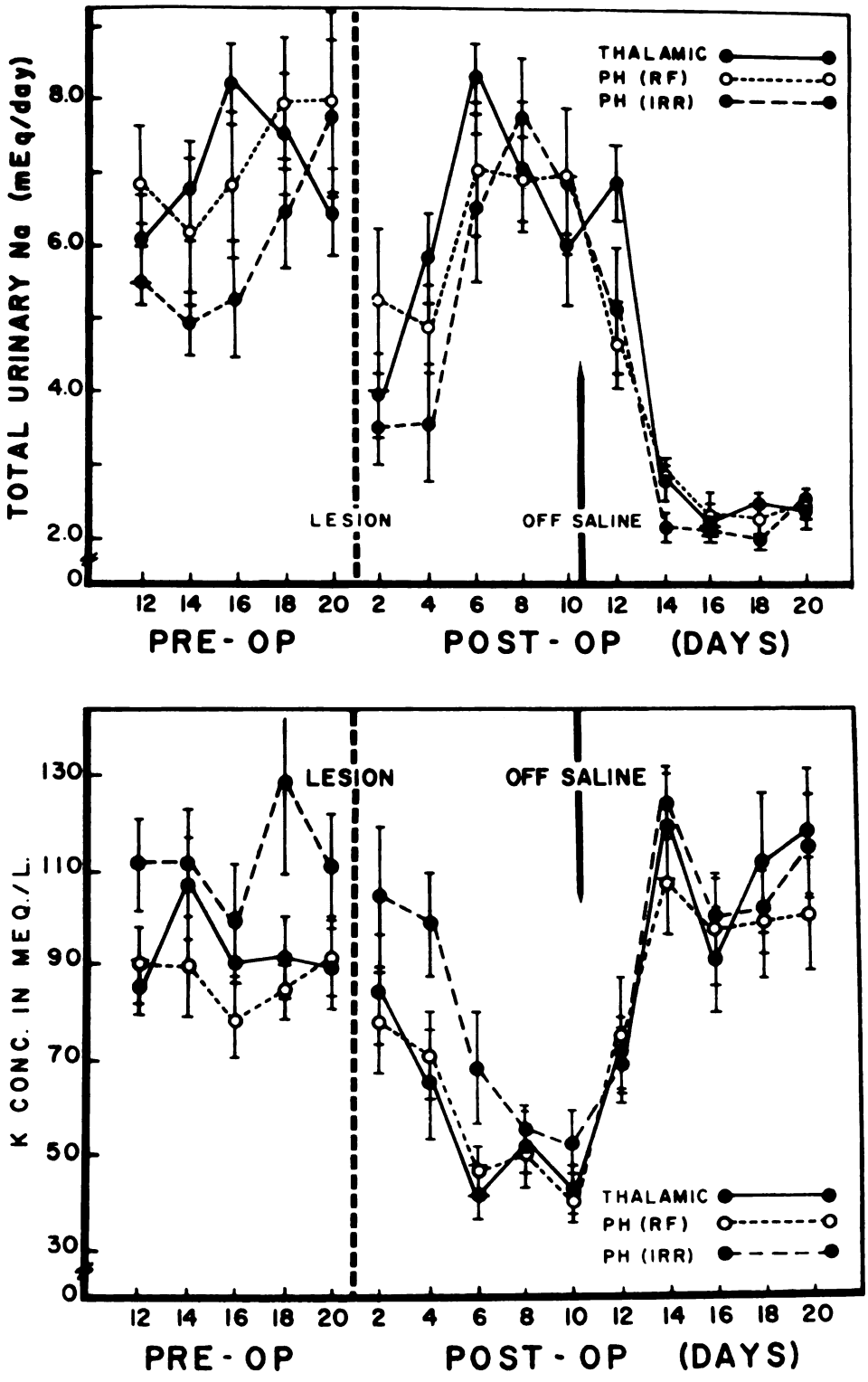
urine output, sodium concentration and total sodium. There were significant differences between the three groups during the ten day pre-operative period ($F = 13.41$, df 2/12, $p < 0.01$). There were non-significant differences between the groups during the post-operative twenty day period ($F = 0.59$, df 2/27, $p > 0.10$). The trend of urinary potassium concentration, however, was the opposite of that for the sodium concentration during this post-operative period. That is, while on both saline and tap water the potassium concentration fell significantly below the pre-operative level. Once the saline was removed the potassium concentration rapidly recovered to a level not different from the pre-lesion values.

With reference to the total solids index (specific gravity, Figure 28) pre-operatively there were differences between the groups ($F = 14.28$, df 2/12, $p < 0.01$). Post-operatively there were non-significant differences between the groups ($F = 2.48$, df 2/27, $p > 0.10$). The trend was very similar to that of the urinary potassium concentration in that there were post-operative decreases in specific gravity after the lesion with a noticeable recovery upon the removal of the saline for all groups.

The groups were non-significantly different in terms of body weight (Figure 29) during the ten day pre-operative and twenty day post-operative periods ($F = 2.50$, 6.87, df 2/12, 2/27 respectively, $p > 0.10$). There was a substantial decrease in body weight for the groups following the surgery with a combined group mean loss of 29.22 grams from 391.72 to 362.50 grams. A constant weight recovery followed the lesion; but the weight gain appeared to be less than the pre-operative rate. At the completion of the twenty day post-operative

Figure 26. Mean (± S.E.) total urinary sodium excreted per 24 hour units for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 27. Mean (± S.E.) urine potassium concentration for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


TOTAL URINARY Na (mEq/day)
8.0
6.0
4.0
2.0
0
THALAMIC
PH (RF)
PH (IRR)
LESION
OFF SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)
K CONC. IN MEQ./L.
130
110
90
70
50
30
0
LESION
OFF SALINE
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

period the combined group mean body weight lacked 9.59 grams of being equal to the pre-operative day 20 mean weight.

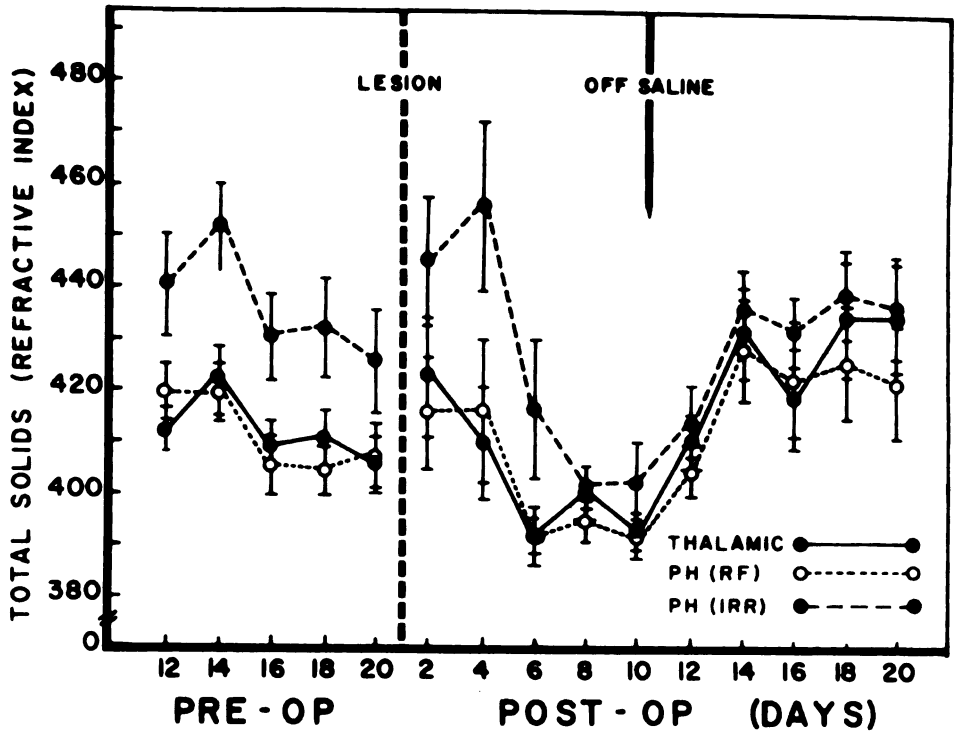
Mean serum sodium values (Figures 30, 31 and 32) for the three groups were non-significantly different from one another at the pre-operative sample with a mean between 132 and 134 mEq/L. On post-operative day 11 the thalamic and PH-IRR groups were not different but the mean sodium level of the PH-RF group was significantly lower than the other groups. On post-operative day 22 the three groups registered increased sodium levels to mean values between 138 and 139 mEq/L. The serum potassium changes (Figures 33, 34 and 35) corresponded relatively well with those of sodium across the three blood samples for the groups.

Mean serum protein levels for the three groups on each of the three blood sample days are given in Figures 33, 34 and 35. There were no differences between the three samples for the two posterior hypothalamic lesion groups which had means of about 6.3 grams/100 ml. The thalamic lesion group registered a serum protein mean of 6.4 pre-operatively and then fell significantly to 6.1 by post-operative day 11 and 6.2 for post-operative day 22.

With regard to serum osmolality the three groups were alike evidencing significant changes from pre-lesion osmolality of about 267-271 mOsm/Kg. and post-operative day 11 osmolality increases to about 275 to 280 mOsm/Kg. However the post-operative day 22 serum osmolality found the thalamic and PH-IRR groups dropping back to mean values of between 271-273 while the PH-RF group's mean was 279 mOsm/Kg.

Figure 28. Mean (± S.E.) urine specific gravity for the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 29. Mean (± S.E.) body weights exhibited by the three lesioned groups for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


TOTAL SOLIDS (REFRACTIVE INDEX)
480
460
440
420
400
380
0
LESION
OFF SALINE
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

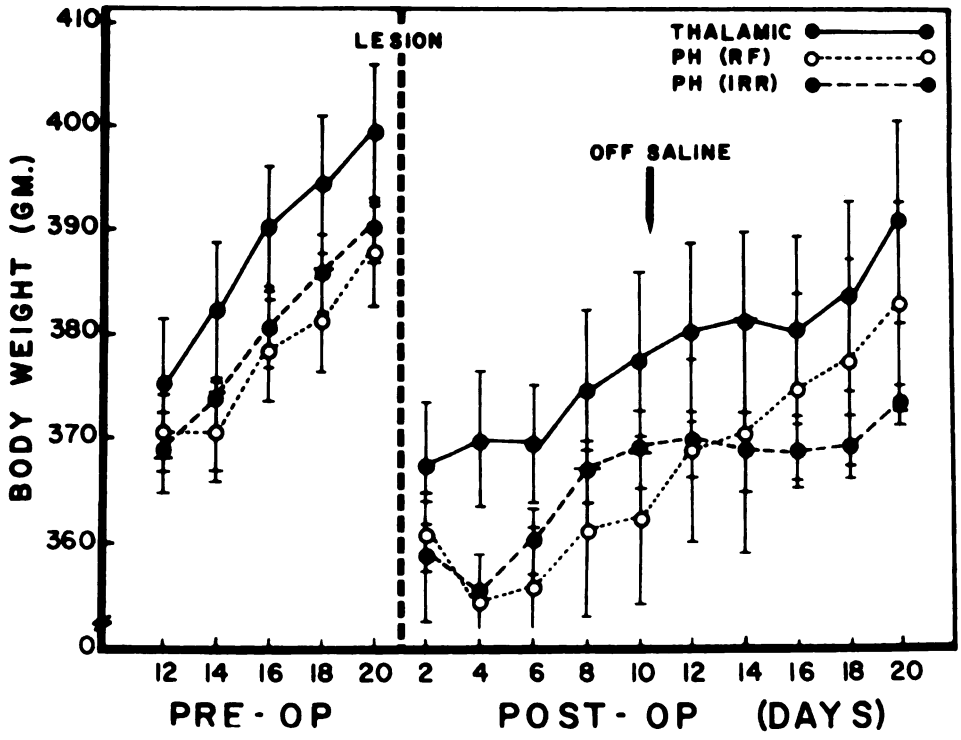

BODY WEIGHT (GM.)
410
400
390
380
370
360
0
LESION
OFF SALINE
THALAMIC
PH (RF)
PH (IRR)
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 30. Mean (± S.E.) serum sodium and potassium concentration for the control lesioned group on one pre-lesion and two post-operative blood sample days.

Figure 31. Mean (± S.E.) serum sodium and potassium concentrations for the posterior medial radio frequency hypothalamic lesioned group on one pre-lesion and two post-operative blood sample days.

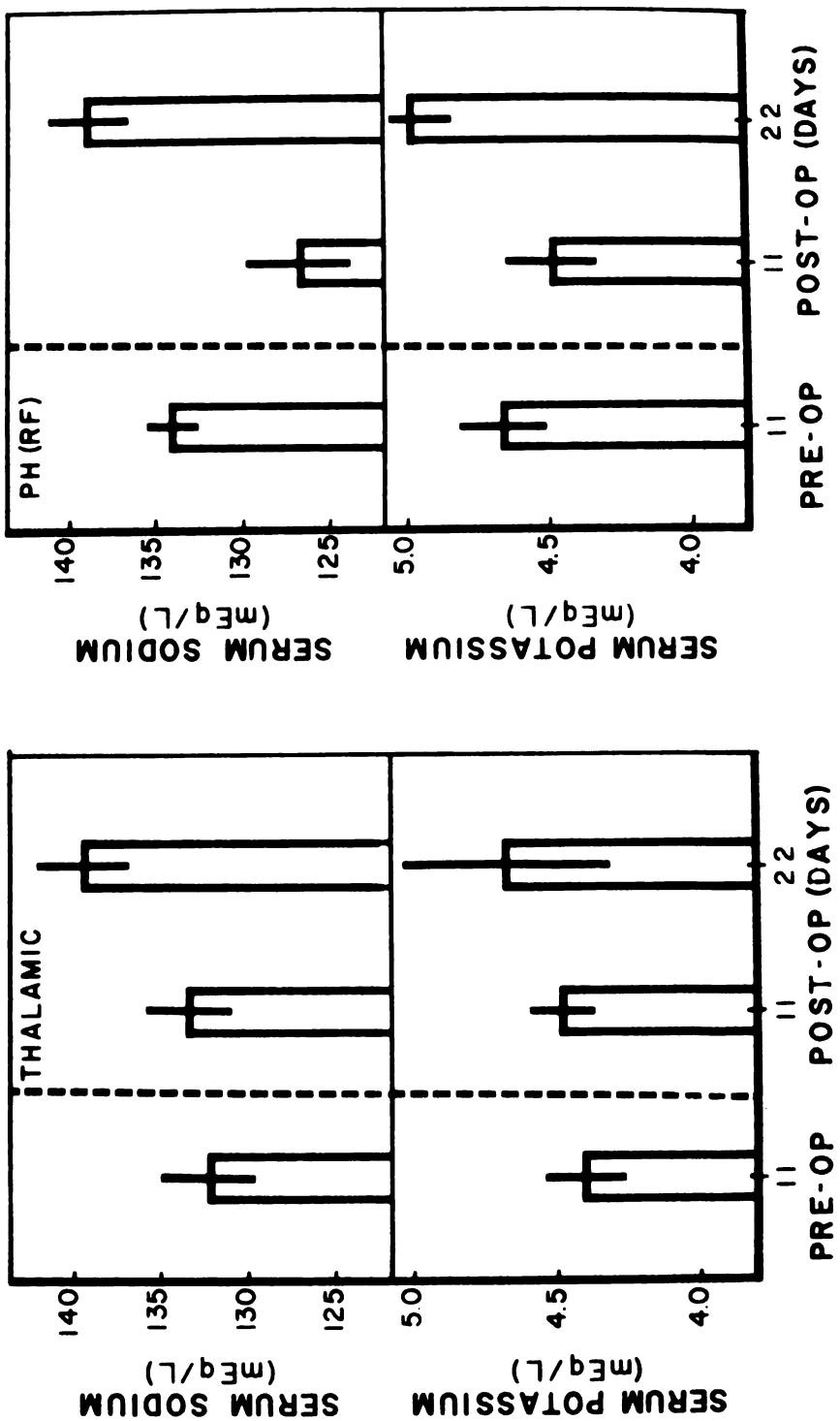
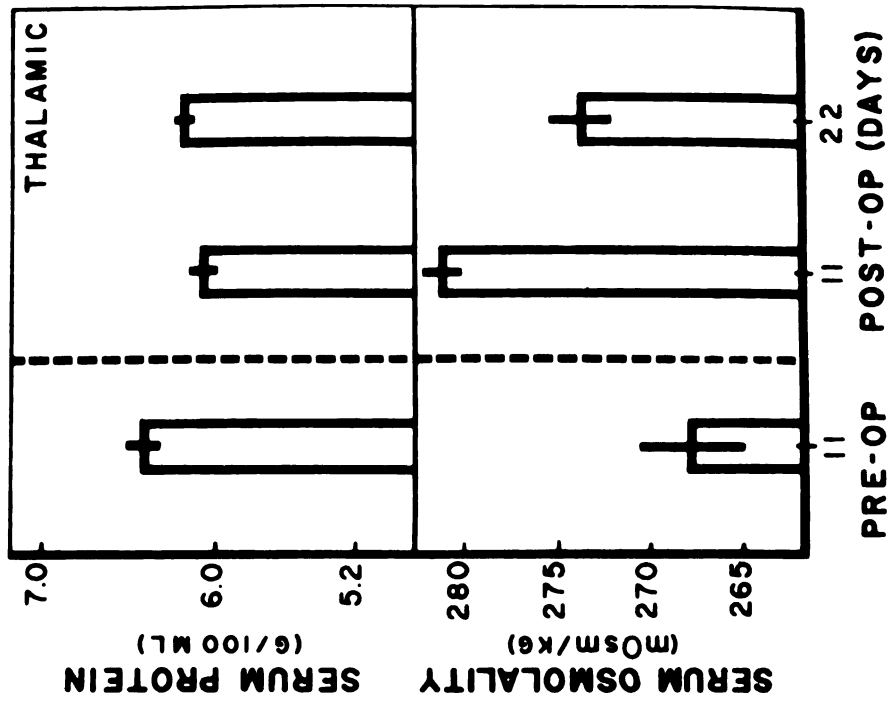

PH (RF)
THALAMIC
SERUM SODIUM (mEq/L)
SERUM POTASSIUM (mEq/L)
140
135
130
125
5.0
4.5
4.0
11
22
PRE-OP
POST-OP (DAYS)

Figure 32. Mean (± S.E.) serum sodium and potassium concentrations for the posterior medial hypothalamic electrolytically lesioned group on one pre-lesion and two post-operative blood sample days.

Figure 33. Mean (± S.E.) serum protein and osmolality for the control lesioned group on one pre-lesion and two post-operative blood sample days.


THALAMIC
7.0
6.0
5.2
SERUM PROTEIN
(G/100 ML)
280
275
270
265
SERUM OSMOLALITY
(mOSM/KG)
11
PRE-OP
11
POST-OP
22
POST-OP (DAYS)

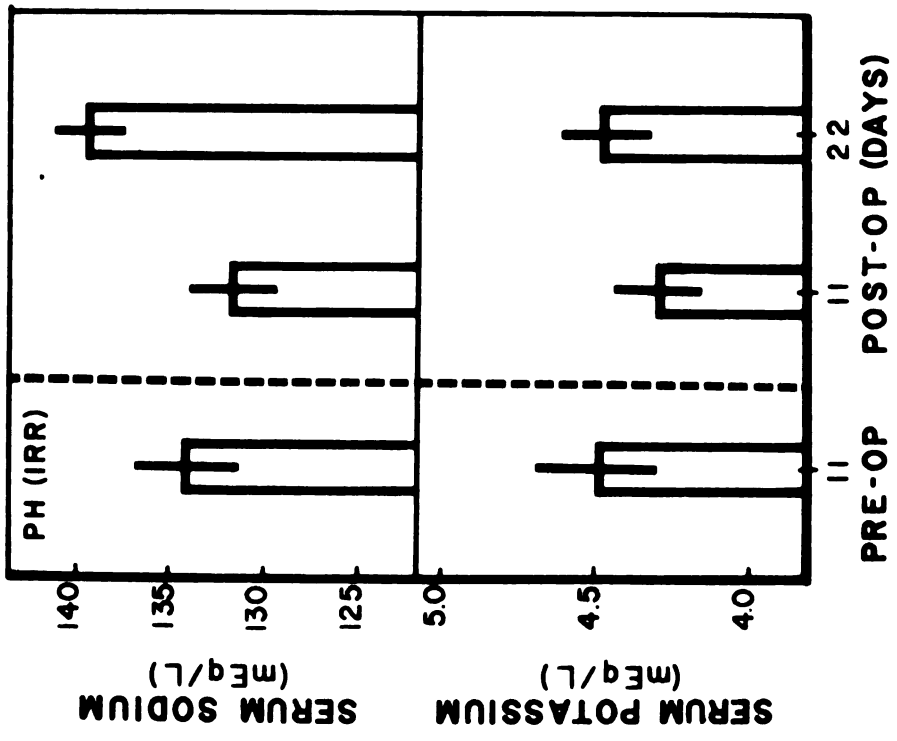
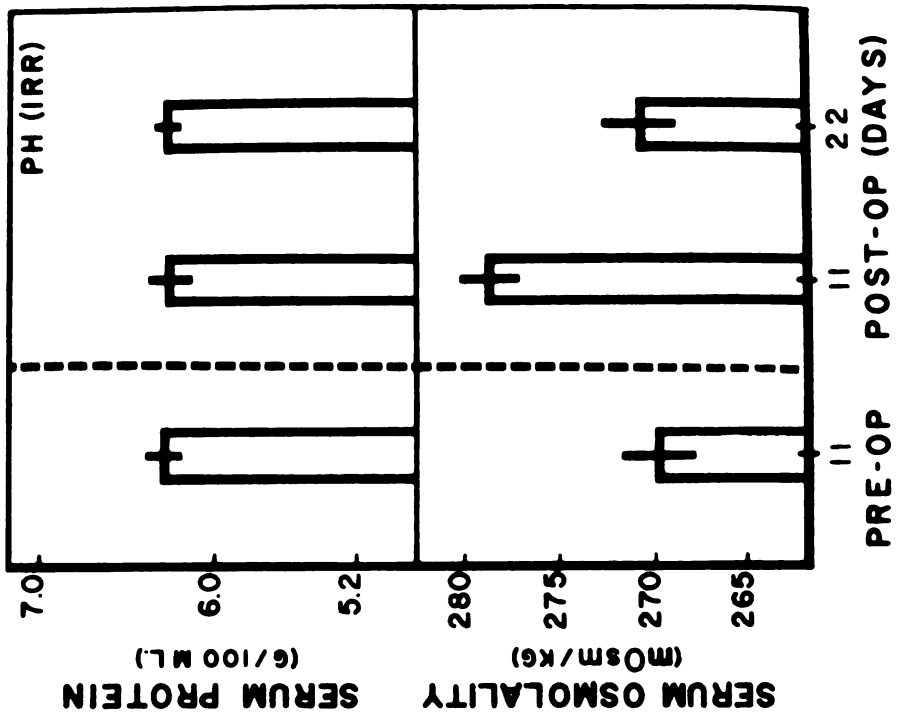
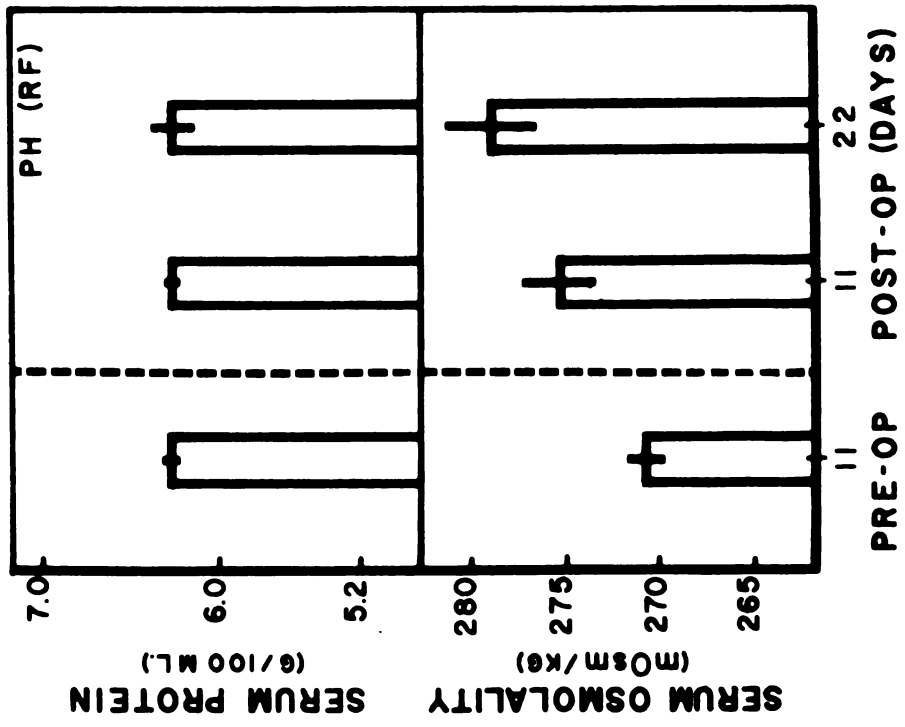

PH (IRR)
140
135
130
125
SERUM SODIUM
(mEq/L)
5.0
4.5
4.0
SERUM POTASSIUM
(mEq/L)
11
PRE-OP
11
POST-OP
22
POST-OP (DAYS)

Figure 34. Mean (± S.E.) serum protein and osmolality for the posterior medial hypothalamic radio frequency lesioned group on one pre-lesion and two post-operative blood sample days.

Figure 35. Mean (± S.E.) serum protein and osmolality for the posterior medial hypothalamic electrolytically lesioned group on one pre-lesion and two post-operative blood sample days.


PH (IRR)
SERUM OSMOLALITY
(mOsm/kg)
280
275
270
265
SERUM PROTEIN
(g/100 mL)
7.0
6.0
5.2
PRE-OP
11
POST-OP (DAYS)
11
22


PH (RF)
SERUM OSMOLALITY
(mOsm/kg)
280
275
270
265
SERUM PROTEIN
(g/100 mL)
7.0
6.0
5.2
PRE-OP
11
POST-OP (DAYS)
11
22

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Anatomical Findings

Photomicrographs of coronal sections prepared for the animals of each of the three groups, i.e., control, experimental (Radio Frequency), experimental (Electrolytic) utilized in the experiment are presented in the Appendix. Next to each photomicrograph is the corresponding de Groot (1959) atlas section. The stain employed for each section presented in the photomicrographs is indicated. The area destroyed in each hemisphere of each animal presented in frozen section was estimated by the use of the compensating polar planimetery method and is included in Table 1. The extent of the lesions with reference to the de Groot atlas is also included.

The control lesions were generally well placed bilaterally (refer to the Appendix). There was a tendency, however, for the lesions to be slightly skewed toward the left hemisphere. Lesion damage in the control group was confined to the medial and lateral habenular nuclei, the stria medullaris of the thalamus, the paraventricular thalamic nucleus and is some cases the dorsomedial thalamic nucleus and hippocampal fissure. The mean volume damage to the right hemisphere of the control animals was 1.93 cubic mm and to the left hemisphere 1.94 cubic mm.

The experimental lesions were not as well placed bilaterally and in no case completely destroyed the posterior medial hypothalamus. However some damage was consistently inflicted upon the target area as well as nearby structures including the dorsal premamillary nucleus, fornix and occassionally the dorsal longitudinal fasciculus, and arcuate nucleus of the hypothalamus. In almost all instances there was third ventricle damage.

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Table 1

Volume and Extent of Lesions

Animal	Area Destroyed (mm^3) Left Hemisphere	Right Hemisphere	Extent of Damage (de Groot Atlas)
controls			
3	1.18	1.36	A 4.4-5.4
6	0.29 (1.19)	0.72 (1.20)	A 3.0-4.4
15	Celloidin Embedded		A 3.6-4.4
16	1.96 (0.27)	1.84 (0.54)	A 4.0-4.6
19	0.86 (2.02)	0.35 (1.69)	A 4.0-4.6
21	Celloidin Embedded		A 4.2-4.8
experimentals (RF)			
5	0.44	0.70	A 4.2-6.0
9	Celloidin Embedded		A 4.4-4.8
10	0.32 (0.15)	0.84 (0.08)	A 4.0-5.0
11	0.21 (0.30)	0.10	A 3.8-4.8
12	Celloidin Embedded		A 4.6-5.0
14	0.31 (0.12)	0.32 (0.09)	A 4.4-4.6
experimentals (Elect.)			
1	0.87	0.74	A 4.2-5.4
7	1.20 (0.20)	1.41 (0.10)	A 4.0-5.0
8	Celloidin Embedded		A 4.2-5.2
17	0.77 (0.37)	1.14 (0.14)	A 4.2-5.0
18	1.32 (0.17)	0.30 (0.04)	A 4.2-5.0
20	Celloidin Embedded		A 4.4-5.0

The extent of gliosis for those animals in which it was encountered has been placed in parentheses. Total effects of the lesion would, therefore, be obtained by adding the area of the direct lesion and the gliosis area.

With reference to the experimental Radio Frequency group the mean volume damage to the right hemisphere was 0.51 cubic mm and to the left 0.46 cubic mm. For the experimental Electrolytic group the mean damage to the right hemisphere was 0.97 cubic mm and to the left 1.23 cubic mm.

Discussion

This experiment failed to replicate Cort's (1963a) findings concerning "salt wasting" following posterior hypothalamic lesions. The post-lesion urinary total sodium output did not increase as predicted by Cort nor did the potassium concentration increase. However it is clear that such lesions result in a dramatic change in isotonic saline preference with a nearly two-fold increase while tap water intake decreased by almost one-half. The question immediately arises as to the location of the sodium and chloride in the bodies of these animals. Presumably storage of these ions must be occuring given the substantial increase in ingestion with no apparent change in urinary output after recovery from surgery. The "sodium reservoir" hypothesis as offered by Stricker and Wolf (1967; Wolf and Stricker, 1967) may warrant considerable attention.

It is important to note that food intake remained constant during this removal thus eliminating it as a source of sodium change. An additional consideration at the time of saline removal conerns the potassium concentration that increased. This pattern may be interpreted as a body stress reaction with the liberation of intracellular potassium from cells, however, the concentration quickly approaches and levels off at values very similar to pre-lesion concentrations.

The Electrolytic and Radio Frequency groups were comparable in their post-lesion fluid metabolic patterns thus casting doubt on the possibility of attributing this increased isotonic saline intake to the irritative quality of the electrolytic lesion technique as demonstrated for the lateral hypothalamus by Barbara Rolls (1970). The increased isotonic saline intake by the dorsomedial thalamic lesioned group was of course unexpected for it was intended that these animals serve as a control group. It may be that electrolytic lesioning of this structure with possible damage to nearby and/or related areas, actually does precipitate alterations in body water regulation. This suggestion is indeed open to skepticism for there appears to be a complete lack of support for such findings in the literature. In several animals there was damage to the medial habenular nucleus. Lengvari et al. (1969) have reported alterations in salt and water metabolism with lesions of this area. Specifically such lesions reduced aldosterone secretion while adrenalectomy resulted in the enlargement of cell nucleus size in the medial habenular nucleus.

In summary, this attempted replication of earlier findings concerning changes in body water balance following electrolytic posterior hypothalamic lesions was at best only partially successful. Increased isotonic saline intake was noted with post-lesion recovery; however, accompanying increases in urine sodium and potassium excretion did not occur as would be expected on the basis of Cort's hypothesis. Also the removal of the isotonic saline for a period of twelve experimentally monitored days and several additional post-experiment days prior to sacrificing the animals revealed no predisposition on the part of these subjects

toward an unhealthy physical condition, nor did the animals increase their food intake which represented the only remaining source of sodium.

A preliminary analysis of the available data to this point would likely suggest an alteration in saline preference with posterior hypothalamic damage that persists in the form of increased tap water intake with saline removal but does not appear to have a noticeable absolute "set point" for the sodium or potassium intake-output ratios.

EXPERIMENT 3

Replication Lesions of the Posterior Medial Hypothalamus

Experiment 2 established consistent and comparable post-lesion effects for damage to the area posterior medial hypothalami by both the electrolytic and radio frequency lesion methods. This supports the contention that the increased ingestion of physiological saline is not simply due to the irritative qualities of the electrolytic lesion method. However, the dorsomedial thalamic and medial habenular nucleus lesioned animals evidenced equally impressive physiological saline solution intakes thus cancelling their utility as a control group. The present study was designed as a replication attempt of Experiment 2 and focused conern upon the post-operative body sodium accumulation that was encountered in Experiment 2.

Subjects

Thirty-six male albino rats of the Holtzman strain approximately 120 days of age were adapted to metabolism cages under constant light for a pre-lesion period of 20 days. The animals were maintained on *ad libitum* tap water and/or 0.87% saline and/or 2.00% saline and powdered Wayne Breeder Blox. The fluid cylinders were switcheddaily on the cages in a random sequence.

Procedure

The animals were stratified into three groups of 12 animals each. Each of these groups were further split into 6 experimental and 6 control subjects. The first group was maintained on tap water only. The second group on tap water and a 0.87% saline solution; and the third group 0.87% and 2.00% saline solutions. Groups 1 and 2 were composed of normal animals while Group 3 subjects had been adrenalectomized a minimum of seven days prior to the initiation of pre-operative day 1.

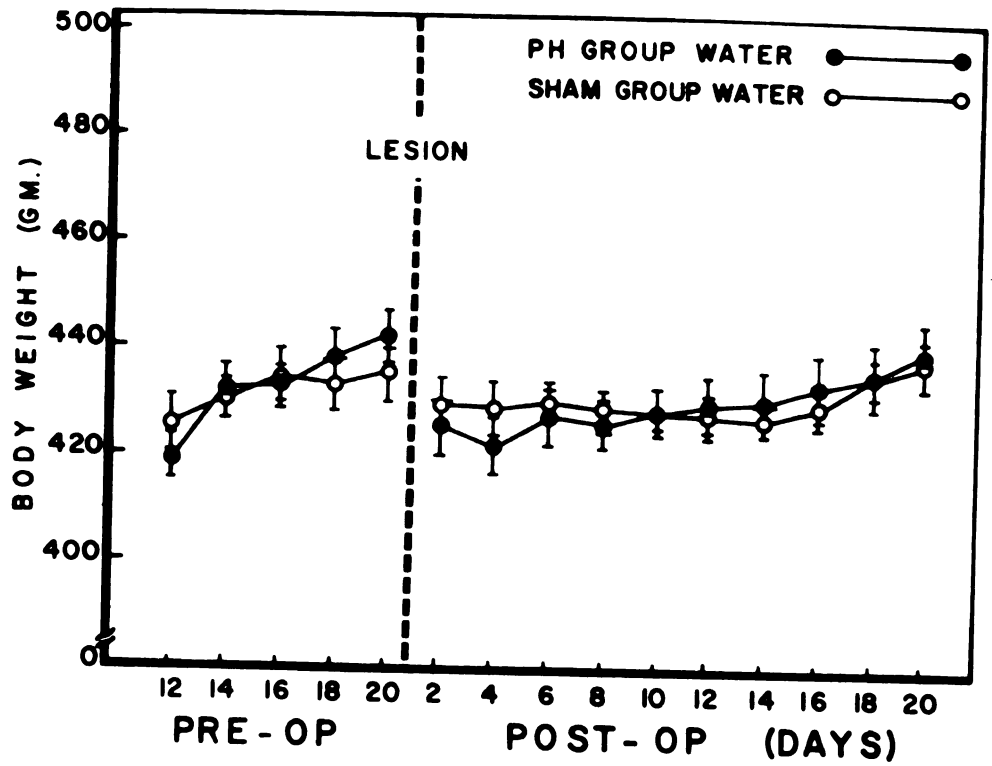
The six experimental animals in each of the three groups received bilateral electrolytic lesions to the area posterior medial hypothalami. The six control animals were treated in the same mannor as the experimentals, however, sham lesions were administered, consisting of sterile dura punctures without the infliction of lesion damage. During the 20 days of adaptation to the metabolism cages and subsequent 20 post-lesion days a urine sample was retained from each animal and body weight determined once a day between 0800-0830 hours. At this time as well as at 2000-2030, water, saline and food consumption and urine volume excretion were also recorded. Urine samples were prepared in the same fashion as described in the previous experiments with regard to analysis. Also 4-hour readings on pre-operative day 15 and post-operative day 6 followed the pattern described in Experiment 2. Blood samples were taken on pre-operative day 10 and post-operative day 10 and also conformed with the procedures described in the previous studies.

Results

Metabolism data have been grouped across two day intervals. The last ten days of the twenty day pre-operative basal rate period is presented along with the entire twenty day post-operative period.

Figure 36. Mean (± S.E.) body weights for posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 37. Mean(± S.E.) body weights for posterior medial hypothalamic and sham lesioned groups maintained on tap water and isotonic saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
SHAM GROUP WATER
LESION
BODY WEIGHT (GM.)
500
480
460
440
420
400
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

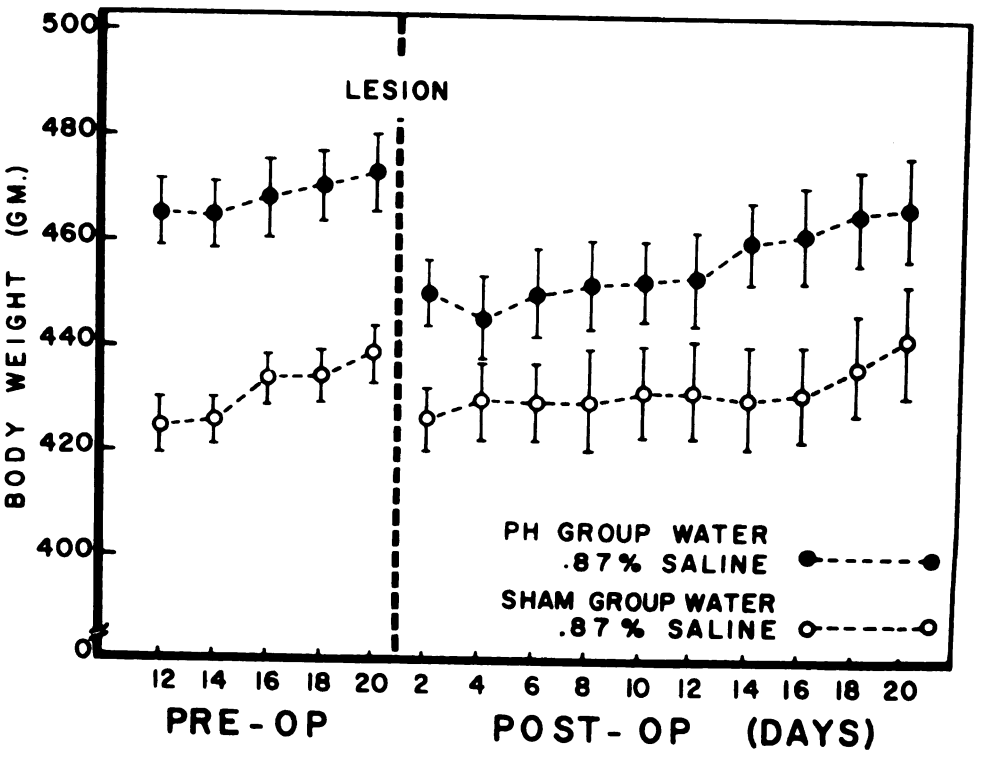
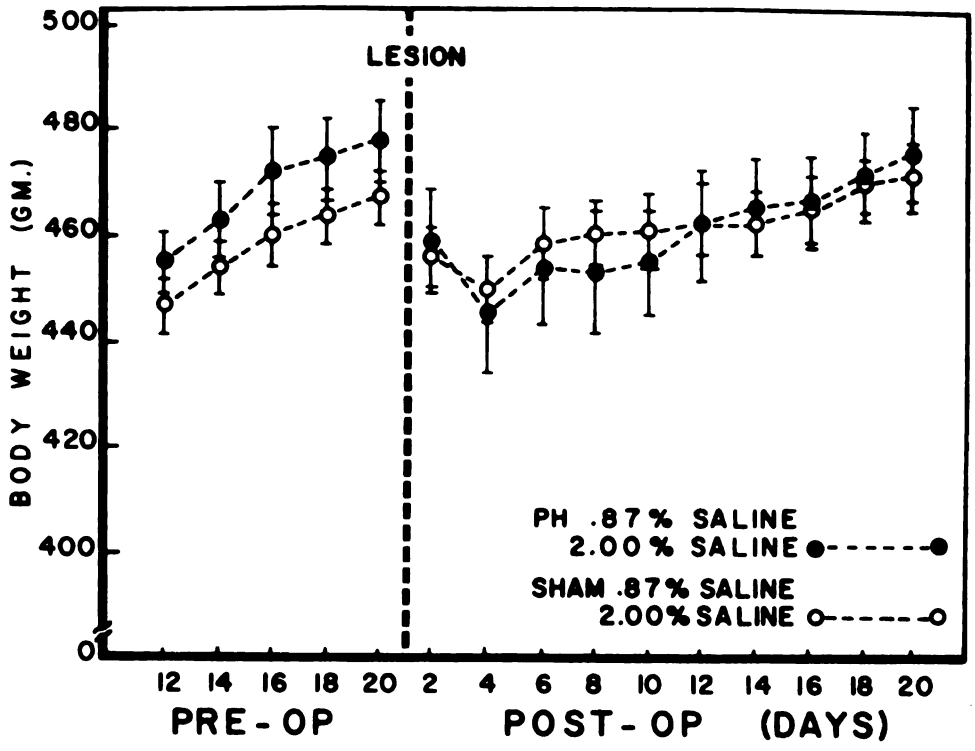
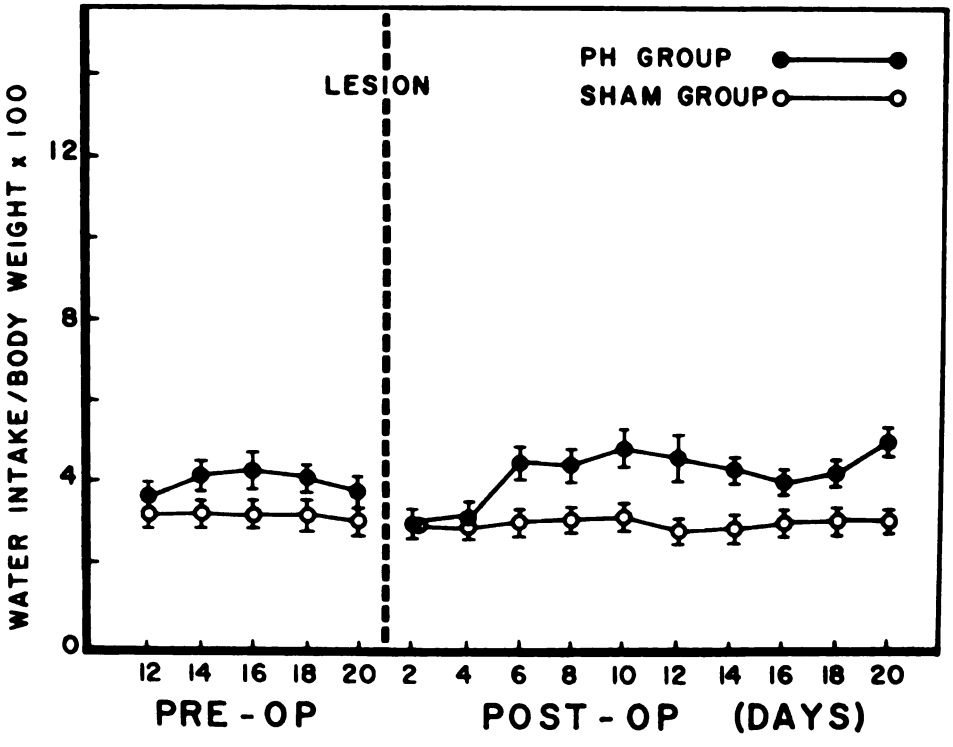

LESION
BODY WEIGHT (GM.)
500
480
460
440
420
400
0
PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 38. Mean (± S.E.) body weights for the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on isotonic and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-lesion.

Figure 39. Mean (± S.E.) tap water intake corrected for body weight differences by the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


LESION
BODY WEIGHT (GM.)
500
480
460
440
420
400
0
PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

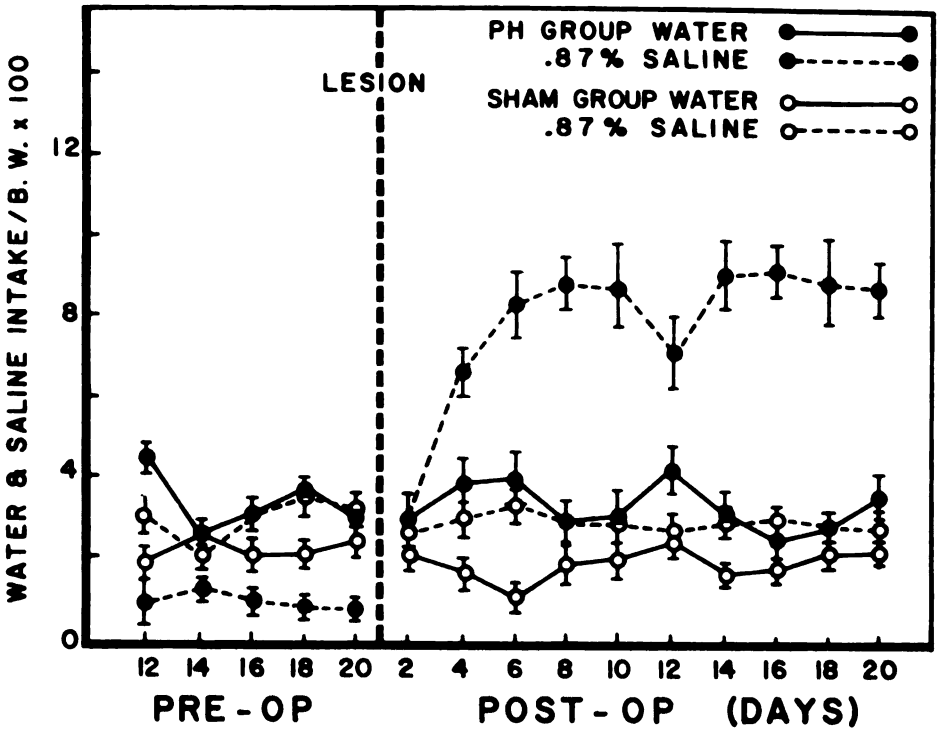

LESION
PH GROUP
SHAM GROUP
WATER INTAKE/BODY WEIGHT x 100
12
8
4
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

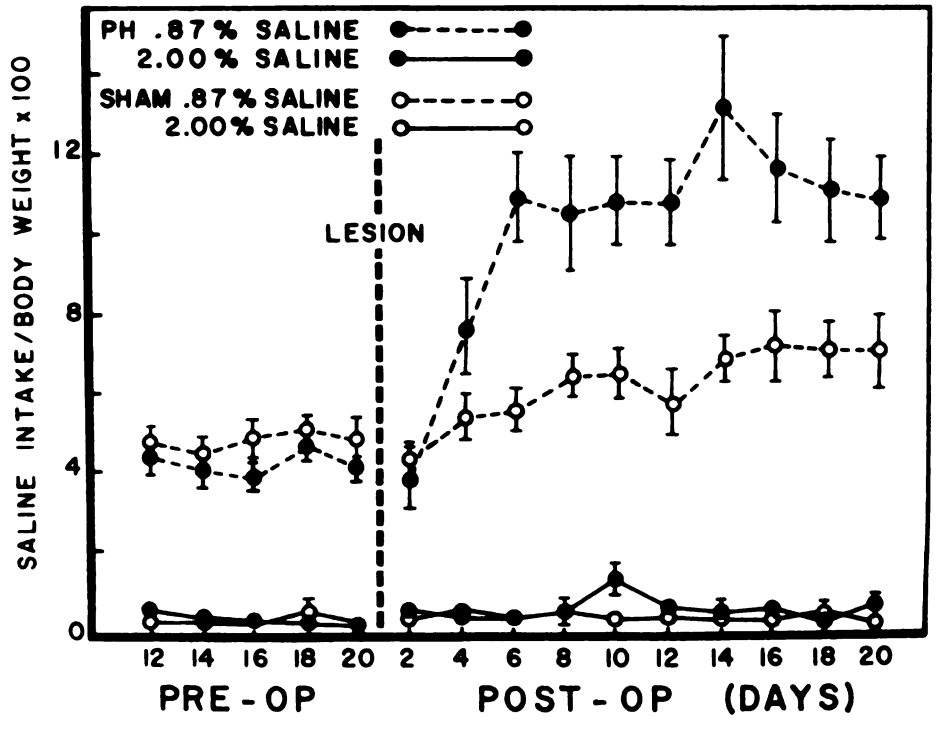
The means and standard errors of the means are plotted across days for each of the six groups. Four out of the six groups revealed significant drops in body weight following surgery (Figures 36, 37 and 38) therefore all subsequent intake-output measures were corrected for body weight differences.

Liquid intakes are shown for each of the three experimental groups and their corresponding sham lesion control groups in Figures 39, 40 and 41. In that these intakes have been corrected for differences in body weight they are presented as percentages. The points on these figures represent the means of the percentages calculated for each animal for each reading period and grouped over two day periods for a given group. The pre-lesion water intake values (Figure 39) for the posterior medial hypothalamic lesioned group (PH group) versus the sham lesioned group were statistically different ($t=5.80$, $p<0.01$). Post-operatively the water intakes between the two groups were also different ($t=8.53$, $p<0.01$). Comparing the water intakes of the sham lesioned animals pre- and post-operatively there was no difference (t for related measures=0.57, $p>0.10$) however, the same comparison with the experimental lesioned group revealed significant increases in water intake during the post-lesion period (t for related measures=6.98, $p<0.01$). This increment in water intake is relatevely small representing a mean change of from 3.89% pre-op to 4.25% post-operatively. The tap water and 0.87% saline solution intakes of the second posterior medial hypothalamic (PH) lesioned group and its sham lesion control group is offered in Figure 40. The tap water intake of the PH group was not different pre- and post-operatively.

Figure 40. Mean (± S.E.) tap water and isotonic saline solution consumption corrected for body weight differences by the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 41. Mean (± S.E.) fluid intakes corrected for body weight differences of the adrenalectomized groups maintained on isotonic and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
LESION
WATER & SALINE INTAKE/B. W. x 100
0
4
8
12
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

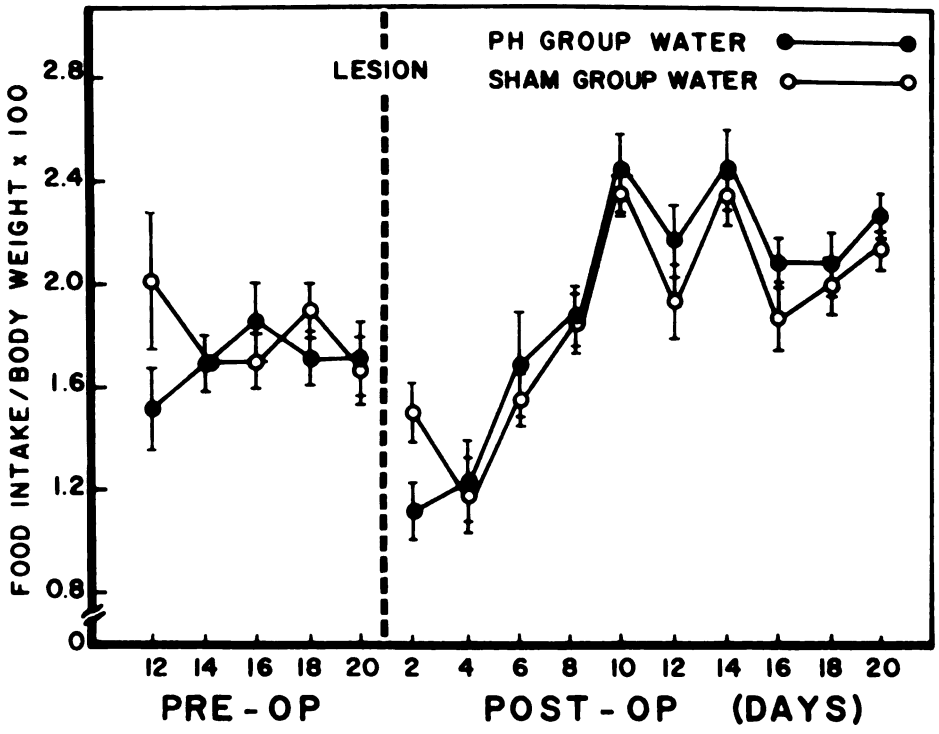

PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
LESION
SALINE INTAKE/BODY WEIGHT x 100
0
4
8
12
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

However the 0.87% saline intake of this group was significantly greater post-operatively (t for related measures=17.32, $p<0.01$) representing a mean change of from 0.68% pre-lesion to 7.65% post-lesion. The sham lesioned animals evidenced no difference comparing pre- and post-lesion water and saline intakes (t for related measures=0.57 and 0.53 respectively, $p>0.10$). The adrenalectomized PH lesioned group (Figure 41) demonstrated a significant increase in 0.87% saline intake during the post-operative period (t for related measures=13.65, $p<0.01$). This represents a mean change of from 4.22% pre-op to 10.21% post-operatively. Their 2.00% saline solution intakes were not different concerning the pre- and post-lesion periods (t for related measures=0.98, $p>0.10$). Although the adrenalectomized sham group indicated no significant change in 2.00% saline intake post-operatively (t for related measures=0.71, $p>0.10$) their 0.87% saline intake did increase significantly (t for related measures=6.22, $p<0.01$), but the mean increase was less than for the adrenalectomized PH group being 4.87% pre-lesion and 6.50% post-lesion.

With regard to food intake corrected for body weight changes the pattern is much the same with the animals of this experiment as established by those of Experiment 2; namely a significant decrease in food consumption following surgery. Significant decreases in post-operative food intake were registered by the PH group maintained on tap water only between day 20 pre-op and day 1 post-op (Figure 42; t for related measures=3.91, $p<0.01$). This was also true for the PH and sham groups maintained on tap water and 0.87% saline (Figure 43; t for related measures=6.56 and 4.45 respectively, $p<0.01$)

Figure 42. Mean (± S.E.) food intakes corrected for body weight differences of the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 43. Mean (± S.E.) food intakes corrected for body weight differences of the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
SHAM GROUP WATER
LESION
FOOD INTAKE/BODY WEIGHT x 100
2.8
2.4
2.0
1.6
1.2
0.8
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

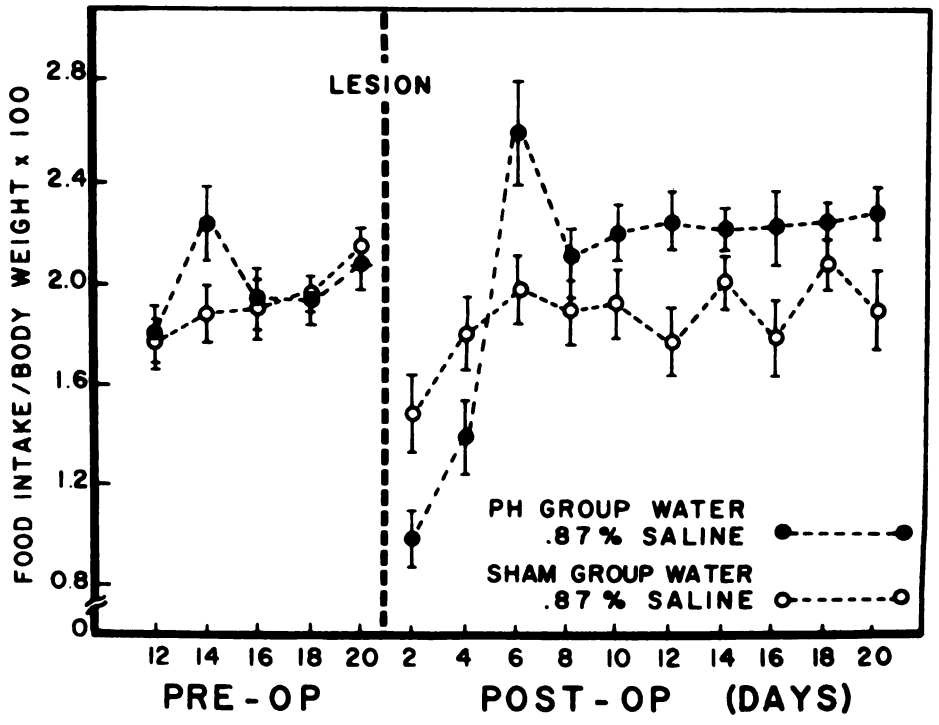
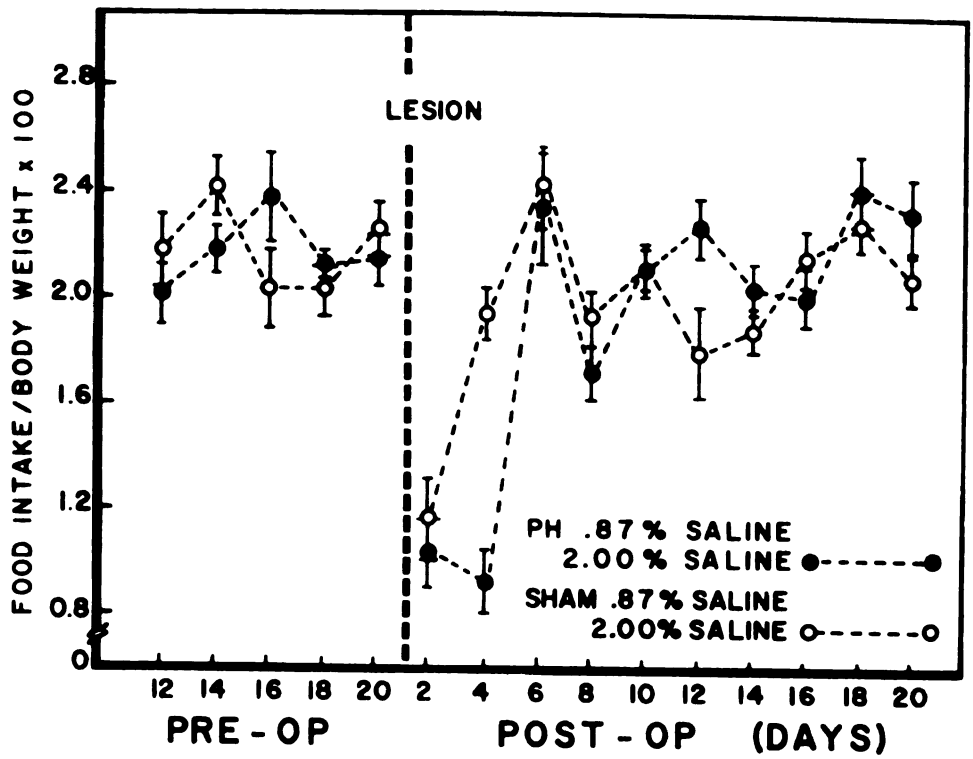
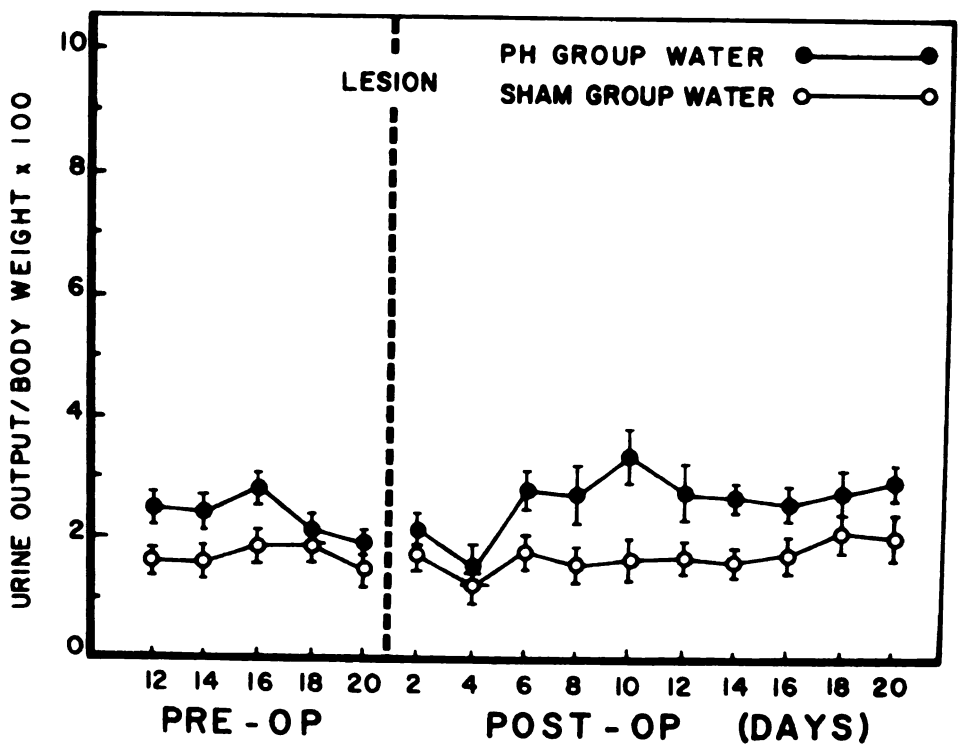

LESION
FOOD INTAKE/BODY WEIGHT x 100
2.8
2.4
2.0
1.6
1.2
0.8
0
PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 44. Mean (± S.E.) food intakes corrected for body weight differences of the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 45. Mean (± S.E.) urine volume outputs corrected for differences in body weight of the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


FOOD INTAKE/BODY WEIGHT x 100
2.8
2.4
2.0
1.6
1.2
0.8
0
LESION
PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)


URINE OUTPUT/BODY WEIGHT x 100
10
8
6
4
2
0
LESION
PH GROUP WATER
SHAM GROUP WATER
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

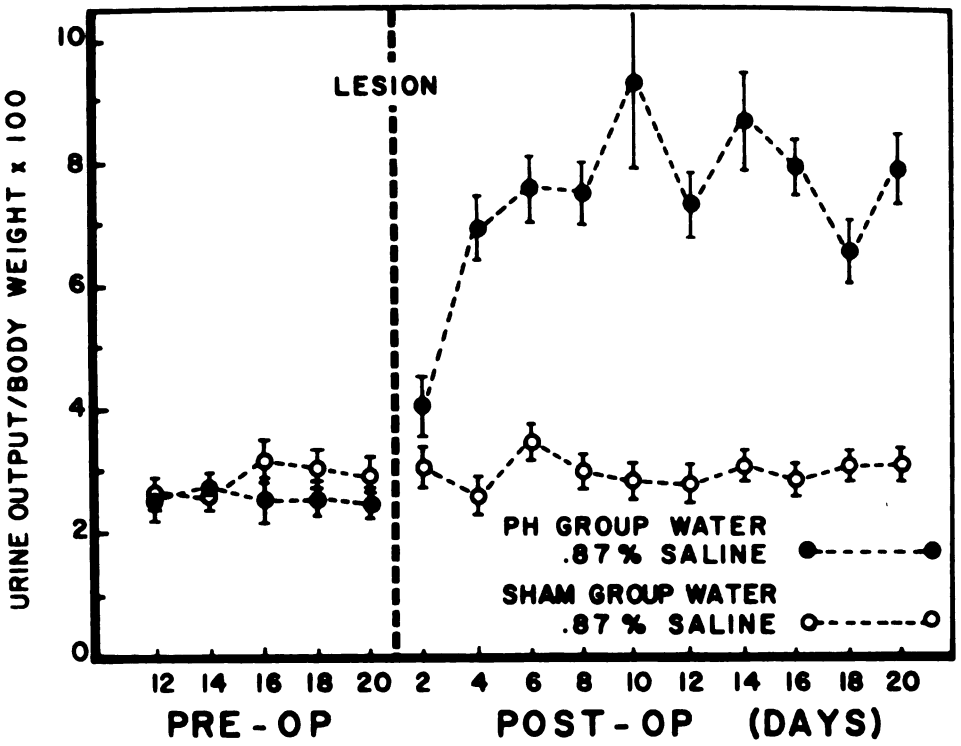
and for the adrenalectomized groups offered 0.87% and 2.00% saline solutions (Figure 44; t for related measures=7.06 and 4.57, PH and sham respectively, $p<0.01$). Recovery of food intake to at least the pre-lesion level of consumption was exhibited by each of the six groups within a period of from four to six days after surgery.

Urine output was also corrected for differences in body weight. The PH group on tap water only (Figure 45) revealed a slight but non-significant increase in urine volume output during the post-lesion period as compared with the pre-lesion basal rate (t for related measures=2.06, $p>0.05$). This was a mean change of from 2.27% pre-op to 2.58% post-op. The tap water sham group demonstrated no difference between pre- and post-lesion urine volume output (t for related measures=1.48, $p>0.10$). This was likewise the case for the sham group maintained on tap water and 0.87% saline solution (Figure 46; t for related measures=0.60, $p>0.10$) while the corresponding PH lesioned group revealed a significant increase in urine volume output post-operatively (t for related measures=14.71, $p<0.01$). This represents a mean change of from 2.47% pre-lesion to 7.33% post-lesion. The adrenalectomized groups (Figure 47) both evidenced increased urine output post-operatively (t for related measures=10.19 and 6.37, PH and sham groups respectively, $p<0.01$). The mean change for the PH lesioned group was from 3.57% pre-op to 7.21% post-op, while the mean change for the sham lesioned group was from 3.42% pre-op to 4.75% post-operative.

Turning to the urine constituent data it must be noted that with regard to sodium concentration there was a significant drop

Figure 46. Mean (± S.E.) urine volume outputs corrected for differences in body weight of the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 47. Mean (± S.E.) urine volume outputs corrected for differences in body weight of the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


URINE OUTPUT/BODY WEIGHT x 100
LESION
PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

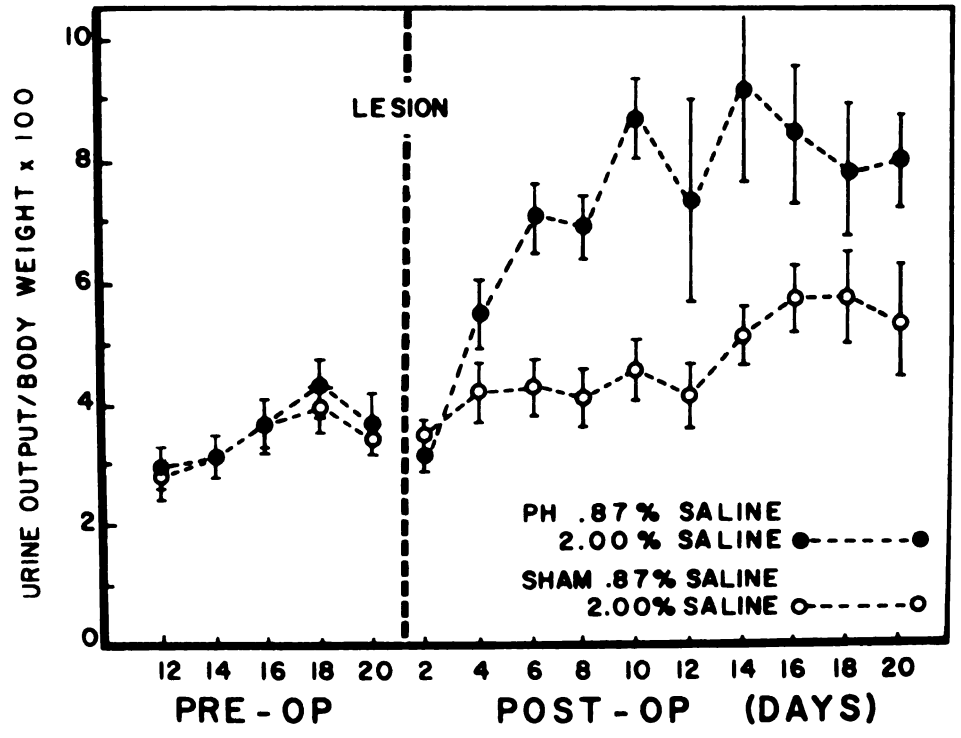
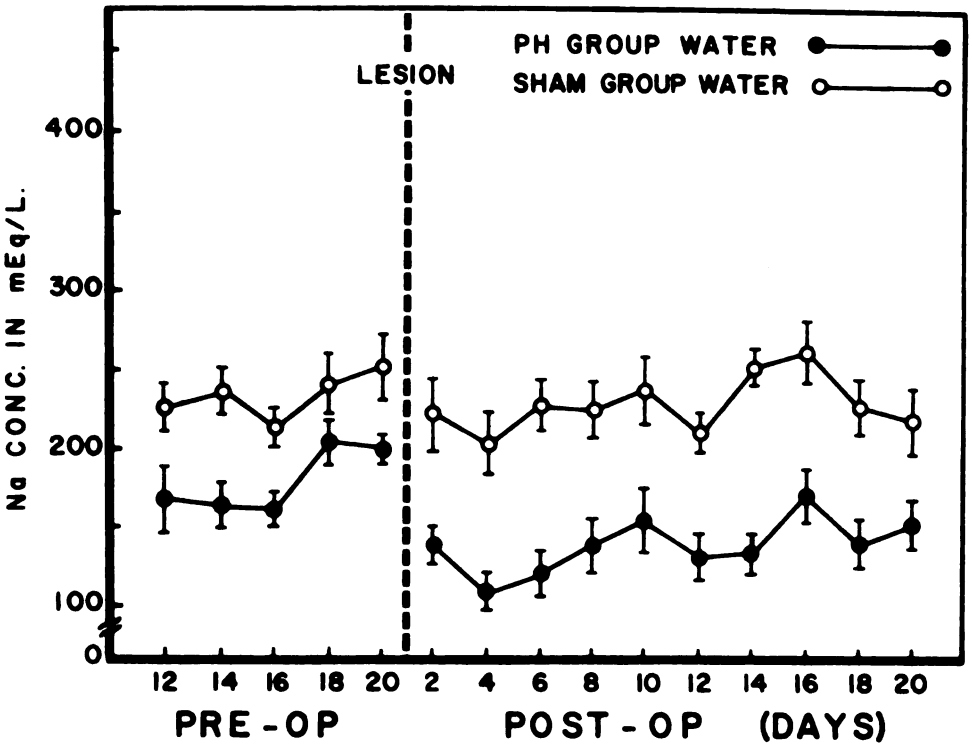
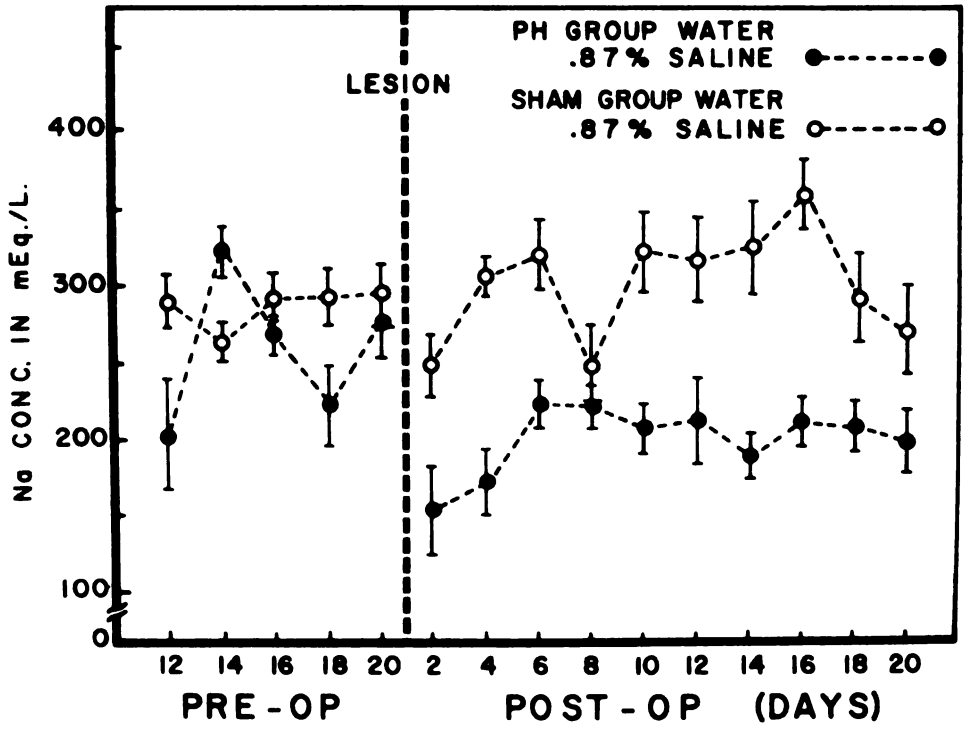

URINE OUTPUT/BODY WEIGHT x 100
LESION
PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 48. Mean (± S.E.) urine sodium concentrations of the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 49. Mean (± S.E.) urine sodium concentrations of the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
SHAM GROUP WATER
LESION
Na CONC. IN mEq/L.
400
300
200
100
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

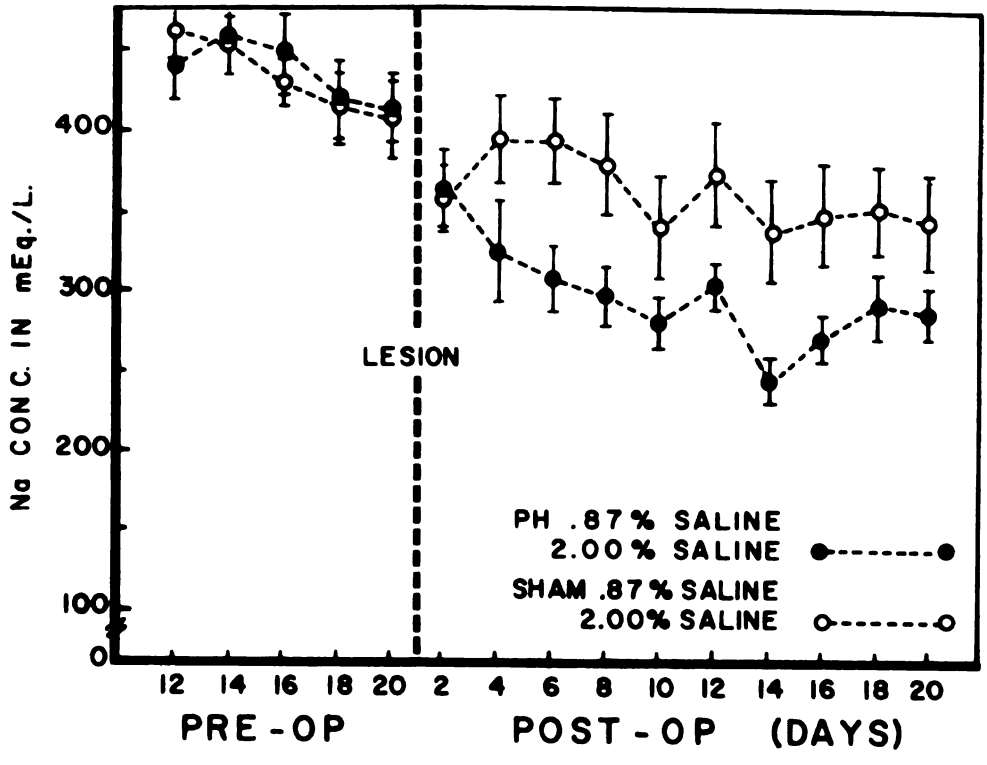

PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
LESION
Na CONC. IN mEq./L.
400
300
200
100
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

post-operatively demonstrated by the PH lesioned tap water only group (Figure 48; t for related measures=3.51, $p<0.01$). The mean change was from 178.02 mEq/L. pre-op to 138.25 mEq/L. post-operative. The corresponding sham lesion group was not different in this respect. There was an initial pre-lesion difference in urine sodium concentration between the experimental and sham groups with the sham group evidencing a significantly elevated mean concentration (238.76 mEq/L. as compared with the PH lesion group's 178.02 mEq/L.; t=10.23, $p<0.01$). The urine sodium concentration for the two groups placed on tap water and 0.87% saline solution (Figure 49) followed a similar pattern in that the sham group showed no difference pre- and post-lesion (t for related measures=1.48, $p>0.10$) while the PH lesion group revealed a decreased mean sodium concentration of from 255.73 pre-op to 200.28 post-op (t for related measures=3.35, $p<0.01$). The urine sodium concentrations for the adrenalectomized groups (Figure 50) are very much different from the other four groups in that both the sham and PH lesioned groups' sodium concentration pre-op was much higher with means of 433.22 and 436.25 mEq/L. respectively, than post-operatively where the means were 359.78 and 294.47 mEq/L. respectively. For both the sham and experimental lesioned groups there were significant decreases in urine sodium concentration (t for related measures=6.75 and 8.34 respectively, $p<0.01$).

The PH and sham lesioned groups maintained on tap water (Figure 51) revealed no change in total urinary sodium excreted pre- and post-operatively (t for related measures 1.42 and 1.03 respectively, $p>0.10$). The PH lesioned group provided tap water and isotonic saline solution

Figure 50. Mean (± S.E.) urinary sodium concentrations of the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 51. Mean (± S.E.) total urinary sodium excreted in 24 hour units by the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


Na CONC. IN mEq./L.
400
300
200
100
0
LESION
PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

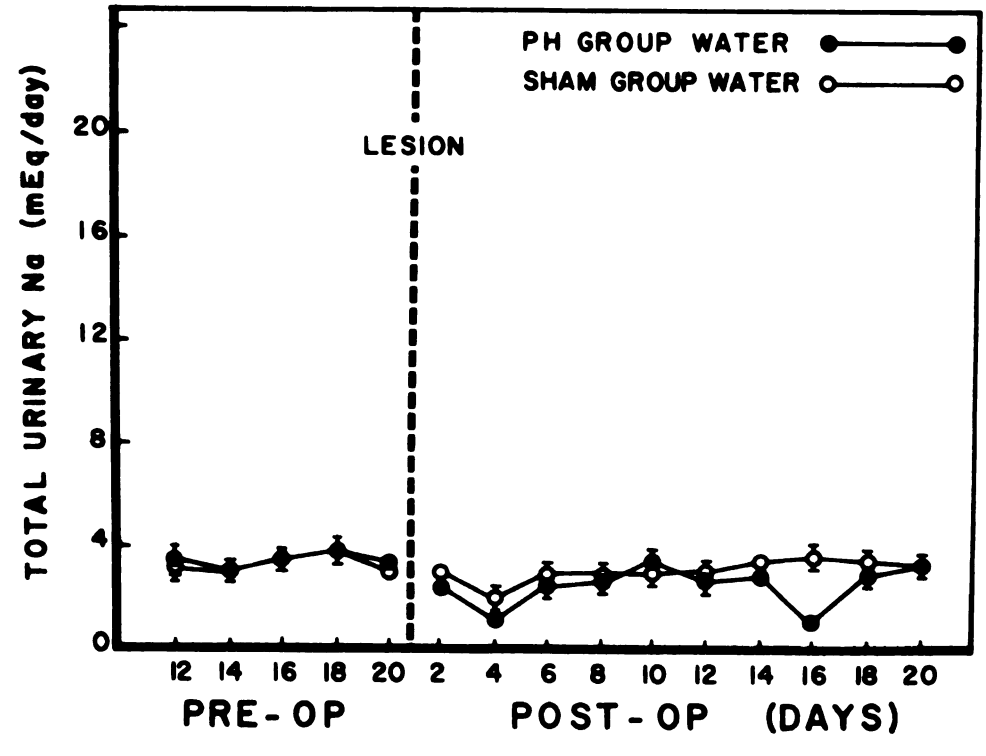
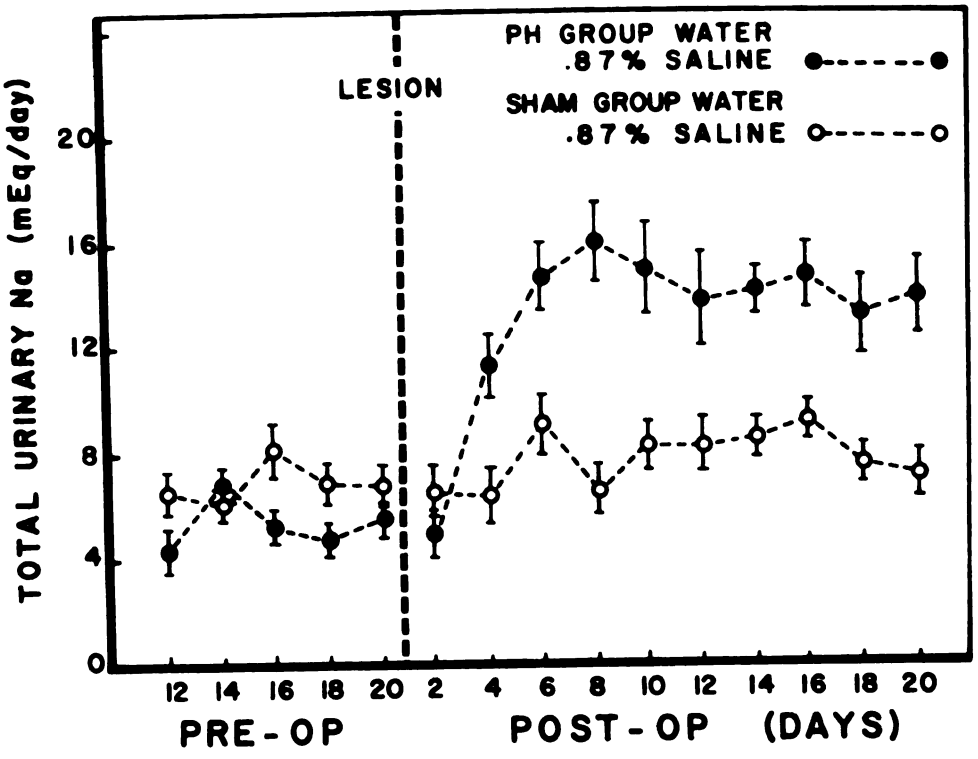
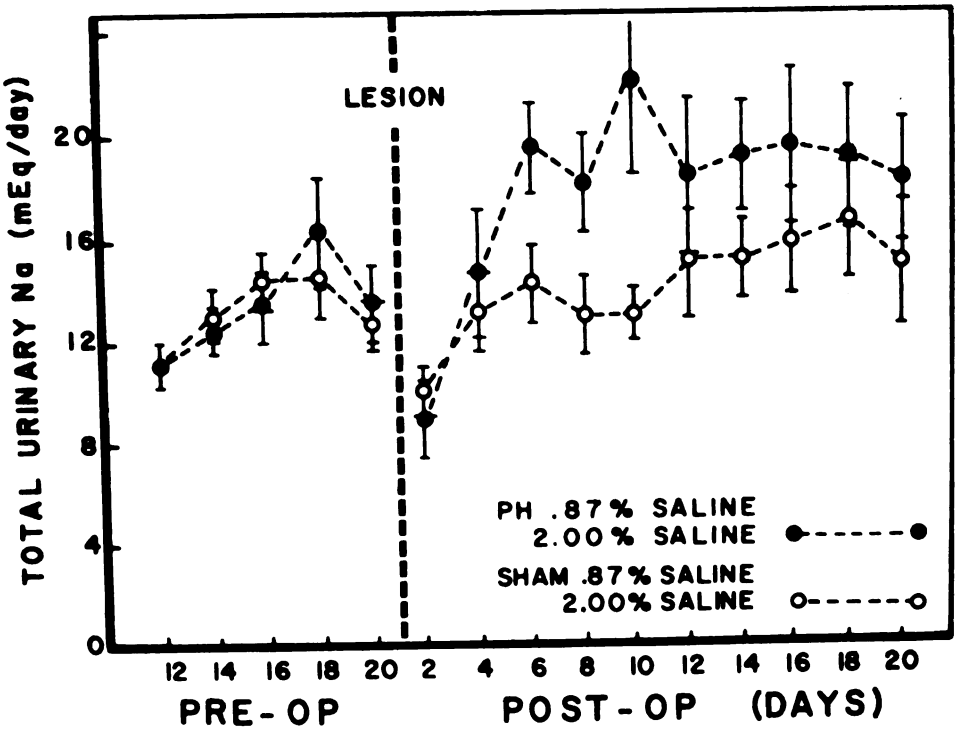

TOTAL URINARY Na (mEq/day)
20
16
12
8
4
0
LESION
PH GROUP WATER
SHAM GROUP WATER
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 52. Mean (± S.E.) total urinary sodium excreted per 24 hour units by the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 53. Mean (± S.E.) total urinary sodium excreted per 24 hour units by the adrenalectomized posterior medial and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
LESION
TOTAL URINARY Na (mEq/day)
0
4
8
12
16
20
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)


LESION
TOTAL URINARY Na (mEq/day)
0
4
8
12
16
20
PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

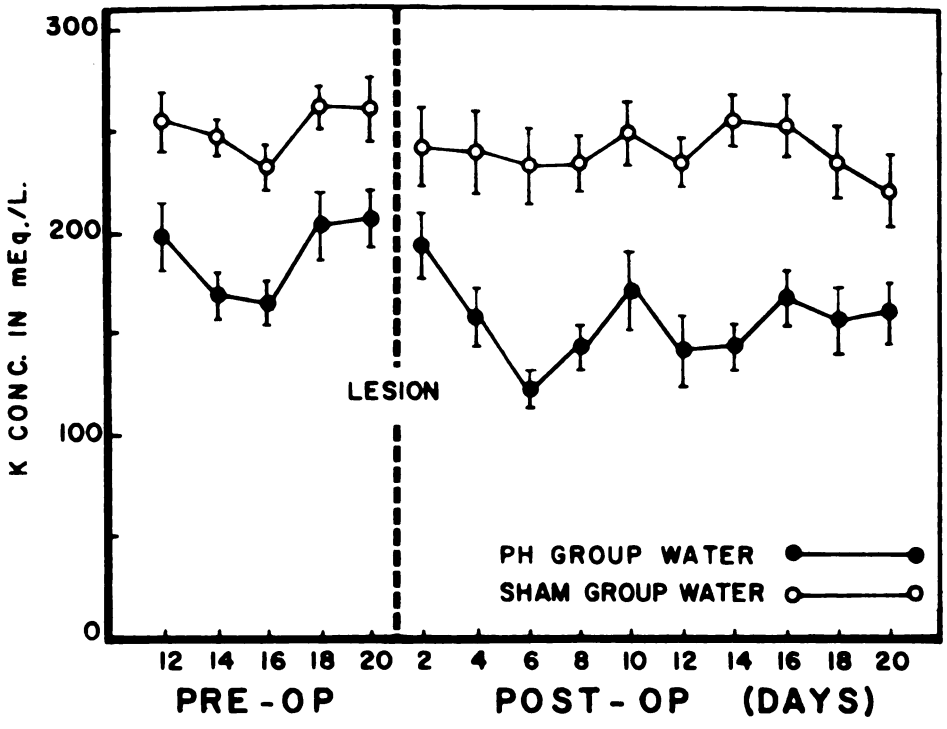
(Figure 52) demonstrated a significantly increased total urinary sodium output post-operatively while the corresponding sham group showed no change (t for related measures=21.99 and 2.05; $p<0.01$ and $p>0.05$ respectively). The adrenalectomized groups maintained on isotonic and 2.00% saline solution (Figure 53) both evidenced increased total sodium outputs post-operatively (t for related measures=7.45 and 6.91 for the PH and sham lesioned groups respectively, $p<0.01$).

The urine potassium concentrations exhibited by the PH lesion group on tap water (Figure 54) was significantly lower post-lesion than pre-lesion (t for related measures=3.42, $p<0.01$) depicting a mean drop of from 187.47 mEq/L. pre-op to 153.90 mEq/L. post-operatively (t for related measures=1.39, $p>0.10$). This same pattern persisted with the two groups maintained upon tap water and 0.87% saline solution (Figure 55). The sham lesion group displayed no change pre- and post-operatively (t for related measures=1.63, $p>0.10$) while the PH lesioned group decreased its potassium concentration significantly (t for related measures=8.30, $p<0.01$) from a mean of 168.15 mEq/L. pre-op to 49.33 mEq/L. post-operative. The adrenalectomized groups (Figure 56) in this instance obeyed the pattern of the other four groups in that the sham group was not different in potassium concentration pre- and post-op (t for related measures=1.79, $p>0.05$), while the PH lesioned group significantly decreased its potassium concentration (t for related measures=6.31, $p<0.01$) from a mean of 134.52 mEq/L. pre-op to 59.05 mEq/L. post-lesion.

There were pre-lesion basal rate differences in specific gravity between the two groups with tap water only available (Figure 57; $t=19.50$, $p<0.01$) with the sham lesion group evidencing a mean

Figure 54. Mean(± S.E.) urinary potassium concentrations of the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 55. Mean (± S.E.) urinary potassium concentrations of the posterior medial hypothalamic and sham lesioned groups maintained on tap water and isotonic saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


300
200
100
0
K CONC. IN mEq./L.
LESION
PH GROUP WATER
SHAM GROUP WATER
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

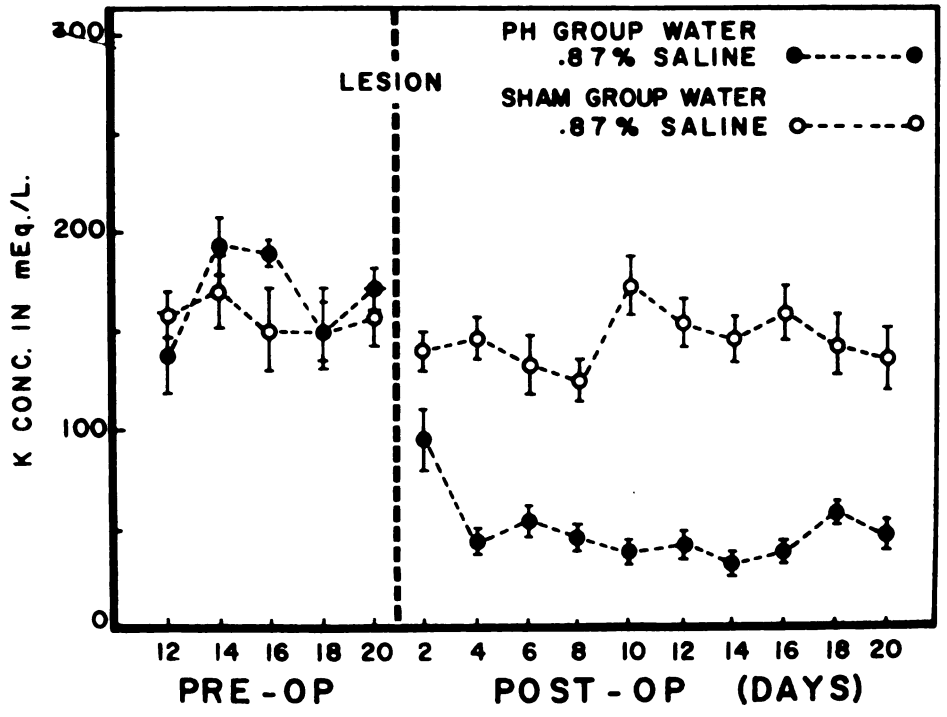
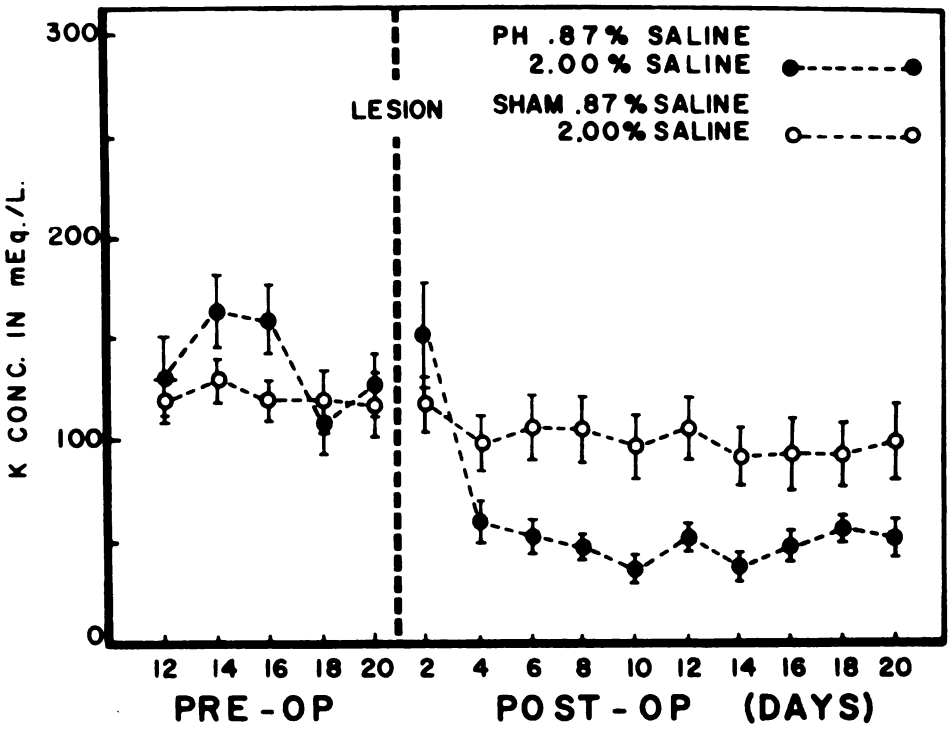

300
200
100
0
K CONC. IN mEq./L.
LESION
PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 56. Mean (± S.E.) urinary potassium concentrations of the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 57. Mean (± S.E.) urinary specific gravity of the posterior medial hypothalamic and sham lesioned groups maintained on tap water for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
LESION
K CONC. IN mEq./L.
300
200
100
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

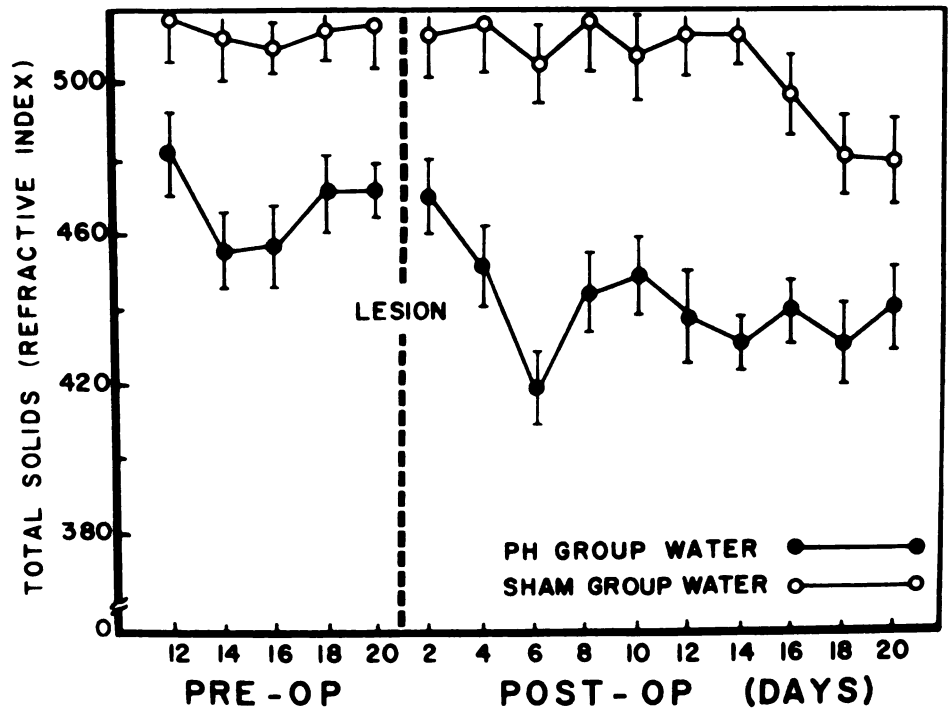
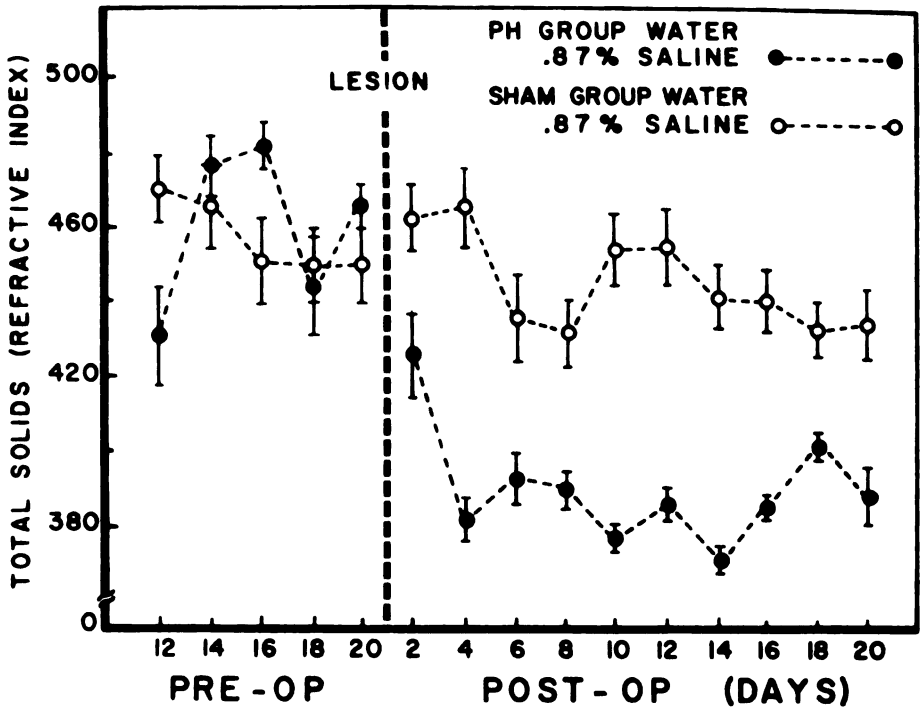
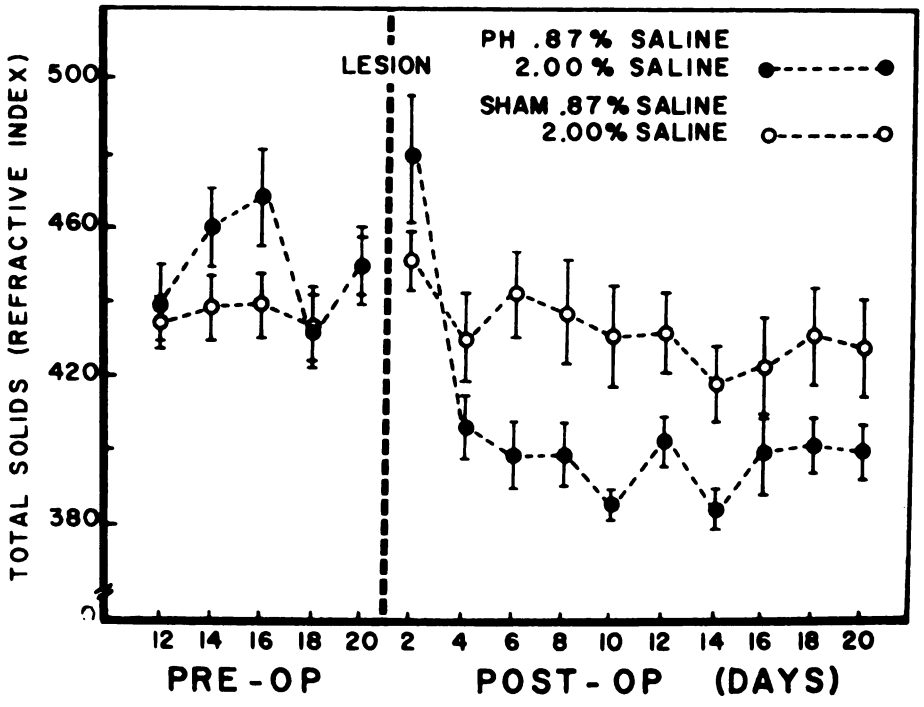

TOTAL SOLIDS (REFRACTIVE INDEX)
500
460
420
380
0
LESION
PH GROUP WATER
SHAM GROUP WATER
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

Figure 58. Mean (± S.E.) urinary specific gravity of the posterior medial hypothalamic and sham lesioned groups maintained on tap water and 0.87% saline solution for the last ten days of a twenty day pre-lesion period and twenty days post-operative.

Figure 59. Mean (± S.E.) urinary specific gravity of the adrenalectomized posterior medial hypothalamic and sham lesioned groups maintained on 0.87% and 2.00% saline solutions for the last ten days of a twenty day pre-lesion period and twenty days post-operative.


PH GROUP WATER
.87% SALINE
SHAM GROUP WATER
.87% SALINE
LESION
TOTAL SOLIDS (REFRACTIVE INDEX)
500
460
420
380
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)


PH .87% SALINE
2.00% SALINE
SHAM .87% SALINE
2.00% SALINE
LESION
TOTAL SOLIDS (REFRACTIVE INDEX)
500
460
420
380
0
12 14 16 18 20 2 4 6 8 10 12 14 16 18 20
PRE-OP
POST-OP (DAYS)

refractive index of 1.3516 and the PH lesion group 1.3468. The PH lesion group revealed significant decreases in specific gravity (t for related measures=6.05, $p<0.01$) with a mean refractive index of 1.3469 pre-op to 1.3442 post-op. The corresponding sham group did drop slightly but not significantly from a mean refractive index of 1.3517 pre-op to 1.3503 post-operative (t for related measures= 1.99, $p>0.05$). The groups placed on tap water and 0.87% saline solution (Figure 58) both indicated drops in specific gravity post-operatively. The sham lesion group decreased from a refractive index of mean 1.3456 pre-op to 1.3445 post-op (t for related measures=3.06, $p<0.01$) while the PH lesion group dropped from a mean refractive index of 1.3461 pre-op to 1.3390 post-op (t for related measures= 5.41, $p<0.01$). The adrenalectomized sham lesion group (Figure 59) revealed no difference between its pre- and post-lesion refractive index (t for related measures=1.77, $p>0.05$) however, the PH lesion group dropped with a mean refractive index of 1.3449 pre-op and 1.3405 post-op (t for related measures=5.77, $p<0.01$).

Measurements taken on blood constituents are offered in Table 2. Serum sodium and potassium mean concentrations pre- (A) and post-lesion (B) are given for each of the six groups as are protein and hematocrit values. The standard errors of the means are also provided.

One of the most interesting aspects of the blood data concerns the pre-lesion depressed sodium, potassium and protein concentrations for the adrenalectomized groups and the recovery of these measures after the experimental or sham lesions. The hematocrit however,

		SERUM Na (mEq./L.)	SERUM K (mEq./L.)	PROTEIN (G./100 ml.)	HEMATOCRIT (% RBC)
TAP PH GROUP	A	143.8 ± 2.65	5.00 ± 0.13	6.55 ± 0.06	46.2 ± 1.62
	B	152.7 ± 2.38	4.60 ± 0.10	6.42 ± 0.09	45.8 ± 0.75
TAP SHAM GROUP	A	151.5 ± 3.18	5.43 ± 0.33	6.65 ± 0.02	47.6 ± 0.51
	B	147.2 ± 2.18	4.13 ± 0.10	6.83 ± 0.09	47.5 ± 0.34
TAP & 0.87% SALINE PH	A	134.6 ± 1.16	4.25 ± 0.32	6.25 ± 0.11	47.3 ± 0.68
	B	145.0 ± 1.59	4.02 ± 0.15	6.28 ± 0.11	44.4 ± 1.17
TAP & 0.87%SALINE SHAM	A	145.0 ± 2.31	5.25 ± 0.16	6.25 ± 0.13	46.6 ± 0.98
	B	145.0 ± 1.71	6.03 ± 1.06	6.53 ± 0.08	46.7 ± 0.62
ADRENALECTOMIZED PH	A	135.8 ± 1.36	4.27 ± 0.15	5.65 ± 0.13	38.5 ± 1.33
	B	146.5 ± 2.06	4.70 ± 0.65	5.68 ± 0.14	41.0 ± 1.75
ADRENALECTOMIZED SHAM	A	138.4 ± 1.69	4.96 ± 0.17	5.65 ± 0.18	38.5 ± 0.88
	B	146.6 ± 1.21	5.12 ± 0.43	6.28 ± 0.14	41.0 ± 0.97

Table 3

Volume and Extent of Lesions

Animal	Area Destroyed (mm^3) Left Hemisphere	Right Hemisphere	Extent of Damage (de Groot Atlas)
tap water group			
A	0.41	1.66	A 4.0-5.0
2	0.20	0.15	A 4.4-5.2
3	0.19	0.16	A 4.6-5.2
4	0.12	0.22	A 4.2-4.8
5	0.39	0.14	A 4.0-4.6
6	0.03	0.21	A 4.2-4.8
tap & 0.87% group			
13b	Celloidin Embedded		
14b	0.82	1.96	A 4.2-4.6
15b	0.17	0.18	A 4.4-4.8
16b	1.79	0.50	A 4.2-5.0
18b	0.81	0.90	A 4.2-4.8
19b	Celloidin Embedded		
0.87 & 2.00% group			
25	0.45	1.34	A 4.0-5.0
26	0.15	0.19	A 4.0-4.8
27	Celloidin Embedded		
28	0.26	0.08	A 4.0-4.4
29	1.42	1.54	A 3.8-4.4
30	0.16	0.41	A 3.8-4.4

increased in value following the surgery but it does not appear to reach the normal values of from 45 to 50% RBC. Also with reference to the tap PH lesion group and tap and 0.87% saline solution PH lesion group there were post-lesion decreases in potassium and hematocrit values while there was a mean increase in serum sodium concentration.

Anatomical Findings

Photomicrographs of coronal sections prepared for the animals of each of the three groups utilized in this experiment are presented in the Appendix. Next to each photomicrograph is placed the corresponding de Groot atlas section. The general format identified for the histological procedural description of Experiment 2 was followed in this presentation. In that the control lesioned animals received dura punctures their brains were not retained for sectioning. The area destroyed in each hemisphere of each experimental animal that underwent frozen sectioning is given in Table 3. The extent of the lesion with reference to the de Groot atlas is also included.

The experimental group maintained on tap water in Experiment 3 evidenced lesion damage to the posterior medial hypothalamus, the dorsal premamillary nucleus and in several cases the fornix, mamillothalamic tract and ventral premamillary nucleus. There was lateral hypothalamic damage in the right hemisphere of animal #5. The mean volume damage incurred by the right hemispheres of these animals was 0.42 cubic mm and to the left hemispheres 0.22 cubic mm.

The animals of the experimental group placed on tap water and isotonic saline solution displayed lesion damage to the posterior medial hypothalamus, dorsal premamillary nucleus and in some instances

the fornix and ventral premamillary nucleus. There was third ventricle damage evident in at least two of these animals. The mean volume damage to the right hemispheres of these animals was 0.89 cubic mm and to the left hemispheres 0.90 cubic mm.

The experimental animals previously adrenalectomized and placed on isotonic and 2% saline solutions received lesion damage to the posterior medial hypothalamus and less frequently to the fornix, mamillo-thalamic tract, the dorsal premamillary nucleus and the dorsal longitudinal fasciculus. The mean volume damage to the left hemispheres of these animals was 0.49 cubic mm and to the right hemispheres 0.71 cubic mm.

In general the lesions of the experimental animals of the present study were much smaller in volume than those of the animals of Experiment 2. The mean difference across all experimental animals for the left hemispheres was 0.84 cubic mm for the animals of Experiment 2 compared with 0.49 cubic mm for the Experiment 3 subjects. Corresponding mean values for the right hemispheres were 0.74 cubic mm for the experiment 2 animals and 0.64 cubic mm for Experiment 3 animals.

Discussion

The present experiment has demonstrated that the posterior medial hypothalamus does exert some control over saline intake in rats. These findings substantiate the results of Experiment 2 concerning an approximately two-fold increase in isotonic saline solution intake following bilateral damage in this neural region. The experimental and sham groups maintained on tap water did not reveal post-operative increases in tap water intake, however, the magnitude of lesion damage

suffered by this PH lesion group was found to be less than that incurred by the other two PH lesion groups (Table 3). It is important to point out that with the PH lesion tap and 0.87% saline solution group there was likewise no change in tap water intake pre- to post-operatively. With reference to the adrenalectomized PH group again there was a significant increase in isotonic saline solution intake post-lesion but no change in 2.00% saline intake. Damage in the posterior medial hypothalamic area, therefore does not appear to affect tap water consumption in normal animals or 2.00% saline solution consumption in adrenalectomized rats but with both preparations the effect is specific to 0.87% saline solution intake. There is a corresponding increase in urine volume output accompanying the increase in isotonic saline solution ingestion.

With regard to urinary constituents there is a general decrease in concentration of the electrolytes measured for the two PH lesion groups given access to 0.87% saline. Specific gravity of the urine also decreased with these groups. The total urinary sodium excreted per day increased significantly after damage to the posterior medial hypothalamus of these two groups. This increase was in contrast to that found in Experiment 2 in which comparable groups revealed no such post-lesion increase in sodium excretion. Analysing individual animals with regard to total sodium consumed versus that excreted, the general conclusion of Experiment 2 is supported, i.e., no "salt loss syndrome" as reported by Cort (1963a). Rather it appears that the excretion of more sodium accompanies an exaggerated imbibition of isotonic saline post-operatively (refer to the individual records in the Appendix). The adrenalectomized groups recorded a very much

elevated pre-operative urinary sodium concentration of approximately 400-450 mEq/L. compared with normal animals' sodium concentration of from 200-300 mEq/L. This has been well documented by Richter (1939) and Bare (1949) who suggest that there is a decrease in sodium and chloride reabsorption from the glomerular filtrate of the kidney resulting in the excretion of increased concentrations of these ions in the urine and a decrease in the concentration of these ions in the extracellular fluids, of the body including the blood. This is supported by the blood serum sodium levels reported in this experiment.

Of additional interest is Richter's suggestion that the animal's attempt to relieve its body salt deficiency in the form of an increased saline solution intake may not be due to a learning process but rather a chemical change in the taste mechanism in the oral cavity. Whatever the physiological characteristics underlying this altered salt appetite it may be further modified by posterior medial hypothalamic lesioning resulting in even greater quantities of isotonic saline solution intake. In that adrenalectomized as well as normal animals reveal this heightened saline intake following posterior medial hypothalamic damage the adrenal corticosteriods may be eliminated as possible contributors to this effect.

The tap and 0.87% saline solution PH group of this experiment subsequently underwent a 35 day period with only tap water available in the interim between Experiment 3 and Experiment 4 and during Experiments 4 and 5. The animals survived well reducing their urinary electrolyte concentrations considerably. Once again as in Experiment 2 there were no signs of ill health as predicted by Cort (1963a) for posterior medial hypothalamic lesioned animals.

Recently Kawamura et al. (1970) have reported cellular unit recording data that may be pertinent to the interpretation of the present findings. Rats were anesthetized with nembutal and then paralyzed with Flaxedil. Data on stomach distention was reported utilizing the ballon method. One ventromedial hypothalamic neuron had a spontaneous discharge of 7 spikes/sec which rose to 25 spikes/sec during distention. This cell, however, did not respond to the application of a 6% saline solution to the tongue. Other units, particularly posterior hypothalamic cells, were very much affected by such stimulation. A cell located in the medial part of the posterior nucleus maintained a spontaneous discharge rate of 5 spikes/sec but with the application of 6% NaCl the rate accelerated to 10 spikes/sec. Electrical stimulation of the tongue also increased firing rate. In contrast a neuron at the same anterior-posterior level but in the lateral hypothalamus evidenced a spontaneous firing rate of 12 spikes/sec which dropped to 6 spikes/sec with the placement of 6% NaCl on the tongue. Electrical stimulation of the tongue in this instance decreased the firing rate of the neuron. There was no response by either neuron when a 1% NaCl solution was placed on the tongue. The saline solutions were rinsed off with water between tongue applications.

Kawamura's results suggest that information from tongue receptors may be channeled directly to the posterior medial and lateral hypothalami concerning salinity concentrations of solutions present in the mouth. Also of interest is the finding that a 1% NaCl solution applied to the tongue resulted in no change with regard to unit discharge activity in these neural regions. It may be that damage inflicted upon the posterior medial hypothalamus in some way elevates existing thresholds governing the quantity of isotonic saline imbibed by rats.

EXPERIMENT 4

Pitressin Influence upon the Experimental Lesion

This experiment investigated the effect of subcutaneous pitressin injections upon the increased fluid intake revealed by posterior medial hypothalamic lesioned animals. There was concern over the possibility of extreme body hydration due to the interaction of pitressin tannate and the exaggerated saline intake. Therefore between post-operative day 20 of Experiment 3 and the first pitressin injection of this study a period of ten days with the non-adrenalectomized animals on tap water was imposed. The adrenalectomized groups were maintained on 0.87% saline solution during this period. Although an increased tap water intake was exhibited by some of the non-adrenalectomized animals the possibility of hyperhydration due to saline ingestion was reduced.

Subjects

The animals of Experiment 3 were employed in this experiment. Their maintenance in metabolism cages was continued and closely approximated the conditions of Experiment 3. The animals were of course older at the initiation of this experiment, approximately 170 days of age.

Procedure

The animals were kept in the same groups as assigned in Experiment 3. On post-lesion day 31, 4 hour readings were begun at 0100. These reading periods obeyed the format described in earlier experiments with the recording of metabolic intake-output measures and the retaining of a urine sample from each animal at each reading period. Urine analysis was also characteristic of the established procedure. Immediately following the 2100 hour reading period of post-operative day 31 one-

half of the animals received a subcutaneous 0.2 U/100 grams dosage[2] of pitressin tannate in oil (Pard-Davis & Company). The other half received an equivalent volume of pure peanut oil. Assignment of the animals to one of the two categories conformed with the following procedure. Each of the three groups had previously been randomly seperated into six control animals which received sham lesions and six experimental animals which received posterior medial hypothalamic lesions. During this experiment, within each of these two subgroups the animals were further randomly divided into three experimental injection animals, to receive pitressin, and three control injection animals to receive peanut oil. During post-lesion day 32, 4 hour readings continued; these readings followed the injections. At the completion of post-lesion day 32 the animals were allowed a three day recovery period during which time metabolic intake-output measures were recorded twice daily at 0800-0830 and 2000-2030. A urine sample was retained and analyzed for each animal at the morning reading period.

On post-lesion day 36, 4 hour readings were once again initiated in the same fashion as five days earlier. Immediately following the 2100 hours reading period subcutaneous injections of pitressin were administered those animals that had earlier served as control injection subjects and an equal volume of peanut oil was provided those animals that had earlier served in the pitressin injection group. In this way each animal received a pitressin injection and a control injection separated by five days. Experimental and control data were thus collected for each animal.

[2]This pitressin tannate dosage represents a compromise between the dosages found to be effective for rats by other investigators (Lubar et al, 1969; Morrison et al., 1967; Smith and McCann, 1964).

Results

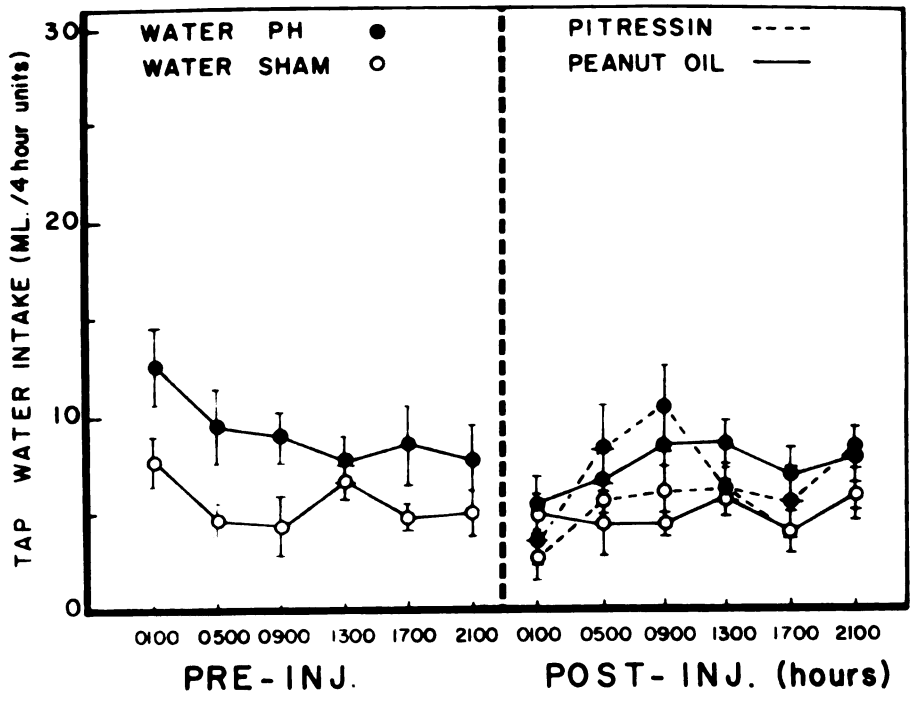
The four hour reading period data for the 24 hour period preceding injections and the 24 hour period immediately following the injections are included in the figures of this section. Each point in these figures represents the mean of the six animals of the designated group. Standard errors of the means are also provided.

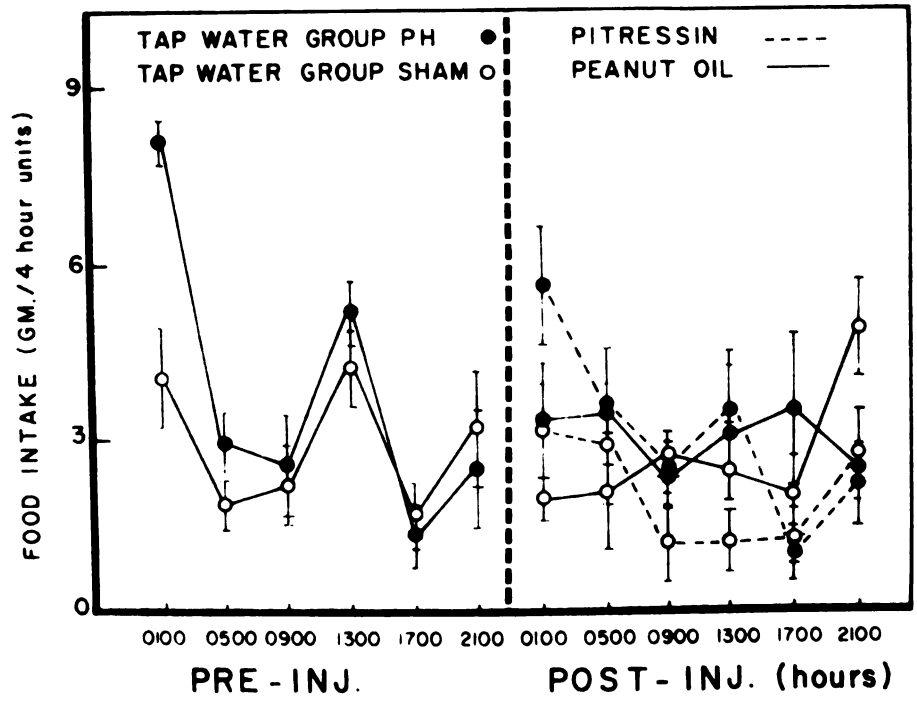
The procedure called for a counter balanced design, i.e., one-half of a group of six animals received the experimental treatment during one 5 day period and the alternate half of the group received the experimental treatment during the next 5 day period. Basal rate data during the pre-injection 24 hour period was thus collected twice for a given group. These pre-injection data were combined for each group for each 4 hour reading period. During the post-injection 24 hour period the results of the pitressin injection are compared with the results derived from the control injection of peanut oil for each group.

Figure 60 represents the tap water intake recorded for the two groups of animals maintained on tap water only through Experiment 3. During the pre-injection 24 hour period there were significant differences between the posterior medial hypothalamic lesioned group and the sham lesioned group with regard to tap water intake ($t=4.44$, $p<0.01$). The PH lesion group yielded a mean of 8.7 ml/4 hour units and the sham group 5.4 ml. Comparing within groups for the pre- and post-peanut oil injection periods there were no differences (t for related measures= 1.60 and 0.52, PH and sham groups respectively, $p>0.10$). There were likewise no differences between the pre- and post-pitressin injection periods within groups (t for related measures=0.67 and 0.33, PH and sham groups respectively, $p>0.10$). There was a difference between

Figure 60. Mean (± S.E.) tap water intake in 4 hour units exhibited by the posterior medial hypothalamic and sham lesioned groups 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 61. Mean (± S.E.) food consumption in 4 hour units for the posterior medial hypothalamic and sham lesioned groups maintained on tap water 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


WATER PH
WATER SHAM
PITRESSIN
PEANUT OIL
TAP WATER INTAKE (ML./4 hour units)
30
20
10
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


TAP WATER GROUP PH
TAP WATER GROUP SHAM
PITRESSIN
PEANUT OIL
FOOD INTAKE (GM./4 hour units)
9
6
3
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

the two groups comparing their reactions to pitressin treatment ($t=3.87$, $p<0.01$). The PH lesion group revealed a mean of 7.12 ml/4 hour units while the sham lesion group's mean was 4.99.

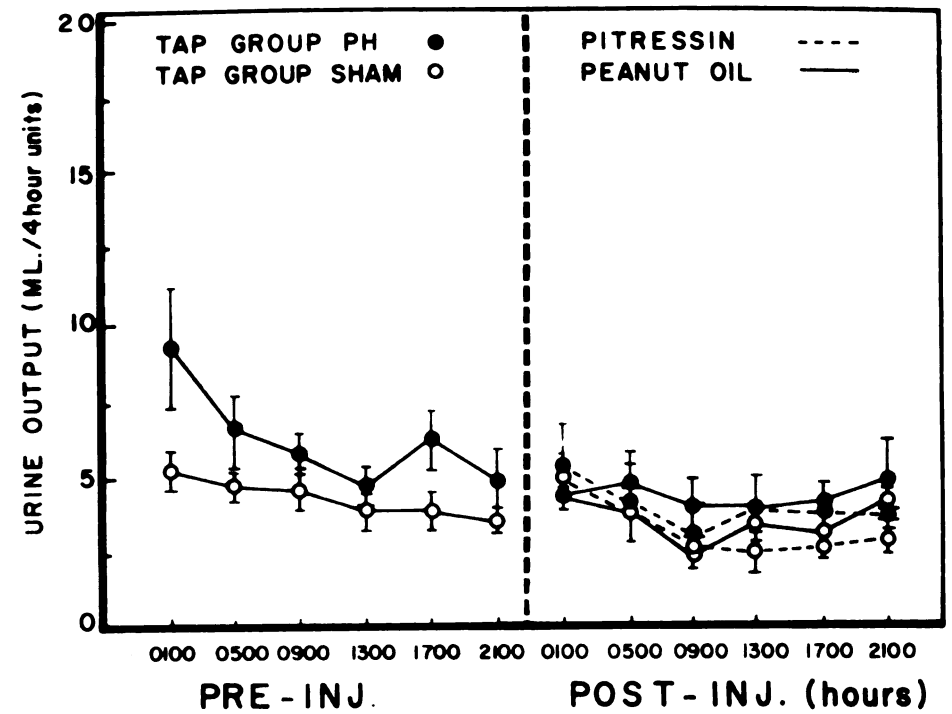
Figure 61 considers food intake for the two groups maintained on tap water. During the pre-injection 24 hours there were no differences between the PH and sham lesioned groups ($t = 1.69$, $p>0.10$). Comparing each group's food intake during pre- and post-peanut oil injection periods there were non-significant differences (t for related measures= 0.87 and 0.10, PH and sham lesion groups respectively, $p>0.10$). Considering each group's reaction to pitressin injection the PH group revealed no change (t for related measures=1.38, $p>0.10$) and the sham lesioned group indicated no difference (t for related measures= 1.38, $p>0.10$). Comparing the two groups during the post-pitressin period the PH group registered a significantly higher food intake than the sham lesioned group ($t=2.28$, $p<0.05$) while the groups were not different during this period following peanut oil injection ($t=0.57$, $p>0.10$).

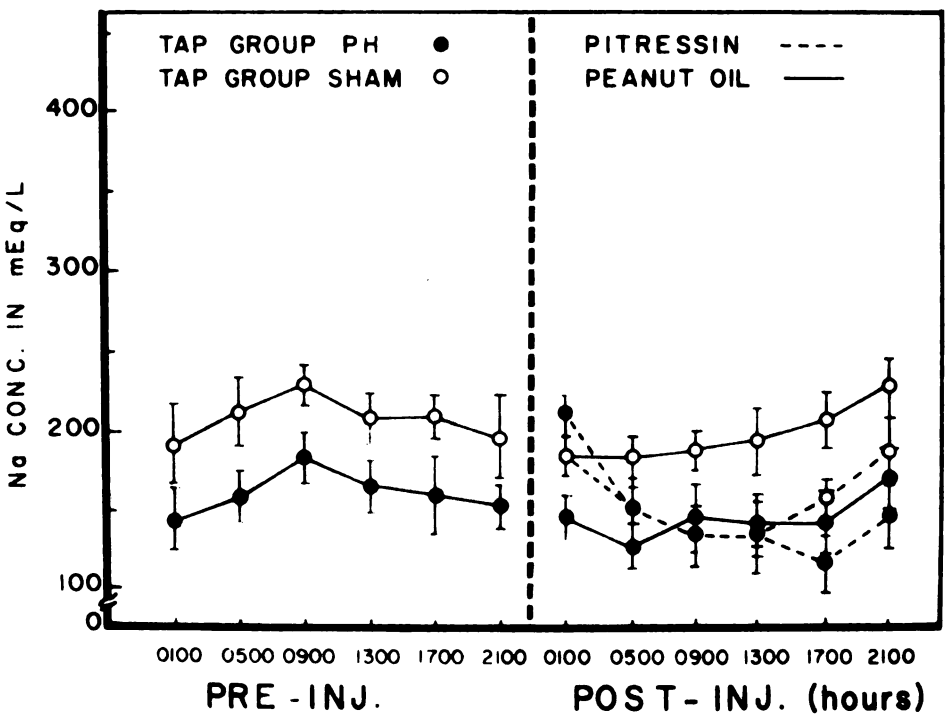
Figure 62 considers urine volume output of the two tap water groups. No differences existed between or within the groups during the post-injection period. Pre-injection the experimental and sham groups were significantly different ($t=4.30$, $p<0.01$). The PH lesioned group's mean urine volume output was 6.10 and the sham lesioned group's mean was 4.22 ml.

Figure 63 indicates the sodium concentrations of the urine in mEq/L. during these two 24 hour periods for the groups maintained on tap water. There were pre-injection differences between the two groups ($t=7.33$, $p<0.01$) with the PH group yielding a mean concentration of

Figure 62. Mean (± S.E.) urine volume excretion in 4 hour units exhibited by the posterior medial hypothalamic and sham lesioned groups maintained on tap water, for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 63. Mean (± S.E.) urinary sodium concentration in 4 hour units for the posterior medial hypothalamic and sham lesioned groups provided tap water, for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


TAP GROUP PH
TAP GROUP SHAM
PITRESSIN
PEANUT OIL
URINE OUTPUT (ML./4hour units)
20
15
10
5
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


TAP GROUP PH
TAP GROUP SHAM
PITRESSIN
PEANUT OIL
Na CONC. IN mEq/L
400
300
200
100
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

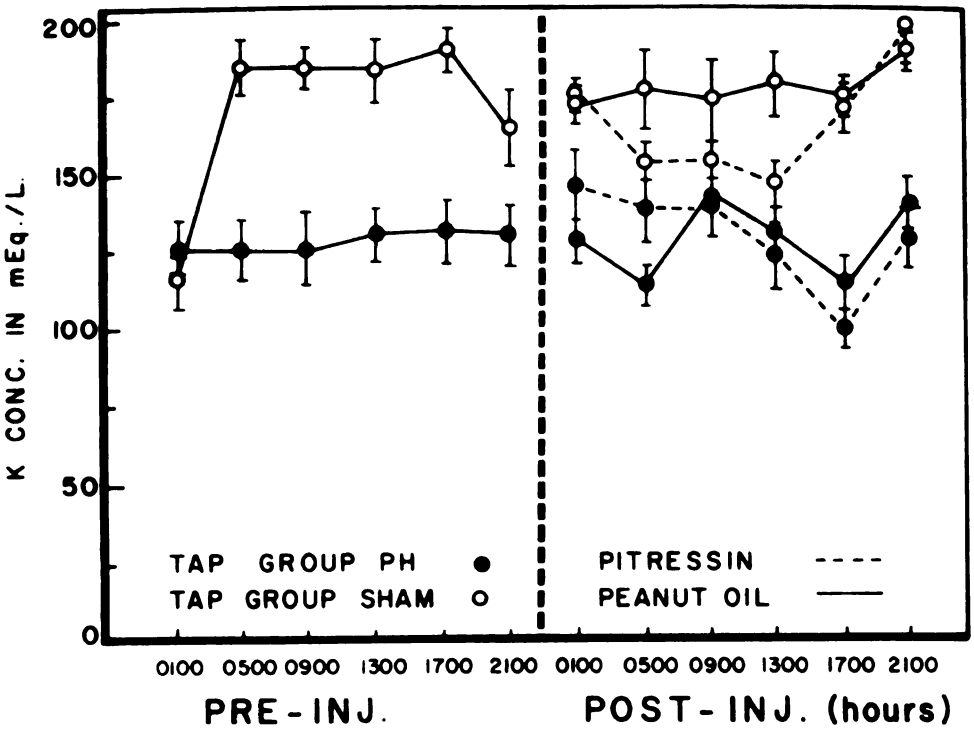
155.75 mEq/L. and the sham lesioned group 204.25 mEq/L. Each group revealed no change comparing pre- and post-peanut oil injection (t for related measures=0.38 and 0.83, PH and sham groups respectively, $p>0.10$). The PH group also registered no change comparing pre- and post-pitressin injection (t for related measures=0.84, $p>0.10$) and the sham lesion group also demonstrated no significant change in sodium concentration during the post-pitressin injection period (t for related measures= 0.73, $p>0.10$). Comparing the two groups during the post-pitressin injection period there were differences indicated ($t=2.73$, $p<0.05$) with the PH group yielding a mean of 147.83 mEq/L. and the sham group 178.83.

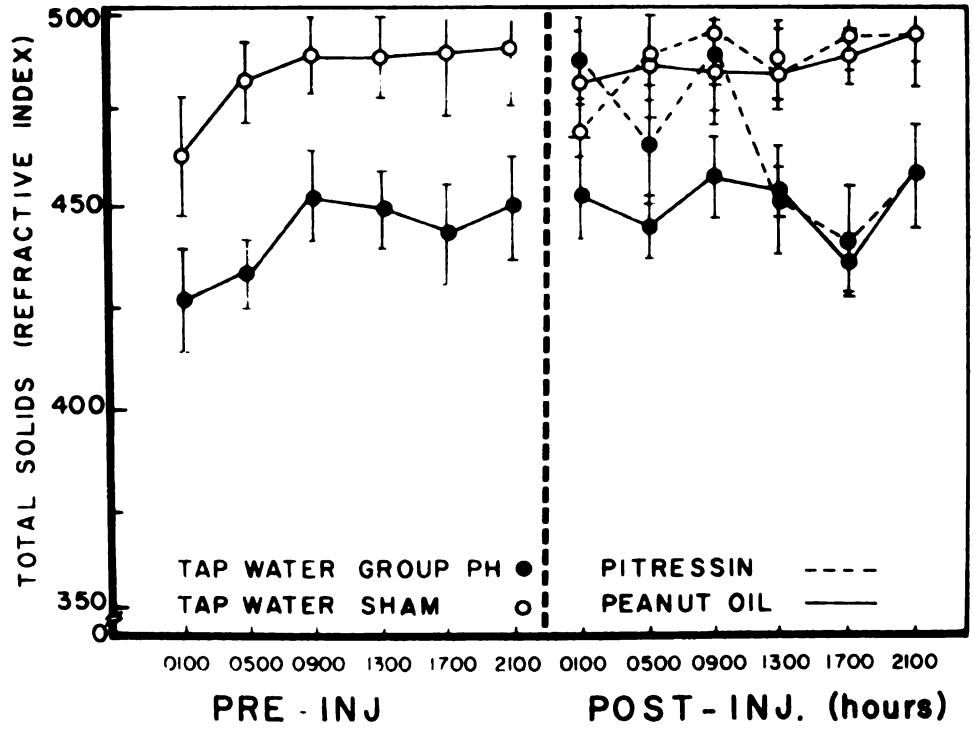
Figure 64 considers the urine potassium concentrations registered by the two tap water groups during the periods under discussion. The groups were significantly different from one another on this measure during the pre-injection period ($t=7.77$, $p<0.01$). The PH lesion group indicated a mean of 128.00 mEq/L. while the sham group revealed a mean of 180.00 mEq/L. potassium. Each group registered no difference comparing pre- and post-peanut oil injection periods (t for related measures=0.87 and 0.89, PH and sham groups respectively, $p>0.10$). This was also true for each group comparing pre- and post-pitressin injection periods (t for related measures=0.15 and 0.55, PH and sham lesion groups respectively, $p>0.10$). Comparing the two groups during the post-pitressin injection period there was a significant difference ($t=3.81$, $p<0.01$) with the PH group yielding a mean of 131.50 and the sham group 168.33 mEq/L.

Figure 65 concerns the urine total solids measures registered for each of the two groups kept on tap water. There was a significant difference between the two groups during the pre-injection period

Figure 64. Mean (± S.E.) urinary potassium concentration in 4 hour units for the posterior medial hypothalamic and sham lesioned groups maintained on tap water, for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 65. Mean (± S.E.) urinary specific gravity in 4 hour units for the posterior medial hypothalamic and sham lesioned groups maintained on tap water, for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


200
150
100
50
0
K CONC. IN mEq./L.
TAP GROUP PH ●
TAP GROUP SHAM o
PITRESSIN ----
PEANUT OIL ——
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

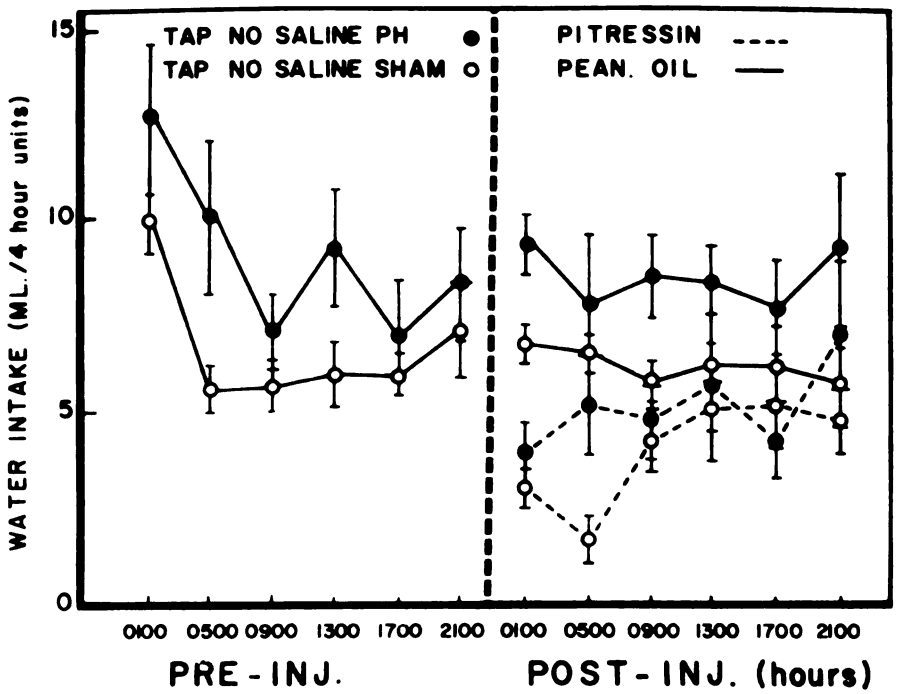

500
450
400
350
0
TOTAL SOLIDS (REFRACTIVE INDEX)
TAP WATER GROUP PH ●
TAP WATER SHAM o
PITRESSIN ----
PEANUT OIL ——
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ
POST-INJ. (hours)

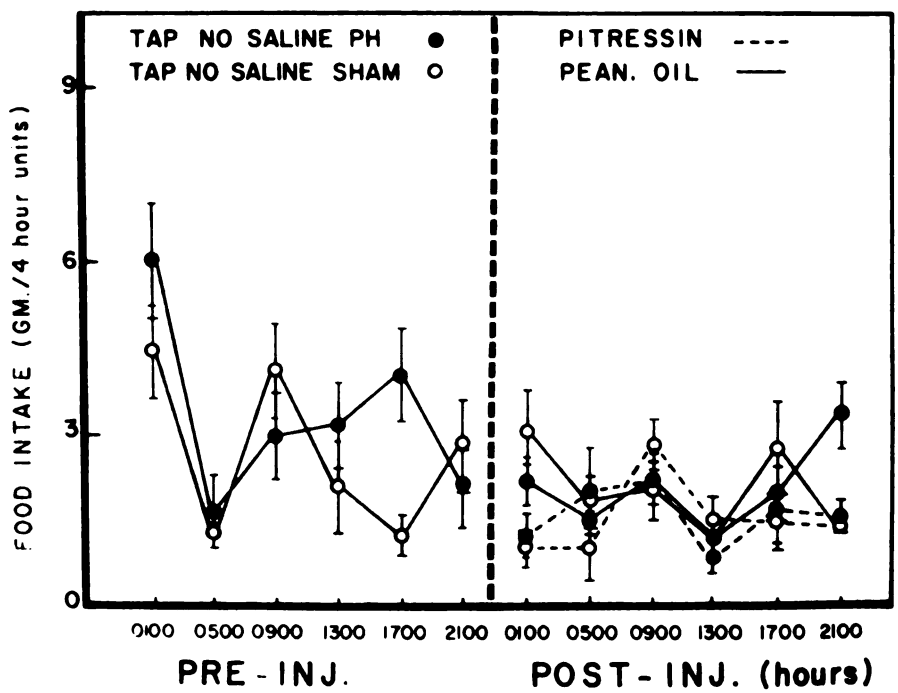
(t=8.75, p<0.01) with the PH group registering a mean of 1.3442 specific gravity and the sham group 1.3485. Comparisons for each group concerning pre- and post-peanut oil injections resulted in a significant difference for the PH lesioned group but not for the sham group (t for related measures=2.43, and 1.96, p<0.01, p>0.05, PH and sham lesioned groups respectively). There was no difference for the sham group pre- to post-pitressin injection (t for related measures=1.71, p>0.10) nor did the PH group reveal a difference (t for related measures=1.77, p>0.10). Although not significant there were urine specific gravity increases for the PH group following pitressin injection that eventially decreased until by 1300 hours, i.e., 16 hours after injection, the measures were equal with pre-injection values. The two groups were significantly different comparing their post-pitressin injection periods (t=2.77, p<0.05) with the PH group yielding a mean specific gravity of 1.3465 and the sham group 1.3489.

Figure 66 begins an analysis of the two groups maintained on tap water and isotonic saline solution during Experiment 3 but placed on tap water only during the present experiment. This figure concerns the water intake recorded for these groups once again during pre- and post-injection periods. Differences existed between the two groups comparing the pre-injection period (t=3.24, p<0.01). The PH group had a mean water intake of 8.88 ml/4 hour periods, the sham group 6.89. Comparing pre- and post-peanut oil injection periods for each group there were no differences (t for related measures= 1.03 and 1.20, PH and sham groups respectively, p>0.10). However

Figure 66. Mean (± S.E.) tap water consumption in 4 hour units exhibited by the posterior medial hypothalamic and sham lesioned groups provided tap water, no saline for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 67. Mean (± S.E.) food intake in 4 hour units for the posterior medial hypothalamic and sham lesioned groups maintained on tap water, no saline for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


TAP NO SALINE PH
TAP NO SALINE SHAM
PITRESSIN
PEAN. OIL
WATER INTAKE (ML./4 hour units)
15
10
5
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


TAP NO SALINE PH
TAP NO SALINE SHAM
PITRESSIN
PEAN. OIL
FOOD INTAKE (GM./4 hour units)
9
6
3
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

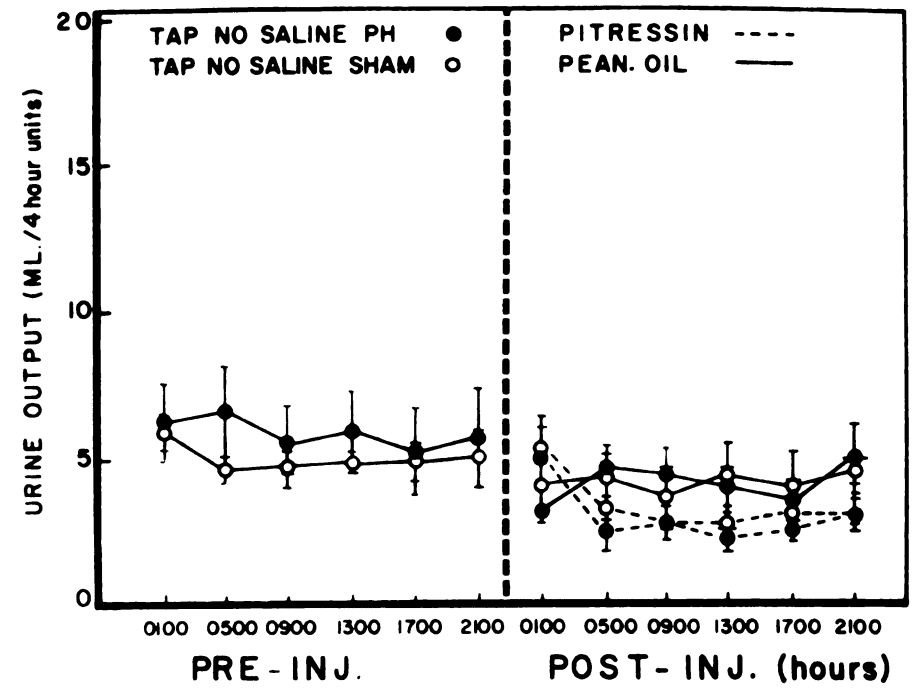
comparing the groups for pre- and post-pitressin injection periods each revealed significant decreases in water intake (t for related measures=2.87 and 2.77, PH and sham respectively, $p<0.05$) with the PH group dropping from a mean of 8.88 ml/4 hour units to 5.08. The sham group dropped from 6.89 to 3.97 ml/4 hour units. No differences existed between the two groups comparing their post-pitressin injection periods (t=1.68, $p>0.10$).

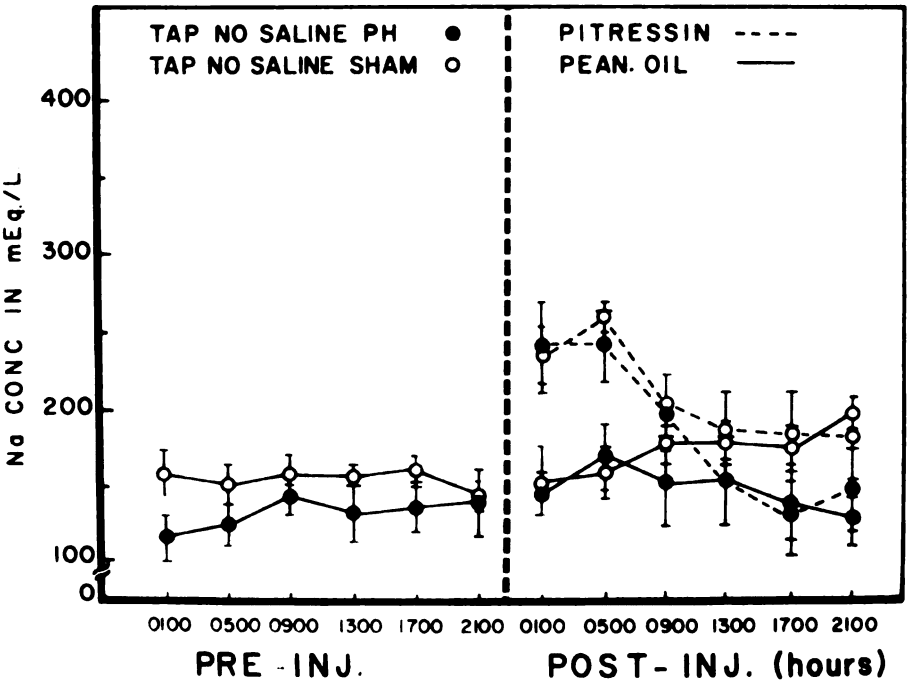
Figure 67 concerns food intake for the two groups under consideration. Few differences existed within or between the groups for the comparisons tested. There were significant drops in food intake pre- to post-pitressin injection (t for related measures=2.27, $p<0.05$); the PH group changed from 3.27 gm/4 hour units to 1.56 post-injection. The sham group's means dropped from 2.45 grams food/4 hour units to 1.53 post-injection however they were not significantly different (t for related measures=1.60, $p>0.10$).

Figure 68 represents the urine volume output for these two groups. Again there were no differences between or within the two groups comparing pre- and post-peanut oil injection periods. Each group did reveal significant decreases in urine output comparing pre- and post-pitressin injection periods (t for related measures=5.32 and 5.84, PH and sham groups respectively, $p<0.01$). This represents a mean drop of from 5.81 to 2.77 ml/4 hour units for the PH group and 5.22 to 3.24 ml/4 hour units for the sham group. There were differences comparing the two groups during the post-pitressin injection period (t=2.97, $p<0.01$). This represents a mean difference of 2.77 ml/4 hour units for the PH group and 3.24 for the sham group.

Figure 68. Mean (± S.E.) urine volume excretion in 4 hour units for the posterior medial hypothalamic and sham lesioned groups provided tap water, no saline for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 69. Mean (± S.E.) urinary sodium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned groups maintained on tap water, no saline for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


TAP NO SALINE PH
TAP NO SALINE SHAM
PITRESSIN
PEAN. OIL
URINE OUTPUT (ML./4 hour units)
20
15
10
5
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

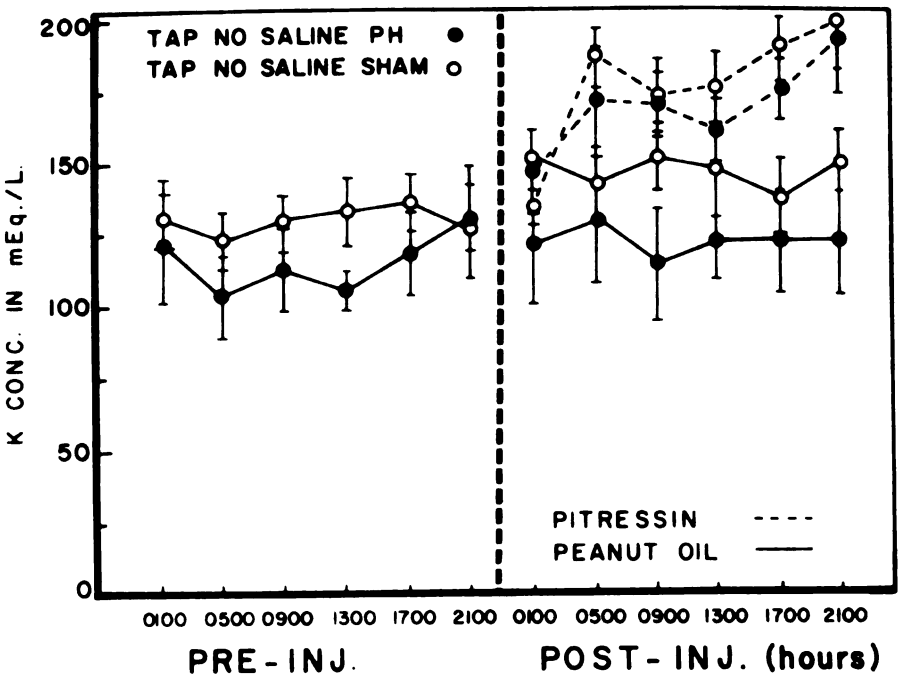

TAP NO SALINE PH
TAP NO SALINE SHAM
PITRESSIN
PEAN. OIL
Na CONC IN mEq/L
400
300
200
100
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

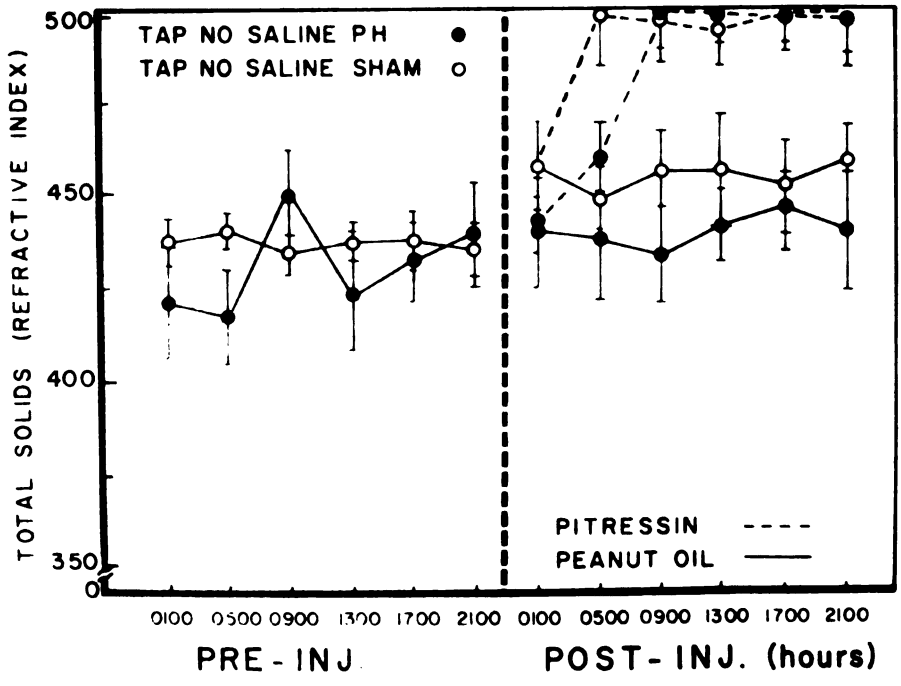
Looking now at the urine sodium concentration for these two groups (Figure 69) there were differences between the groups during the pre-injection period ($t=3.89$, $p<0.01$). The PH group revealed a mean of 133.08 mEq/L. while the sham group's mean was 156.92. Each group demonstrated no differences comparing pre- and post-peanut oil injection periods (t for related measures=1.90 and 1.99, PH and sham groups respectively, $p>0.05$). Comparing each group's pre- and post-pitressin injection period there were differences (t for related measures=2.90 and 3.47, PH and sham groups respectively, $p<0.01$). The PH group increased its sodium concentration from a pre-injection mean of 125.16 to 182.17 mEq/L. The sham group's corresponding means were from 153.33 to 208.67 mEq/L. By approximately 1300 hours or 16 hours after pitressin injection the PH group's urine sodium concentration had decreased to its pre-injection level. Comparing the two groups during their post-pitressin injection periods they were different ($t=4.82$, $p<0.01$) with the PH group indicating a mean of 182.17 mEq/L. and the sham group 208.67.

Figure 70 considers the urine potassium concentration of these two groups. There were differences between the two groups for the pre-injection period ($t=4.22$, $p<0.01$) with the PH group yielding a mean of 115.03 and the sham group 132.33 mEq/L. Comparing each group with respect to pre- and post-peanut oil injection periods there was no difference for the PH group (t for related measures= 1.03, $p>0.10$). The sham group, however, revealed a significant difference (t for related measures=5.17, $p<0.01$). There was a mean change of from 134.33 mEq/L. pre-injection to 149.17 post-injection.

Figure 70. Mean (± S.E.) urinary potassium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned groups maintained on tap water, no saline for the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 71. Mean (± S.E.) urinary specific gravity in 4 hour units for the groups maintained on tap water, no saline, medial posterior hypothalamic and sham lesioned for the 24 hour period prior to and the 24 hours following independent applications of pitressin and peanut oil injections.


TAP NO SALINE PH ●
TAP NO SALINE SHAM o
K CONC. IN mEq./L.
200
150
100
50
0
PITRESSIN ----
PEANUT OIL ——
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


TAP NO SALINE PH ●
TAP NO SALINE SHAM o
TOTAL SOLIDS (REFRACTIVE INDEX)
500
450
400
350
0
PITRESSIN ----
PEANUT OIL ——
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

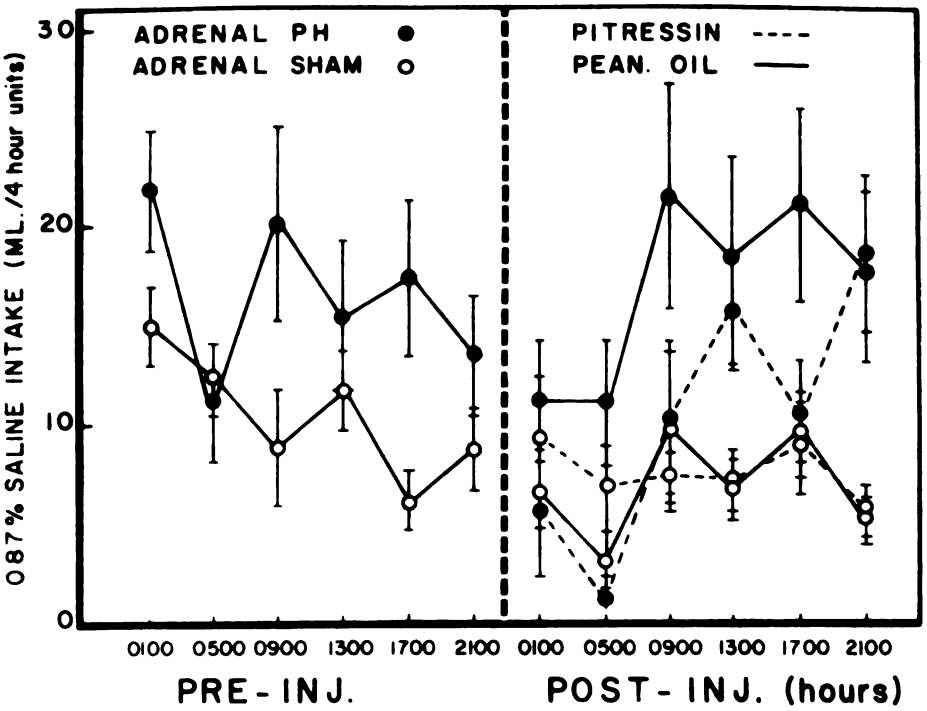
Comparing each group with regard to pre- and post-pitressin injection periods both indicated differences (t for related measures=6.22 and 8.65, PH and sham respectively, $p<0.01$). The PH group changed from a pre-injection mean of 115.03 to a post-injection mean of 170.17 mEq/L. while the corresponding change in the sham group was from a mean of 132.33 to 181.97 mEq/L. post-injection.

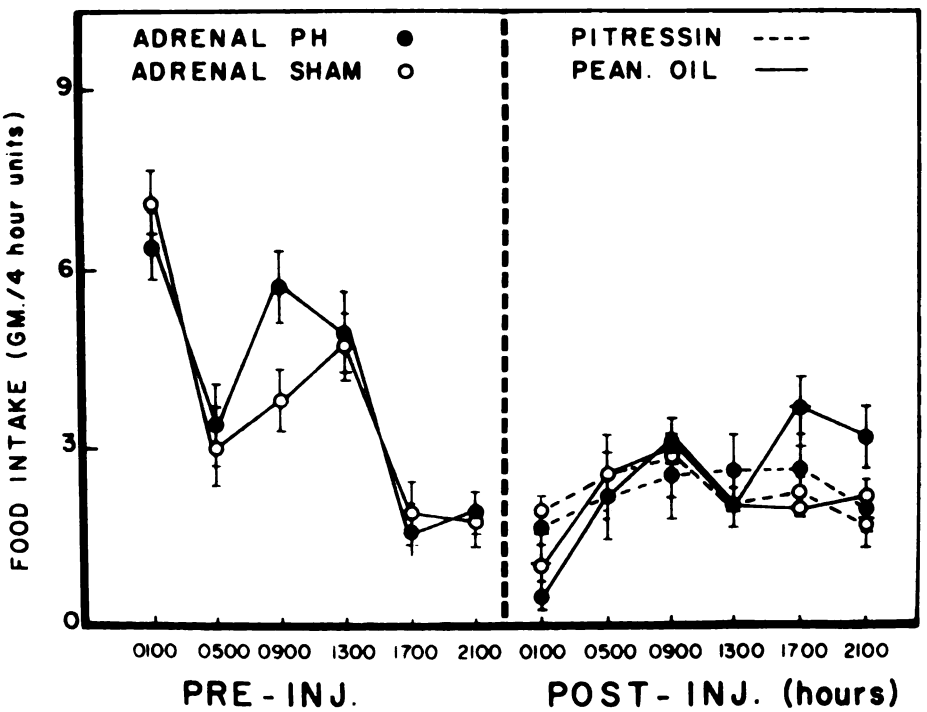
The two groups were different from one another during the post-peanut oil injection period ($t=6.25$, $p<0.01$) with the PH group yielding a mean of 122.33 and the sham group 149.33 mEq/L. During the post-pitressin injection period they were also different ($t=4.05$, $p<0.01$) with the PH group revealing a mean of 170.17 and the sham group's mean was 181.97 mEq/L. During the post-pitressin 24 hour period neither group revealed a tendency to begin falling back to the urine potassium level registered during the pre-injection periods.

Figure 71 concerns the urine total solids recorded for each group during the indicated observation periods. There were pre-injection differences for the two groups ($t=3.89$, $p<0.01$). The PH group had a mean of 1.3425 and the sham group's urine specific gravity mean was 1.3437. Each group similarly registered differences comparing pre- and post-peanut oil injection periods (t for related measures=7.14 and 7.53, PH and sham groups respectively, $p<0.01$). The PH group's mean changed from 1.3425 pre-injection to 1.3439 post-injection while the sham group changed from 1.3437 to 1.3455. Within each group there were significant changes comparing pre- and post-pitressin injection periods (t for related measures=5.44 and 7.10, PH and sham groups respectively, $p<0.01$). The PH group

Figure 72. Mean (± S.E.) isotonic saline consumption in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups maintained on 0.87% saline solution, during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 73. Mean (± S.E.) food consumption in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups maintained on 0.87% saline solution during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


ADRENAL PH
ADRENAL SHAM
PITRESSIN
PEAN. OIL
087% SALINE INTAKE (ML./4 hour units)
30
20
10
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


ADRENAL PH
ADRENAL SHAM
PITRESSIN
PEAN. OIL
FOOD INTAKE (GM./4 hour units)
9
6
3
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

changed from a mean of 1.3425 pre-injection to 1.3492 post-injection. Corresponding alterations for the sham group were from 1.3437 to 1.3504. Comparing the two groups during post-pitressin injection periods they were different ($t=2.10$, $p<0.05$). And they were different during the post-peanut oil injection periods ($t=4.95$, $p<0.01$).

With Figure 72 begins an analysis of the results derived from the two adrenalectomized groups maintained on isotonic saline during this experiment. In this figure 0.87% saline solution intake is considered. There were differences between the two groups during the pre-injection period ($t=3.69$, $p<0.01$). The PH group yielded a mean of 16.54 ml/4 hour units while the sham group's mean was 10.42. Comparing each group during pre- and post-peanut oil injection periods there were no significant changes (t for related measures=0.05 and 1.62, PH and sham groups respectively, $p>0.10$). The sham group revealed no change comparing pre- and post-pitressin injection periods (t for related measures=1.86, $p>0.05$). The PH group revealed a non-significant decrease in isotonic saline intake (t for related measures= 1.37, $p>0.10$) of from a mean of 16.45 to 10.53 ml/4 hour units. There was no difference between the two groups comparing the post-pitressin injection period ($t=1.46$, $p>0.10$).

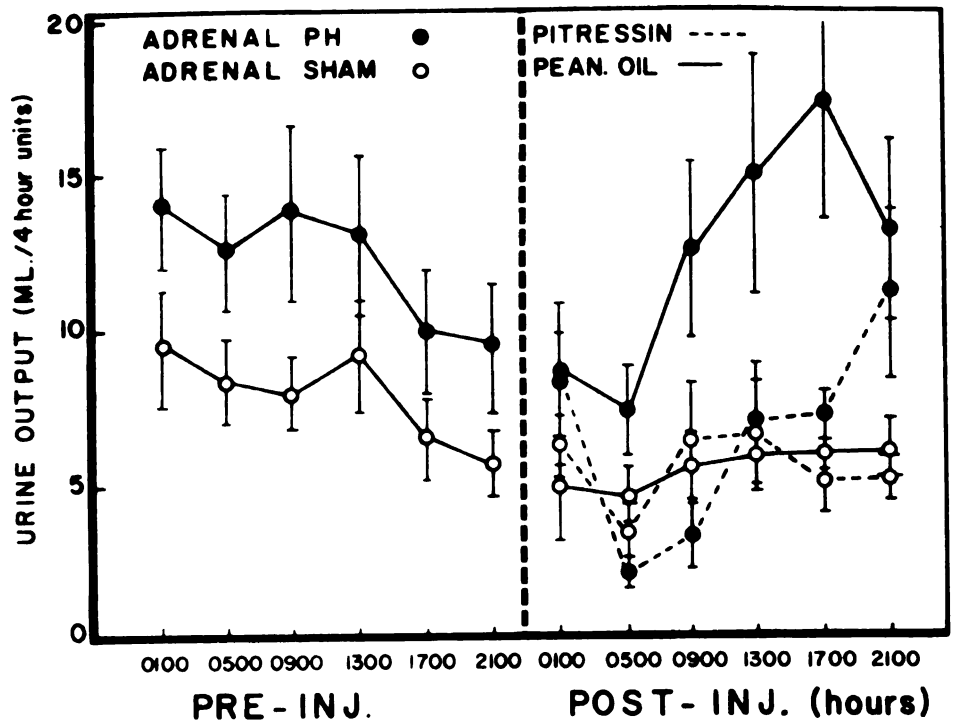
Figure 73 considers the food intakes of these groups. Comparing the groups during the pre-injection periods there was no difference ($t=0.08$, $p>0.10$) with the PH group yielding a mean intake of 3.86 gm/4 hour units and the sham group 3.82. Each group registered non-significant decreases in food intake comparing pre- and post-peanut oil injection periods (t for related measures=1.15 and 1.38, PH and sham groups respectively, $p>0.10$). For the PH group there was a mean

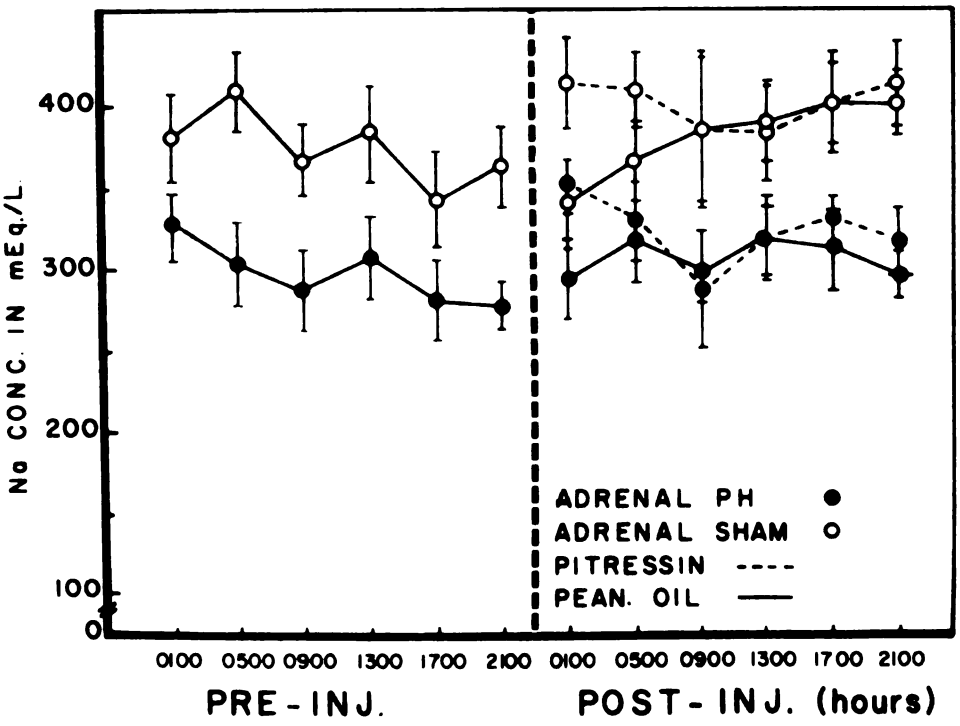
drop of from 3.86 gm/4 hour units pre-injection to 2.56 post-injection. Corresponding values for the sham group were 3.82 to 2.08 gm/4 hour units. There were also non-significant drops for each group comparing pre- and post-pitressin injection periods (t for related measures=1.23 and 1.54, PH and sham groups respectively, $p>0.10$). These changes were from a pre-injection mean of 3.86 to a post-injection mean of 2.33 gm/4 hour units for the PH group and from 3.82 to 2.47 for the sham group. The two groups were not different from one another during the post-peanut oil injection period ($t=1.34$, $p>0.10$) or during the post-pitressin injection period ($t=0.78$, $p>0.10$).

Figure 74 concerns the urine volume output of the two adrenalectomized groups. Comparing the groups during the pre-injection periods there was a significant difference ($t=6.10$, $p<0.01$). The PH group's mean was 12.19 ml/4 hour units while the mean of the sham group was 7.72. Comparing each group during pre- and post-peanut oil injection periods there was no difference for the PH group (t for related measures=0.05, $p>0.10$) with a pre-injection mean of 12.19 and post-injection of 12.44 ml/4 hour units. The sham group did indicate a significant difference comparing these periods (t for related measures=2.65, $p<0.05$). The mean change was from 7.72 pre-injection to 5.61 ml/4 hour units post-injection. Comparing within each group regarding pre- and post-pitressin injection periods there was a significant decrease in urine output registered from the PH group (t for related measures=2.73, $p<0.05$) representing a mean change of from 12.19 to 6.85 ml/4 hour units. While the sham group also indicated a significant change (t for related measures=3.71, $p<0.01$)

Figure 74. Mean (± S.E.) urine volume excretion in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 75. Mean (± S.E.) urinary sodium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


ADRENAL PH
ADRENAL SHAM
PITRESSIN ----
PEAN. OIL
URINE OUTPUT (ML./4 hour units)
20
15
10
5
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


Na CONC. IN mEq./L.
400
300
200
100
0
ADRENAL PH
ADRENAL SHAM
PITRESSIN ----
PEAN. OIL
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)

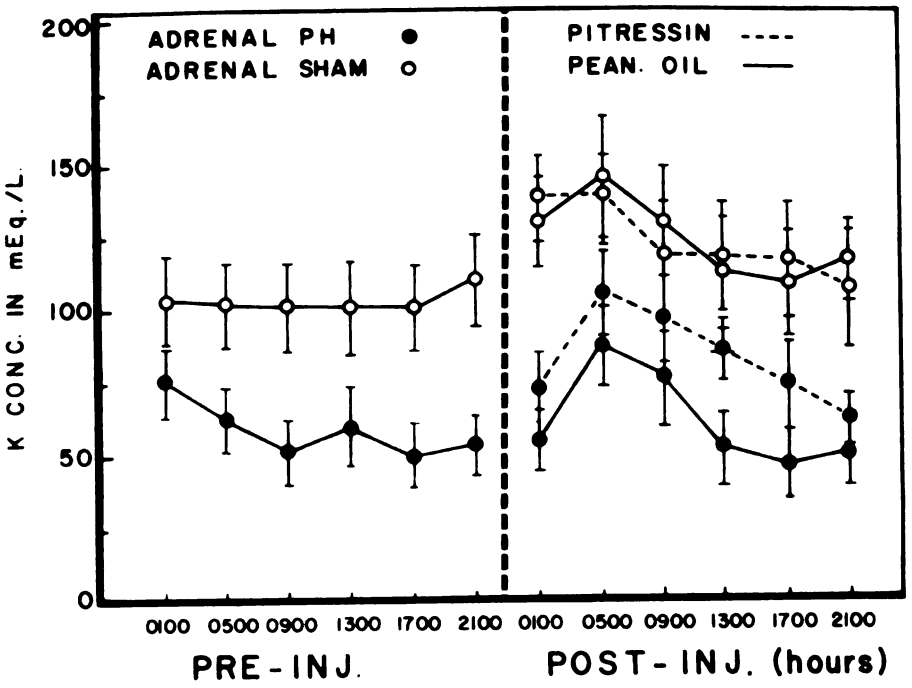
with a mean change of from 7.72 ml/4 hour units to 547 post-injection. The two groups differed significantly from one another during the post-peanut oil injection period ($t=4.85$, $p<0.01$) but not during the post-pitressin injection period ($t=1.07$, $p>0.10$).

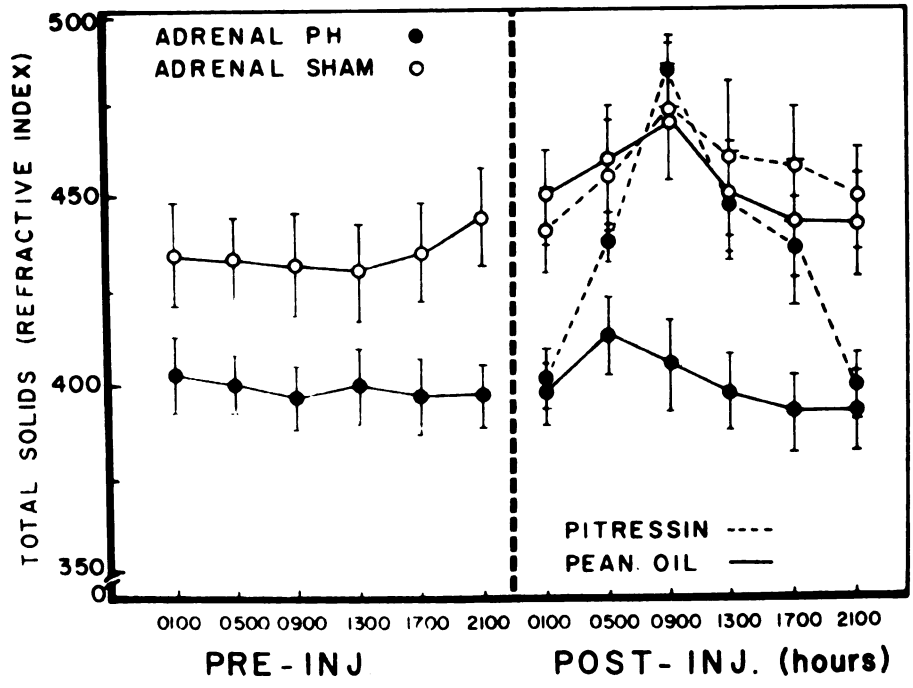
Figure 75 concerns the urine sodium concentrations recorded for the adrenalectomized groups for the periods under consideration. During the pre-injection period the two groups differed significantly from one another ($t=8.80$, $p<0.01$) with the PH group yielding a mean of 298.75 mEq/L. and the sham group 374.83. Comparing each group during pre- and post-peanut oil injection periods there was no difference (t for related measures=0.92 and 0.31, PH and sham respectively, $p>0.10$). There were differences within groups comparing pre- and post-pitressin injection periods (t for related measures= 3.54 and 2.38, PH and sham groups respectively, $p<0.01$, $p<0.05$). This represents a mean change of from 298.75 mEq/L. to 323.83 post-injection for the PH group and corresponding values for the sham group were 374.83 and 402.67. There was a difference between the two groups comparing post-pitressin injection periods ($t=12.21$, $p<0.01$). There was also a difference between the two groups for the post-peanut oil injection periods ($t=7.76$, $p<0.01$).

Figure 76 considers the urine potassium concentrations for the adrenalectomized groups. During the pre-injection period there were significant differences between the two groups ($t=11.44$, $p<0.01$). The PH group's mean was 59.42 mEq/L. while the sham group's mean was 107.33. Comparing each group during pre- and post-peanut oil injection there were no differences indicated for the PH group (t for related

Figure 76. Mean (± S.E.) urinary potassium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.

Figure 77. Mean (± S.E.) urinary specific gravity in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours following independent applications of pitressin and peanut oil injections.


ADRENAL PH ●
ADRENAL SHAM ○
PITRESSIN ----
PEAN. OIL ——
K CONC. IN mEq./L.
200
150
100
50
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ.
POST-INJ. (hours)


ADRENAL PH ●
ADRENAL SHAM ○
TOTAL SOLIDS (REFRACTIVE INDEX)
500
450
400
350
0
PITRESSIN ----
PEAN. OIL ——
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-INJ
POST-INJ. (hours)

measures=0.01, p>0.10). However, the sham group revealed a significant difference (t for related measures=2.06, p<0.05) with a mean change of from 107.33mEq/L. pre-injection to 126.00 post-injection. There was a difference for the sham group comparing pre- and post-pitressin injection periods (t for related measures=4.14, p<0.01). The PH group indicated an increase in potassium concentration (t for related measures=3.65, p<0.01) from a pre-injection mean of 59.42 mEq/L. to 83.67 post-injection. Comparing the two groups during the post-peanut oil injection period there were significant differences (t=21.83, p<0.01) between the groups which were also apparent concerning the post-pitressin injection period (t=6.54, p<0.01).

Figure 77 inspects the urinary total solids measures taken on the adrenalectomized groups during the pre- and post-injection 24 hour periods. Comparing the groups during the pre-injection period there were significant differences (t=12.26, p<0.01) with the PH group revealing a mean of 1.3398 specific gravity and the sham group 1.3436. With reference to pre- and post-peanut oil injection periods the PH group indicated no difference while the sham group did indicate a difference (t for related measures=0.10 and 2.91, PH and sham groups respectively, p>0.10, p<0.01). The mean change for the sham group was from 1.3436 pre-injection to 1.3455 post-injection. Comparing pre- and post-pitressin injection periods for the PH and sham groups there were significant differences registered (t for related measures=3.36 and 3.93, PH and sham groups respectively, p<0.01). For the PH group this was a mean change of from 1.3398 specific gravity to 1.3435 post-injection. For the sham group the change was from 1.3436 to 1.3459

post-injection. There were subsequent fall backs for each group to their respective pre-injection levels by 2100 hours post-injection. Comparing the two groups with respect to post-peanut oil injection periods there were significant differences ($t=22.98$, $p < 0.01$). There was also a significant difference between groups comparing post-pitressin injection periods ($t=3.04$, $p < 0.01$). Between 0100 and 0900 hours the PH group's urinary specific gravity rose quickly from 1.3404 to 1.3482. Subsequently it fell back until by 2100 hours post-injection the measure was 1.3403.

Discussion

This experiment was included as a means of evaluating the effect of exogenous pitressin upon the increased fluid intake revealed by posterior medial hypothalamic lesioned animals. The sham and experimental lesioned groups originally maintained on tap water thus offer little in the way of meaningful data in the present experiment for they did not increase their fluid intake to any substantial degree over that evidenced by their sham lesioned control group. The next two groups designated tap water-no saline and the adrenalectomized animals which were experimentally lesioned, revealed mean fluid intakes significantly above those of their corresponding control groups.

Considering the tap-no saline groups the majority of the statistical tests suggest that the experimental and sham groups responded similarly to peanut oil and pitressin injections. The relative level of water intake is unchanged for each group following peanut oil injection. With pitressin injection both groups decreased their water intake.

Urine volume output appeared to be depressed slightly be peanut oil injection and only slightly more by pitressin injection for the sham and experimentally lesioned groups.

Turning to urinary constituents, sodium concentration revealed a slight increase following peanut oil injection for the sham group and slight increase or no change for the experimental group. Both groups indicated a significant increase in sodium concentration following pitressin injection with a subsequent decline to pre-injection levels by some 16 to 20 hours after the injection. Urinary potassium concentrations were only minimally elevated by peanut oil injection for the two groups but pitressin injection resulted in an increased potassium concentration occuring within 8 hours after injection and persisting throughout the remaining 24 hours post-injection period for both groups. A similar pattern held for the urinary specific gravity measures.

For the majority of indices utilized in this experiment the non-adrenalectomized experimental versus sham lesioned group comparisons were very similar with regard to pitressin injection. From the data thus far considered there is no bases for the contention that exogenous pitressin administration differentially affects the sham and experimentally lesioned groups.

In general the data collected from the sham and experimental lesioned adrenalectomized groups conform with the established pattern if allowance is made for the altered values of the measures taken due to the effects of adrenalectomy.

Urinary specific gravity offers the only sound difference between the sham and experimental animals' responses to pitressin injection. The sham group evidenced very similar reactions to both peanut oil and pitressin injection. The PH group revealed a substantial increase in urinary specific gravity following pitressin injection which was not apparent with peanut oil injection.

The conclusion to be offered from Experiment 4 is that exogenous pitressin administration results in only superficial response differences between the experimental and sham lesion groups. It may therefore be suggested that bilateral posterior medial hypothalamic lesions do not impair the antidiuretic hormone system's influence upon kidney water reabsorption. However, this experiment did not address the question of whether the heightened isotonic saline intake noted to follow such lesions may be due to a direct impairment of ADH synthesis and/or release. The employment of an ADH bioassay method (Gilmore and Vane, 1970) would, presumably be valuable in such an analysis.

EXPERIMENT 5

The Effect of Water Deprivation

To this point it has been demonstrated that posterior medial hypothalamic lesioned animals increase their isotonic saline solution intake to a significantly greater degree than sham lesioned controls. Such lesions applied to previously adrenalectomized animals likewise result in increased isotonic saline solution intake. The experimentally lesioned groups responded to exogenous pitressin administration with an increased urine concentration. The sham lesioned groups

evidenced a similar urine concentrating ability. A water deprivation regimen is a stressful condition in that body water and electrolyte saving mechanisms are required to function rather well in maintaining ECF volume and tonicity. Comparisons between the experimental and sham lesioned groups in response to a 23½ hour water deprivation schedule was thus employed as an additional means of identifying body water regulatory changes due to the experimental lesions.

Subjects

The animals of Experiments 3 and 4 were used in the present experiment. At the initiation of this experiment the animals were approximately 184 days of age.

Procedure

Following the conclusion of Experiment 4 a five day recovery period was provided. Experiment 5 directed attention upon the imposition of a 23½ hour water deprivation schedule and concomitant changes in metabolic measures. On post-lesion day 43, 4 hour readings began following the design earlier described. Immediately after the 2100 hours reading period water was removed from all three major groups of animals and 4 hour reading periods continued through post-lesion day 44. With the conclusion of day 44, 12 hour readings were initiated and water provided for a 30 minute period once a day immediately following the 0900 reading period.

Results

The format established in the results section of Experiment 4 will be followed in the presentation of these findings. The data for the 4 hour reading periods during the 24 hours preceding water

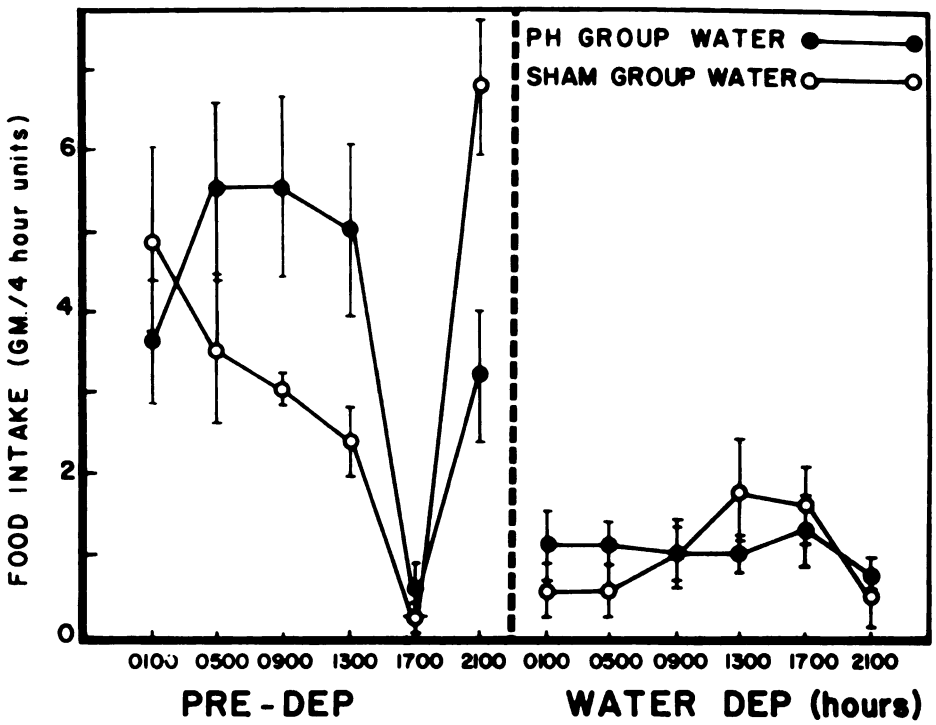
deprivation and the 24 hour period immediately following the initiation of water deprivation are included in the figures of this section. Each point represents the mean of the six animals of the designated group. Standard errors of the means are also provided.

Figure 78 indicates the food intake registered by the two groups maintained on tap water prior to and after the application of water deprivation. During the 24 hour pre-deprivation period the posterior medial hypothalamic lesioned group and the sham lesioned group were not significantly different with regard to food intake ($t=0.41$, $p>0.10$). Each group revealed significant decreases in food intake comparing the pre- and post-deprivation periods (t for related measures=3.43 and 2.22, PH and sham groups respectively, $p<0.01$, $p<0.05$). However, the two groups were not different during the first day of water deprivation with reference to food intake ($t=0.57$, $p>0.10$). The food intakes of the groups earlier supplied both tap and 0.87% saline solution (Figure 79)evidenced no difference during the pre-deprivation period ($t=1.53$, $p>0.10$). Comparing each group's food intake during the pre-deprivation and deprivation periods the posterior medial hypothalamic group demonstrated no difference (t for related measures= 0.26, $p>0.10$).

Again with the two adrenalectomized groups maintained on 0.87% saline solution (Figure 80) there were no differences between their food intakes during the pre-deprivation period ($t=0.84$, $p>0.10$) or during the isotonic saline deprivation period ($t=1.27$, $p>0.10$). However each group demonstrated a significantly decreased food intake comparing pre-deprivation and first day fluid deprivation periods

Figure 78. Mean (± S.E.) food consumption in 4 hour units of the medial posterior hypothalamic and sham lesioned groups maintained on tap water during the 24 hours prior to, and 24 hours of fluid deprivation.

Figure 79. Mean (± S.E.) food consumption in 4 hour units of the medial posterior hypothalamic and sham lesioned groups provided tap water, no saline during the 24 hours prior to, and 24 hours of fluid deprivation.


PH GROUP WATER
SHAM GROUP WATER
FOOD INTAKE (GM./4 hour units)
0
2
4
6
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

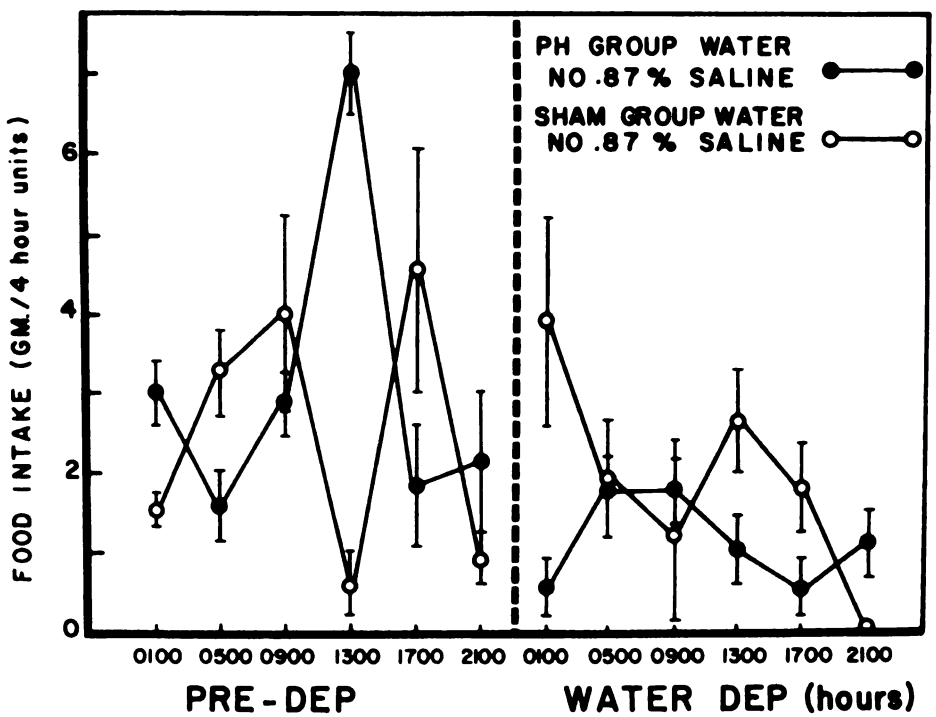
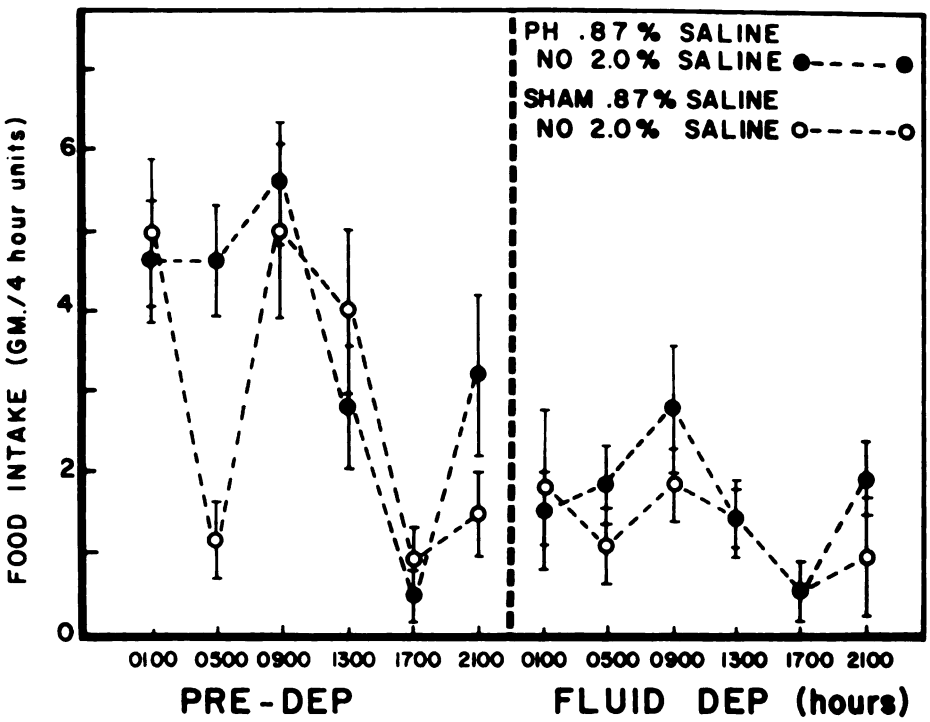
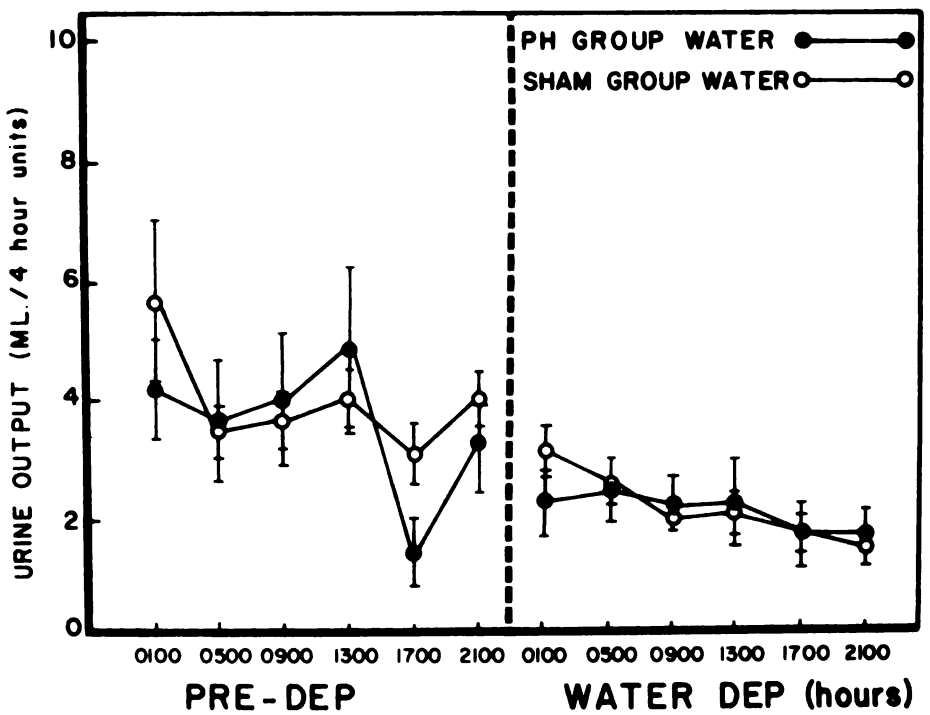

PH GROUP WATER
NO .87 % SALINE
SHAM GROUP WATER
NO .87 % SALINE
FOOD INTAKE (GM./4 hour units)
0
2
4
6
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

Figure 80. Mean (± S.E.) food consumption in 4 hour units of the medial posterior hypothalamic and sham adrenalectomized groups maintained on 0.87% saline solution during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 81. Mean (± S.E.) urine volume output in 4 hour units for the medial posterior hypothalamic and sham lesioned groups provided tap water during the 24 hours prior to and 24 hours of fluid deprivation.


PH .87% SALINE
NO 2.0% SALINE
SHAM .87% SALINE
NO 2.0% SALINE
FOOD INTAKE (GM./4 hour units)
0
2
4
6
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
FLUID DEP (hours)


PH GROUP WATER
SHAM GROUP WATER
URINE OUTPUT (ML./4 hour units)
0
2
4
6
8
10
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

(t for related measures=3.77 and 2.76, PH and sham lesioned groups respectively, $p<0.01$, $p<0.05$).

Turning to urine volume output, for the two groups maintained on tap water (Figure 81) there were no differences comparing them during the pre-deprivation period ($t=0.66$, $p>0.10$) and the same was true for the first day of water deprivation ($t=0.03$, $p>0.10$). Each group did reveal significantly decreased urine output comparing pre-deprivation and first day deprivation values (t for related measures= 6.37 and 6.68, PH and sham lesioned groups respectively, $p<0.01$).

The urine volume output of the two groups originally maintained on tap water and isotonic saline solution (Figure 82) indicated significant differences during the pre-deprivation period ($t=2.72$, $p<0.05$). This probably reflected the heightened intake of tap water by the PH group. The mean output for the PH lesioned group was 6.87 ml/4 hour units while the sham group's mean was 4.58 ml/4 hour units. These two groups were however not different with respect to urine output volume during the first day of water deprivation ($t=0.31$, $p>0.10$). Comparing pre-deprivation and first day deprivation periods the PH lesioned group registered a significantly decreased urine volume output during water deprivation from a mean of 6.87 ml/4 hour units to 2.62 ml/4 hour units (t for related measures= 4.42, $p<0.01$). The sham lesioned group also indicated a change from a mean of 4.58 ml/4 hour units pre-deprivation to 2.72 during deprivation (t for related measures=6.67, $p<0.01$).

The urine formation of the adrenalectomized groups (Figure 83) also revealed differences between the two groups during the pre-

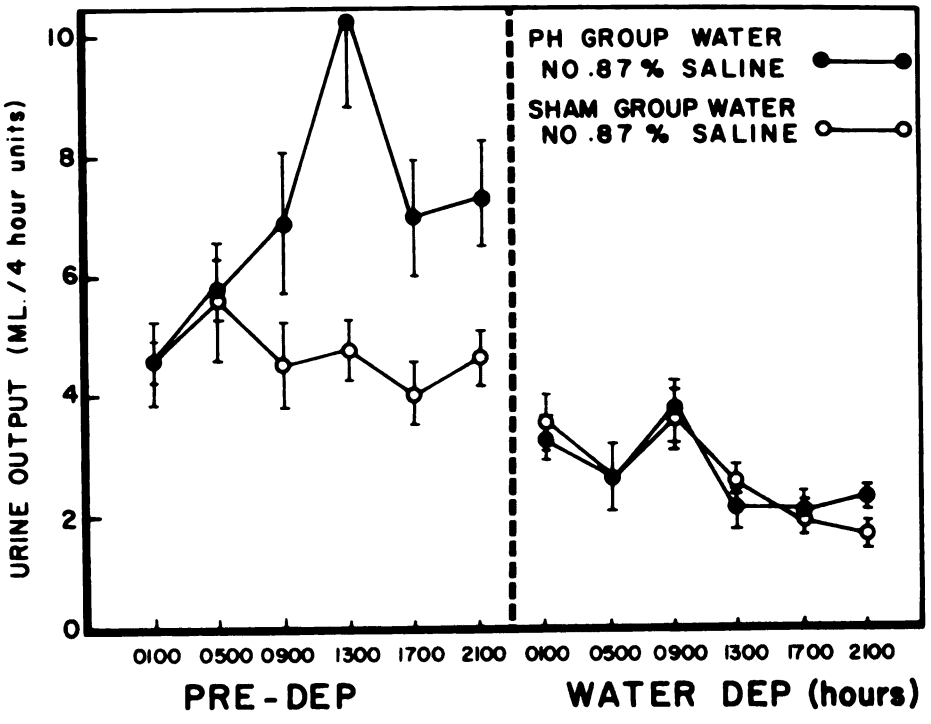
deprivation period ($t=13.82$, $p<0.01$). There were significant drops in urine volume output for the PH lesioned group from 9.83 ml/4 hour periods pre-deprivation to 3.02 ml/4 hour units during the first day of deprivation (t for related measures=12.06, $p<0.01$). This was true of the sham lesioned group as well, from a mean of 6.35 ml urine/ 4 hour period pre-deprivation to 2.56 ml/4 hour units during the first day of deprivation (t for related measures=8.56, $p<0.01$).

The urine constituents in general revealed a pattern of increased concentration during the imposition of fluid deprivation. At odds with this statement were the adrenalectomized groups particularly with reference to sodium concentration.

Figure 84 displays the sodium concentrations in mEq/L. of the urine excreted by the groups placed on tap water during the pre-deprivation and first day of water deprivation. There was a significant difference indicated between these two groups during the pre-deprivation period with the sham lesion group averaging 194.83 mEq/L. sodium and the PH lesion group yielding a mean of 142.33 mEq/L. sodium ($t=49.72$, $p<0.01$). The two groups were, likewise different during the first day of deprivation. The sham group's urinary sodium concentration registered a mean of 289.63 mEq/L. while the PH group averaged 227.83 mEq/L. ($t=9.80$, $p<0.01$). Each group evidenced significant increases in sodium concentration comparing pre-deprivation and first day deprivation periods, with the sham group moving from a mean of 194.83 mEq/L. to 289.63 mEq/L. (t for related measures=5.36, $p<0.01$) and the PH group from 142.33 mEq/L. to 227.83 mEq/L. (t for related measures=5.77, $p<0.01$).

Figure 82. Mean (± S.E.) urine volume output in 4 hour units for the medial posterior hypothalamic and sham lesioned groups provided tap water, no saline, during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 83. Mean (± S.E.) urine volume output in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups maintained on 0.87% saline solution during the 24 hours prior to and 24 hours of fluid deprivation.


PH GROUP WATER
NO .87 % SALINE
SHAM GROUP WATER
NO .87 % SALINE
URINE OUTPUT (ML./4 hour units)
0
2
4
6
8
10
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

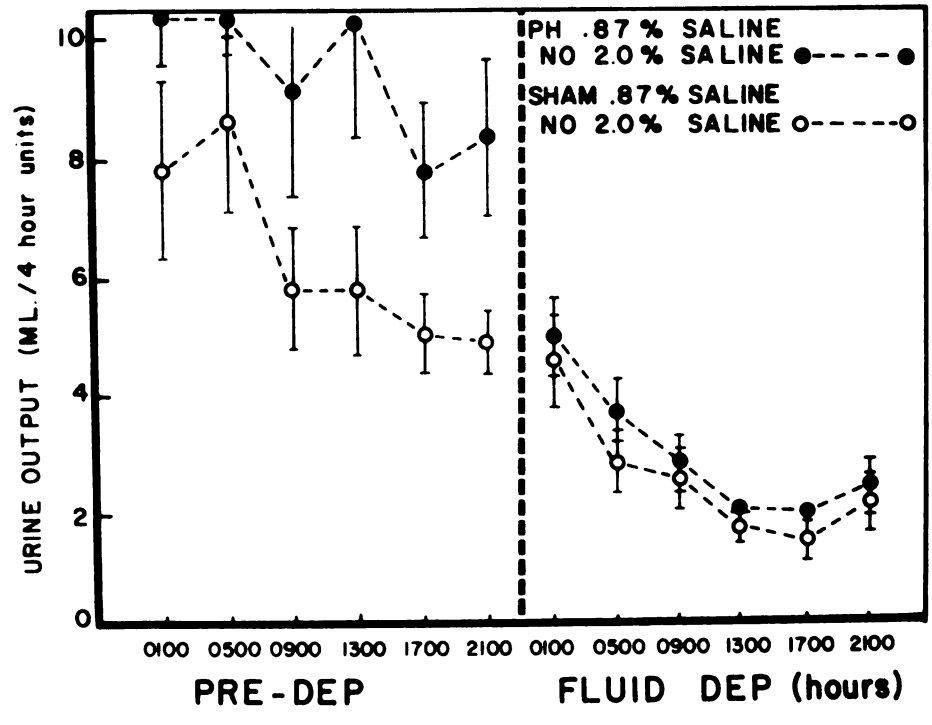
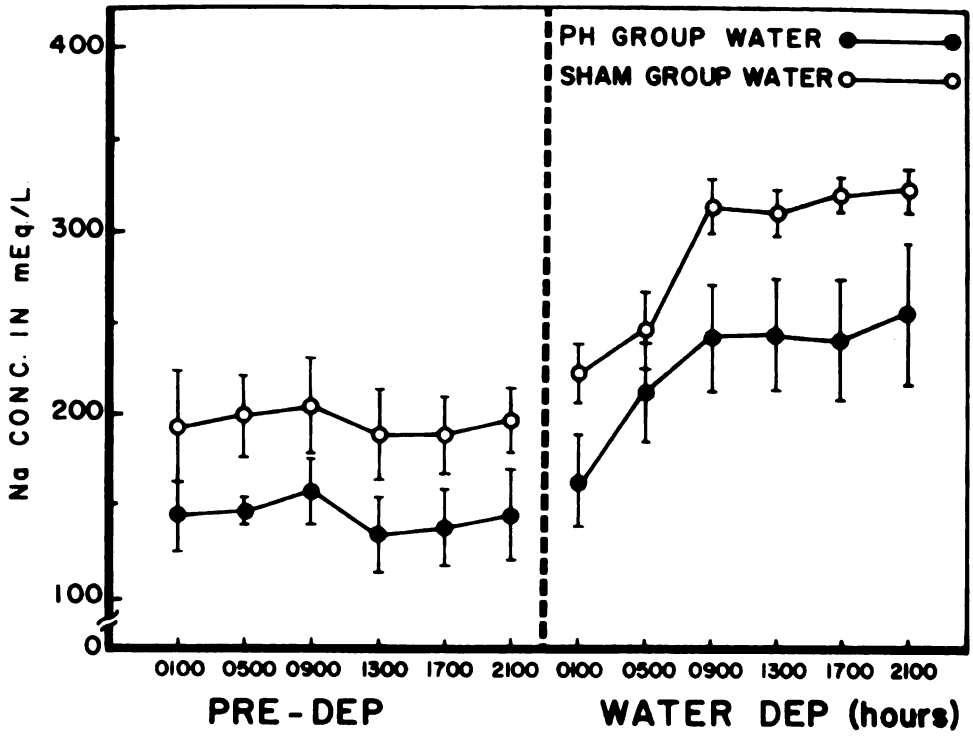
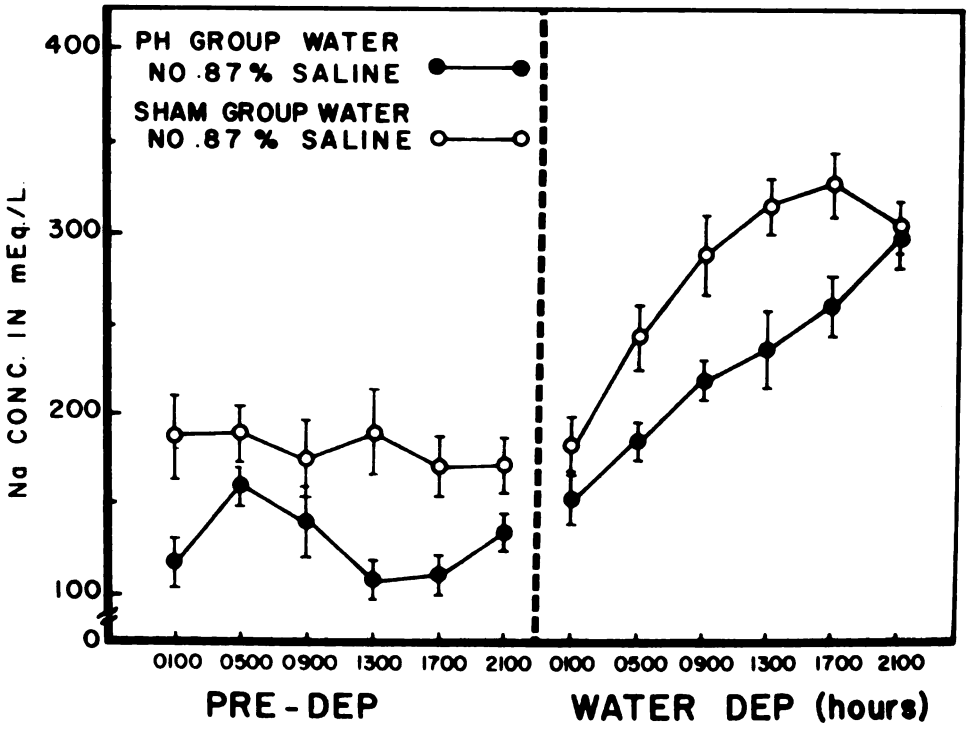

PH .87 % SALINE
NO 2.0 % SALINE
SHAM .87 % SALINE
NO 2.0 % SALINE
URINE OUTPUT (ML./4 hour units)
0
2
4
6
8
10
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
FLUID DEP (hours)

Figure 84. Mean (± S.E.) urinary sodium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned groups provided tap water during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 85. Mean (± S.E.) urinary sodium concentration in 4 hour units of the medial posterior hypothalamic and sham lesioned groups provided tap water, no saline, during the 24 hours prior to and 24 hours of fluid deprivation.


PH GROUP WATER
SHAM GROUP WATER
Na CONC. IN mEq/L
400
300
200
100
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)


PH GROUP WATER
NO .87 % SALINE
SHAM GROUP WATER
NO .87 % SALINE
Na CONC. IN mEq/L
400
300
200
100
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

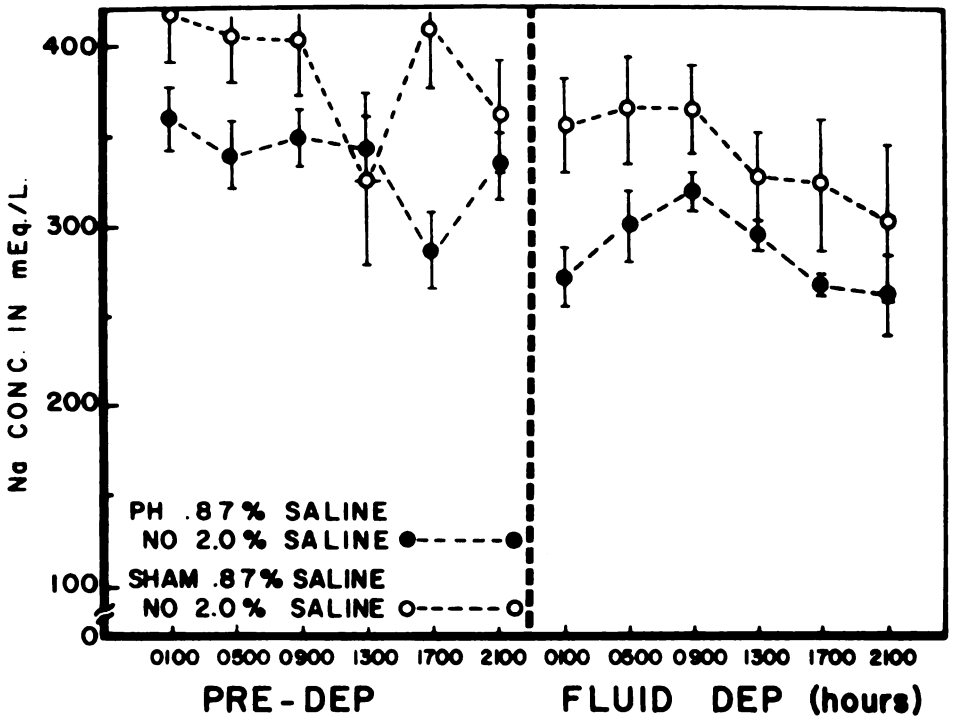
This same pattern was established by the groups earlier maintained on tap water and 0.87% saline solution (Figure 85). There were significant differences between the groups during the pre-deprivation period ($t=5.99$, $p<0.01$) with the sham group displaying a mean urine sodium concentration of 177.33 mEq/L. and the PH group 126.67 mEq/L. Differences also existed within each group comparing pre-deprivation and deprivation periods (t for related measures=4.22 and 3.95, sham and PH groups respectively, $p<0.01$). The sham group changed from a pre-deprivation level of 177.33 mEq/L. to 279.67 mEq/L. sodium while the PH group altered its mean urinary sodium concentration from 126.67 mEq/L. pre-deprivation to 224.75 mEq/L. during the first day of deprivation. The two groups were also different from one another during the deprivation periods ($t=4.61$, $p<0.01$).

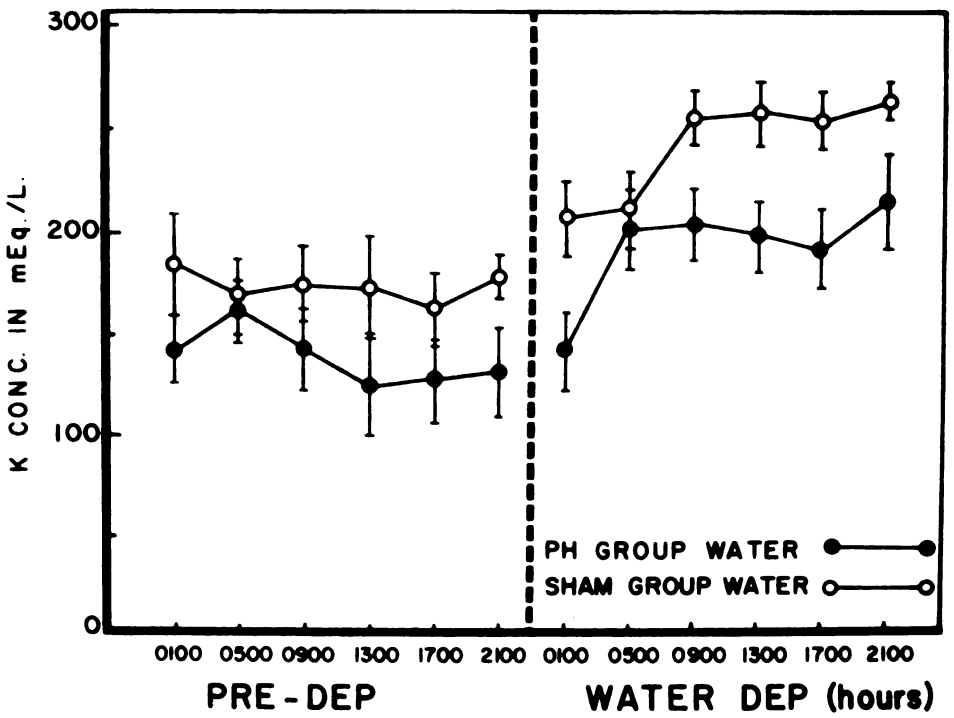
The adrenalectomized groups (Figure 86) did not subscribe to the format thus far established in that both groups while demonstrating between differences during the pre-deprivation period ($t=2.90$, $p<0.01$), revealed decreased urinary sodium concentrations during the deprivation period. The sham group dropped from a mean of 387.33 mEq/L. sodium pre-deprivation to 344.17 during deprivation (t for related measures= 3.30, $p<0.01$). And the PH group dropped from a mean sodium concentration pre-deprivation of 331.33 mEq/L. to 286.17 mEq/L. during deprivation (t for related measures=4.29, $p<0.01$). Again the groups were different from one another during the first day of fluid deprivation ($t=8.35$, $p<0.01$).

The urinary potassium concentrations displayed by the two groups provided tap water is given in Figure 87. There were no differences between the sham and PH lesioned groups during the pre-deprivation

Figure 86. Mean (± S.E.) urinary sodium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 87. Mean (± S.E.) urinary potassium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned groups maintained on tap water during the 24 hours prior to and 24 hours of fluid deprivation.


Na CONC. IN mEq./L.
400
300
200
100
0
PH .87% SALINE
NO 2.0% SALINE
SHAM .87% SALINE
NO 2.0% SALINE
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
FLUID DEP (hours)


K CONC. IN mEq./L.
300
200
100
0
PH GROUP WATER
SHAM GROUP WATER
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

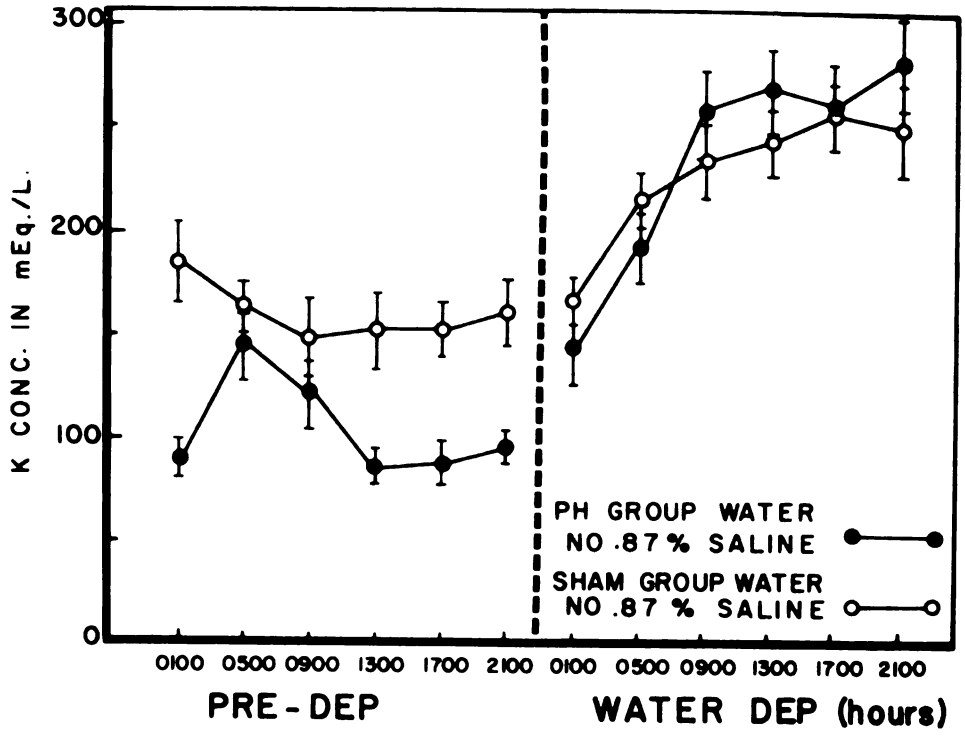
period ($t=0.93$, $p>0.10$). Each group evidenced significant changes from pre-deprivation to the deprivation period. The sham group altered its potassium concentration from a mean of 174.33 mEq/L. pre-deprivation to 231.17 mEq/L. during the deprivation period (t for related measures=5.97, $p<0.01$). The PH group also increased its concentration from a mean of 102.00 mEq/L. potassium pre-deprivation to 234.83 mEq/L. during the deprivation period (t for related measures=4.91, $p<0.01$).

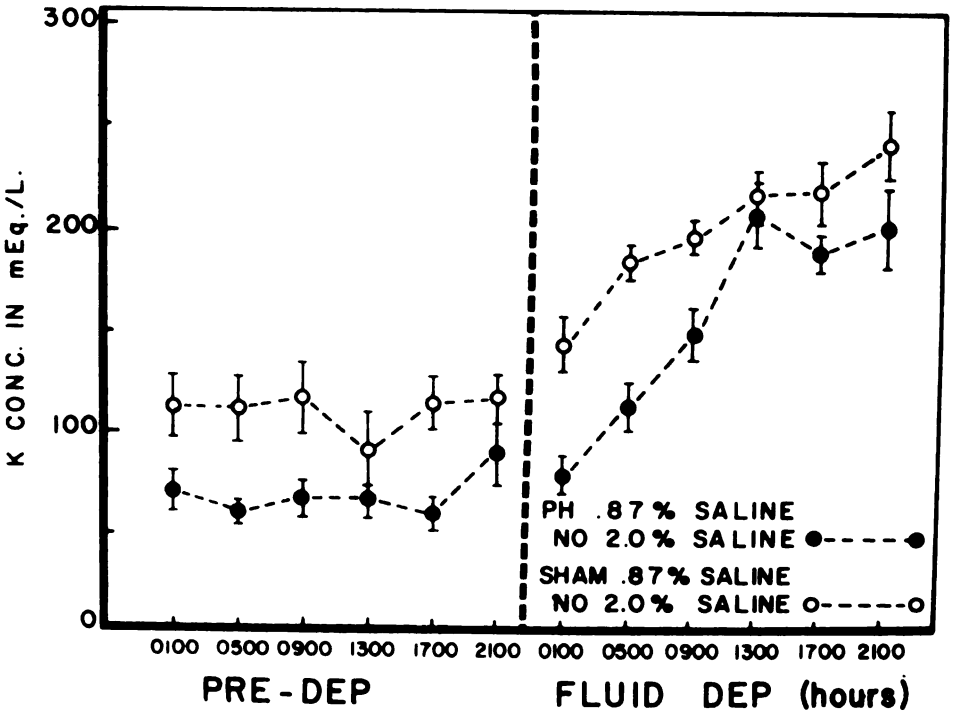
There were no differences between the two groups during the first day of water deprivation ($t=0.42$, $p>0.10$). The adrenalectomized animals (Figure 89) more closely followed the pattern established by the other groups for altered urinary potassium concentration during fluid deprivation. The sham and PH lesion groups were different from one another during the pre-deprivation period ($t=9.41$, $p<0.01$). The sham group altered its potassium concentration from a mean of 110.00 mEq/L. pre-deprivation to 200.50 during fluid deprivation (t for related measures=5.98, $p<0.01$). The PH lesioned group also showed a difference with a potassium concentration mean of 69.83 mEq/L. pre-deprivation to 154.33 during the first day of deprivation (t for related measures=3.81, $p<0.01$). The two groups were different from one another during the first day of fluid deprivation ($t=5.19$, $p<0.01$) with the sham group evidencing a higher potassium concentration in its urine.

Changes in specific gravity were in the same direction for all three major groups. The two groups supplied tap water (Figure 90) revealed differences during the pre-deprivation period ($t=4.59$, $p<0.01$).

Figure 88. Mean (± S.E.) urinary potassium concentration in 4 hour units for the medial posterior hypothalamic and sham lesioned groups provided tap water, no saline during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 89. Mean (± S.E.) urinary potassium concentration in 4 hour units of the medial posterior hypothalamic and sham lesioned adrenalectomized groups provided 0.87% saline solution during the 24 hours prior to and 24 hours of fluid deprivation.


300
200
100
0
K CONC. IN mEq./L.
PH GROUP WATER
NO .87% SALINE
SHAM GROUP WATER
NO .87% SALINE
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)


300
200
100
0
K CONC. IN mEq./L.
PH .87% SALINE
NO 2.0% SALINE
SHAM .87% SALINE
NO 2.0% SALINE
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
FLUID DEP (hours)

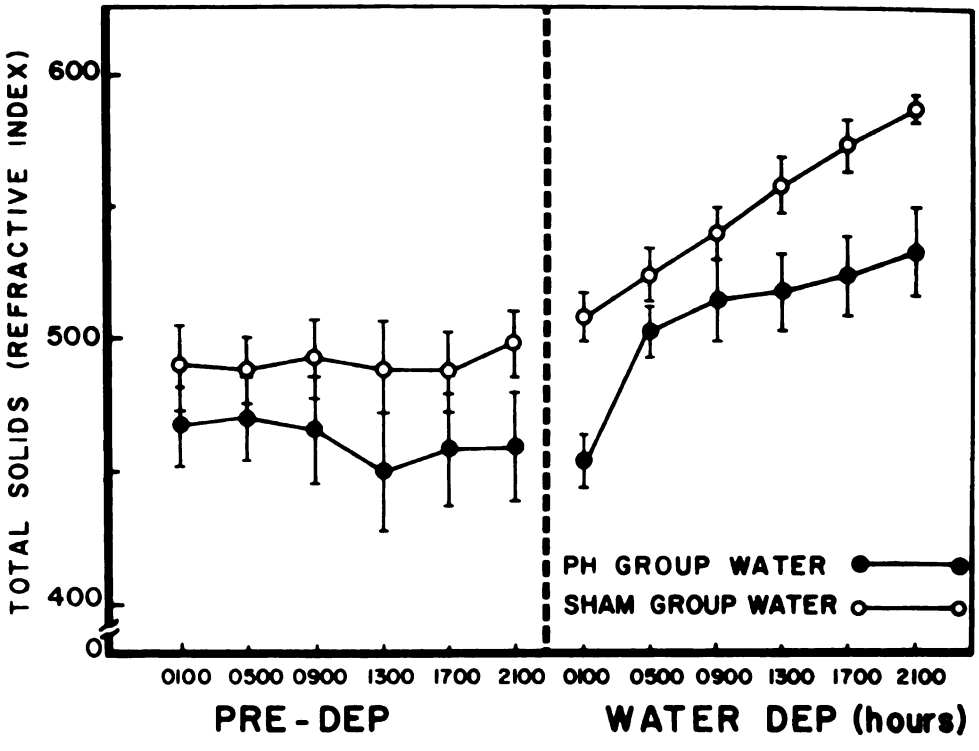
Each group indicated significant increases in specific gravity comparing pre-deprivation and deprivation periods (t for related measures=5.87, and 3.20, sham and PH groups respectively, $p<0.01$). The sham group changed from a mean specific gravity of 1.3486 pre-deprivation to 1.3549 during deprivation and the PH lesioned group from 1.3464 to 1.3509 during the first day of deprivation.

The two groups originally maintained on tap water and isotonic saline solution (Figure 91) were different during the pre-deprivation period with regard to urinary specific gravity ($t=7.29$, $p<0.01$). Each group evidenced a significant increase in specific gravity comparing the pre-deprivation and deprivation periods (t for related measures=4.35 and 4.99, sham and PH groups respectively, $p<0.01$). The changes were in the magnitude of from a mean of 1.3472 to 1.3531 during deprivation for the sham group and from 1.3411 pre-deprivation to 1.3511 during deprivation for the PH lesioned group. The two groups revealed differences for the first day of deprivation with regard to urinary specific gravity ($t=3.65$, $p<0.01$).

Figure 92 represents the urinary specific gravity for the two adrenalectomized groups maintained on isotonic saline solution during this experiment. These groups were significantly different during the pre-deprivation period ($t=11.78$, $p<0.01$). Each group increased its urinary specific gravity during the first day of fluid deprivation (t for related measures=6.47 and 4.54, sham and PH lesioned groups respectively, $p<0.01$). The sham lesioned group changed from a mean of 1.3440 to 1.3508 during the deprivation

Figure 90. Mean (± S.E.) urinary specific gravity in 4 hour units of the medial posterior hypothalamic and sham lesioned groups provided tap water during the 24 hours prior to and 24 hours of fluid deprivation.

Figure 91. Mean (± S.E.) urinary specific gravity in 4 hour units of the medial posterior hypothalamic and sham lesioned groups provided tap water, no saline during the 24 hours prior to and 24 hours of fluid deprivation.


TOTAL SOLIDS (REFRACTIVE INDEX)
600
500
400
0
PH GROUP WATER
SHAM GROUP WATER
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

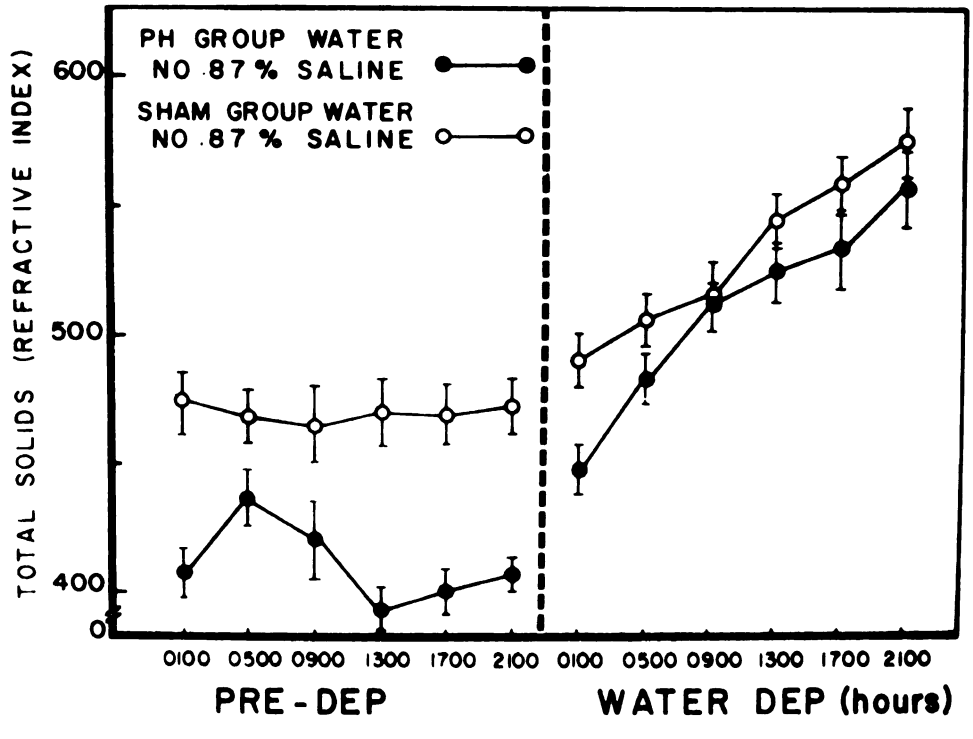
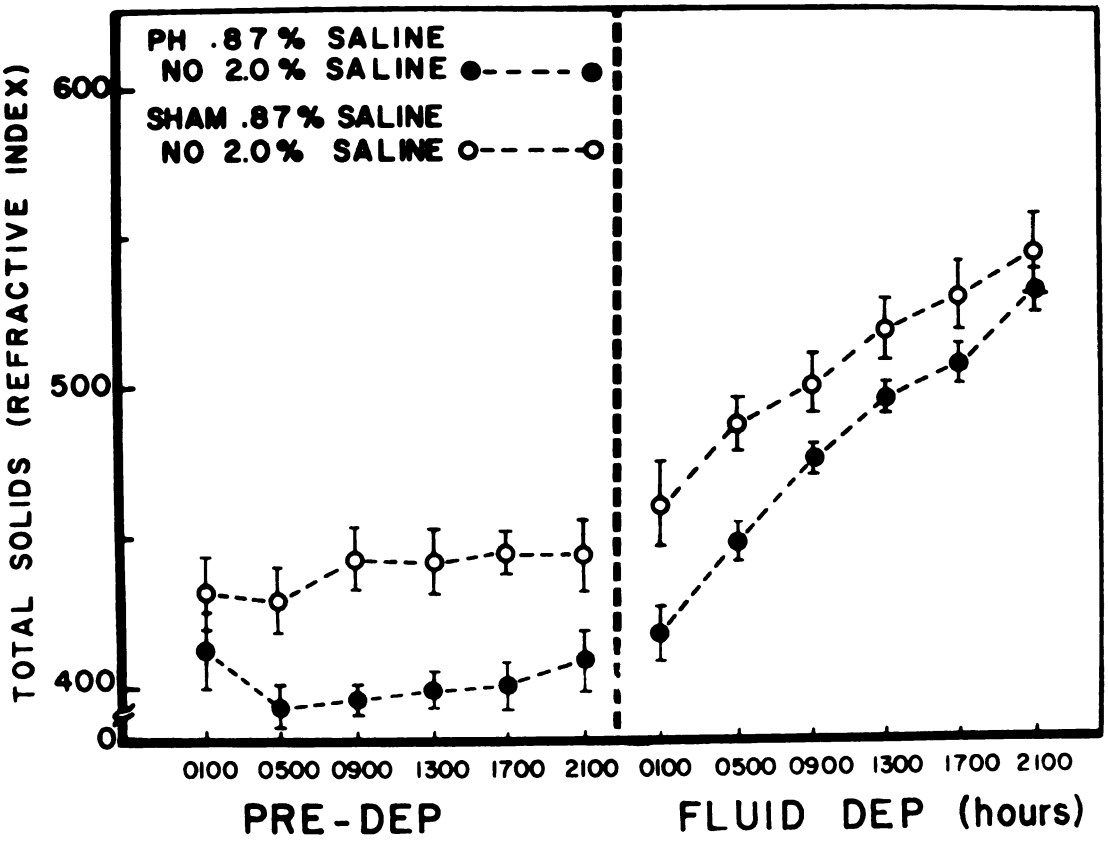

TOTAL SOLIDS (REFRACTIVE INDEX)
600
500
400
0
PH GROUP WATER
NO .87 % SALINE
SHAM GROUP WATER
NO .87 % SALINE
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
WATER DEP (hours)

Figure 92. Mean (± S.E.) urinary specific gravity in 4 hour units of the medial posterior hypothalamic and sham lesioned adrenalectomized groups maintained on 0.87% saline solution during the 24 hours prior to and 24 hours of fluid deprivation.


PH .87% SALINE
NO 2.0% SALINE
SHAM .87% SALINE
NO 2.0% SALINE
TOTAL SOLIDS (REFRACTIVE INDEX)
600
500
400
0
0100 0500 0900 1300 1700 2100 0100 0500 0900 1300 1700 2100
PRE-DEP
FLUID DEP (hours)

period while the PH lesioned group altered its urinary specific gravity from 1.3402 pre-deprivation to 1.3480 during the first day of fluid deprivation. There were differences between the two groups during the first day of deprivation with the sham group displaying a higher urinary specific gravity ($t=6.11$, $p<0.01$).

Discussion

During the first day of water deprivation, food intake significantly decreased for all groups. This is in contrast with water intake during the first day of food deprivation (O'Kelly and Wright, 1971). The urine volume also declined during water deprivation for all groups. All three major groups revealed no difference between urine volume output during the first day of water deprivation comparing the experimental and control subgroups. This is of some interest in that the experimentally lesioned animals of the water-no 0.87% saline group and of the adrenalectomized group revealed significantly higher urine volumes prior to deprivation compared with their control groups. This, of course, reflected the heightened fluid intake of these animals.

Regarding urinary sodium concentrations the experimentally lesioned animals were consistently below the values indicated by their corresponding control groups both prior to and during the first day of water deprivation. The non-adrenalectomized groups maintained on tap water revealed increases in sodium concentration during water deprivation. The experimentally lesioned animals, however, did not concentrate their urine to the same degree as the controls. This may represent an altered kidney ability due to the hypothalamic lesions. This interpretation is not supported by the

potassium ion results, however, for the experimental water-no 0.87% saline group was not different from its control group during the deprivation period in spite of its depressed potassium concentration level during the pre-deprivation period.

The adrenalectomized animals maintained on 0.87% saline solution actually decreased their urinary sodium concentration during deprivation. This may reflect a decreased food intake. Wayne Breeder Blox have about a 1% sodium chloride content. The sodium intake provided by the isotonic saline solution was, of course, not available during fluid deprivation. Their potassium concentration, however, did increase substantially with the experimentally lesioned animals again revealing a low concentration during the pre-deprivation period and an inhibited ability to increase its urinary potassium concentration as compared with its control group.

The urinary specific gravity data closely approximated the potassium results. For all groups the experimentally lesioned animals demonstrated low pre-deprivation urinary specific gravities and during deprivation maintained levels somewhat below their corresponding control groups.

Thus even though the three major groups revealed few differences in urine volume output during deprivation, comparing each experimental group with its control group, the former in general failed to concentrate its urine as efficiently as the control groups. This may represent an impaired kidney concentrating ability. These results may deny support for Cort's "Substance X" in that damage to the presumed production site of this neurohumor should result in a natriuresis or urinary sodium loss that would probably be unchanged or enhanced during water deprivation rather than being inhibited as demonstrated in this experiment.

General Discussion

Given the intricate nature of the ADH-Aldosterone systems in maintaining body water balance the voluntary ingestion of isotonic or near isotonic saline soltuion in excess of actual need is puzzling (Bare, 1949; Nelson, 1947; O'Kelly, 1954; Stellar and McCleary, 1952; Young and Chaplin, 1949). The present series of experiments has identified a contribution to this saline ingestion at the site of the posterior medial hypothalamus. Bilateral lesions at this locus by either electrolytic or radio frequency procedures yields two- to three-fold increases in isotonic saline solution imbibition. Adrenalectomy prior to lesioning does not alter this pattern thus eliminating the influence of corticosteriods. The adrenalectomized animals were ingesting more isotonic saline than normal animals due to the decreased ability of their kidneys to reabsorb sodium. In spite of this increased intake, lesions of the posterior medial hypothalamus caused a still greater intake of saline soltuion.

This isotonic saline polydipsia evidenced by the experimentally lesioned animals was not due to an inability of the kidney to conserve fluids for the administration of exogenous pitressin tannate resulted in a decreased fluid intake and increased urinary specific gravity and electrolyte concentrations not appreciably different from control animals. A close examination of day 1 of a 23½ hour water deprivation schedule reflected differences between the experimentally and control lesioned subjects. With the initiation of water deprivation all animals evidenced decreased food intake and urine volume output. There were urinary constituency differences between the control and

experimental groups. In general the experimental subjects revealed a reduced ability to concentrate their urinary electrolytes, as compared with their corresponding control groups. Urinary specific gravity showed a similar trend.

The possibility of changes upon aldosterone secretion is relevant to the following discussion and therefore several brief summary paragraphs concerning the adrenal-pituitary system have been included.

Steroids which function as mineralocorticoids increase reabsorption of sodium not only at the tubule level but also from sweat, saliva and gastric juice. The primary mineralocorticoid influencing renal tubular exchange of K^+ and H^+ for Na^+ ions is aldosterone (Ganong, 1967). It is certainly true that sodium excretion is affected by other factors in addition to aldosterone such as glomerular filtration rate, osmotic diuresis and fluctuations in tubular sodium reabsorption independent of aldosterone. However, with chronic mineralocorticoid excess, i.e., hyperaldosteronism (Conn's Syndrome) there is a marked ECF volume expansion, potassium depletion and hypernatremia. With continued potassium depletion there is kidney damage resulting in a loss of concentrating ability (hypokalamic nephropathy) and a polyuric-polydipsic condition results (Nocenti, 1968, p. 979).

Adrenalectomy or adrenal insufficiency (Addison's Disease) causes severe loss of both sodium and chloride by the kidneys due to a lack of aldosterone thus a serum hyponatremia and increased potassium level ensues. There is also a decreased plasma volume with increased blood viscosity. With the initial sodium loss goes an obligatory water diuresis and consequently a dehydration state occurs. Following this

initial diuresis water loss begins to lag behind salt loss and cellular overhydration occurs with the subject becoming oliguric (Ganong, 1967, pp. 978-979).

The effect of adrenalectomy upon the isotonic saline polydipsia shown to accompany bilateral posterior medial hypothalamic lesions is an important consideration for several reasons. It has been shown that the polyuria-polydipsia accompanying food deprivation (O'Kelly and Wright, 1971) may be prevented in rabbits by prior adrenalectomy. If these adrenalectomized animals are provided exogenous hydrocortisone acetate during food deprivation the polyuric-polydipsic syndrome appears. Thus an adrenocortical hormone seems to be responsible for a polydipsic condition with urinary sodium loss during food deprivation in rabbits (Nocenti and Cizek, 1970).

As mentioned earlier in this paper it is clear that brain lesions may have an affect upon the adrenal gland. Medial habenular lesions result in a reduction in the nucleus size of the zona glomerulosa cells suggesting a decreased aldosterone secretion (Lengvari, et al., 1970). The above considerations serve as a prelude to an alternative interpretation of Cort's (1963a) findings that bilateral electrolytic lesions of the posterior nucleus of the hypothalamus result in a "salt wasting" syndrome due to the absence of a natriuretic substance, released from the diencephalon (Cort et al., 1966; 1968). In that Cort's 1963a article is his sole and often referenced attempt to monitor this "salt wasting" syndrome for any extended length of time, seven post-operative days, it deserves particular attention.

Cort (1963a) indicated a serum hyponatremia following the lesions, no data were given for serum potassium. There was also a urinary sodium loss increasing three to five-fold by the end of post-operative day 1. The animals increased their intake of isotonic saline and returned their serum sodium level from a low of 135 mEq/L. on day one to 145 by post-operative day five. Cort's conclusion was that with a post-lesion sodium depletion the animals drank isotonic saline in response to an ECF volume depletion rather than 3% saline to correct a pure sodium loss. No histological data were provided. There is nothing in the article to suggest that these lesions did not interfere with aldosterone secretion in a similar way as Lengvari's MHN lesions. Cort's animals displayed the symptoms evidenced by hypoaldosterone animals, i.e., hyponatremia, urinary sodium loss, increased isotonic saline intake which prevented Cort's animals from revealing severe sodium depletion terminating in death. In other papers, however, Cort and his colleagues appear to have ruled out changes in endogenous aldosterone secretion as a factor in the "salt wasting" syndrome for a carotid occlusion stimulus in cats (Cort and Lichardus, 1963a; Lichardus and Cort, 1963). Following adrenalectomy the animals were maintained on daily injections of 5 mg DOCA and 15 mg Hydrocortisone (Fac). Nocenti and Cizek's Fac dosage for rabbits was 2.5 mg/day. With this replacement therapy Cort has failed to control the influence of hydrocortisone upon the increased tubular rejection fraction and urinary natriuresis accompanying carotid occlusion. Of greater significance is his failure to test the interaction of posterior hypothalamic lesions with prior adrenalectomy stretched over several days.

The present series of experiments failed to replicate Cort's "salt wasting" syndrome following posterior hypothalamic lesions. This may have been a function of the small lesions used by the present investigator. However, an increase in isotonic saline ingestion was demonstrated in the lesioned animals, and prior adrenalectomy did not prevent this increased physiological saline intake. Thus Cort is supported on his assumption that adrenalectomy does not affect the heightened isotonic saline intake noted to occur following posterior hypothalamic lesions, but his proposal that the intake comes in response to a decrease in ECF volume accompanying acute sodium depletion is not supported. In fact the present results seriously question Cort's lesion findings with rats for it is clear that small bilateral posterior hypothalamic lesions may yeild isotonic saline polydipsia with no true"salt wasting" syndrome. If Cort's lesions were larger than these it could be that neural efferents passing through the mesencephalon were severed possibly in a similar manner with those presumably interrupted by Lengvari et al. (1970) lesions of the MHN. Thus in addition to the isotonic saline polydipsia resulting from PH lesions Cort in some way may actually have interfered with aldosterone secretion.

An alternative possible explanation concerns adrenocorticotropic hormone (ACTH). It is known that the hypothalamus has some influence over ACTH release by the anterior pituitary (Goldfien and Ganong, 1962). ACTH stimulates aldosterone secretion, however, hypophysectomy, and thus the loss of ACTH, does not result in the profound body fluid electrolyte disturbances present with adrenalectomy (Pitts, 1968, p.218). Even so ACTH influence should not be overlooked.

ADH also may play a role in that with the liberation of ADH from the median eminence there is passage of the hormone into the portal system of the adenohypophysis which stimulates ACTH. ACTH then activates the zona glomerulosa of the adrenal gland and aldosterone output increases (Sawyer, Munsick and Van Dyke, 1960). Arginine vasopressin influences the adrenal gland to increase its secretion of both aldosterone and hydrocortisone (Hilton, 1960).

Sufficient damage to any one of the above systems could possibly result in a reduced level of aldosterone secretion. It is of some importance that posterior hypothalamic stimulation yeilds increased gastric secretion presumably by influencing the pituitary-adrenal systems in the form of increased corticoid release and thus a greater tubular rejection fraction for sodium. This would also explain Cort's "salt wasting" syndrome.

The present observation of an increased fluid intake following the lesioning of a medially located brain area is certainly not original with this investigator. In fact there are indications from the literature that relatively homogenous neural regions may be functionally divided into medial versus lateral subdivisions that are complementary with respect to body water maintanence. Bilateral lesions of the ventromedial hypothalamus (VMH) yield an increased 1.0% saline solution intake (Kawamura et al., 1970). Lesions of the arcuate nucleus of the hypothalamus have been shown to result in an increased 2.0% saline intake (Covian and Antunes-Rodrigues, 1963). And bilateral lesions of what this author would like to refer to as the posterior lateral hypothalamus (LHP), i.e., the region immediately lateral

to the area under investigation in this dissertation, resulted in an adipsic condition with post-operative excretion of a dilute urine (O'Kelly and Hatton, 1969).

There are indications that the amygdaloid complex may also be similarly divided on such a basis. Bilateral lesions of the corticomedial nucleus result in an increased 1.5% saline intake while such lesions of the lateral nucleus, with some damage to the medial and basolateral nuclei, yielded decreased 1.5% saline intake (Gentil et al., 1968).

The application of lesions to the septal area has not been conclusive. It appears that very large lesions affecting both the lateral and medial septal areas result in an increased intake of 0.87% (Donovich et al., 1969) and 1.5% (Vilar et al., 1967) saline solutions. Although Wolf (1967) claims to have lesioned only the lateral septal area and found no change in 2.0% saline solution intake, several of his lesions also damaged the medial septum and none of his lesions included more than about two-thirds of the lateral septal (due to the elongated curving shape of this structure complete destruction is indeed a difficult task).

An unexpected result of the control lesions utilized in Experiment 2 of this dissertation was increased isotonic saline intake. It appears that these bilateral lesions in addition to damaging the intended target, the dorsomedial thalamic nucleus, also affected the medial habenular nucleus (MHN). The subcommissural organ did not appear to be damaged. Although the exaggerated 0.87% NaCl intake following such lesions does not appear to have been previously reported, Lengvari et al. (1970) have demonstrated that such lesions result in a reduction

in nucleus size of the zona glomerulosa cells with no effect upon the zona fasciculata suggesting a reduced aldosterone secretion. Adrenalectomy initiated enlargement of the nuclei of the MHN cells. The results of bilateral destruction of the lateral habenular nucleus (LHN) appears to be unavailable in the literature.

There is neuroanatomical support for the above functional division of these structures into medial and lateral groupings. Fiber tracts appear to arise from the ventromedial, anteriomedial and posteriomedial hypothalamic nuclei and contribute to the periventricular system (Morgane, 1969) at least a portion of which flows into the stria terminalis which courses around the anterior commissure terminating primarily in the central nucleus and medial portion of the basal nucleus of the amygdala. There is also a branching from the stria terminalis in the form of the stria medularis which establishes connections with the medial habenular nucleus (Haymaker et al., 1969). On the other hand, fibers originating from the posterior and anterior lateral hypothalamus pass through the medial forebrain bundle forming the ventral amygdalofugal pathway which appears to terminate in and about the lateral amygdaloid nucleus (Haymaker et al., 1969). Reciprocating fibers have been identified between the posterior lateral hypothalamus and the lateral septal nucleus (Guillery, 1957) and between the lateral septal nucleus and the lateral habenular nucleus (Gurdjian, 1925).

Although the above indications concerning the function of medial and lateral structures are certainly notconclusive there is ample evidence to suggest that the medial portions of the above listed

neural structures may be excercising an inhibitive influence upon fluid intake. When such regions are damaged or destroyed a polydipsic condition ensues which may represent the release of said inhibition. Conversely the lateral portions of these structures may exert a predominately facilitory influence upon fluid intake which, when removed via ablation, yields a marked hypodipsic or adipsic condition.

As previously indicated there remain several major pieces of information lacking from the above schemata. Hopefully knowledge concerning these untested portions of this hypothesis will be made available in the near future.

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APPENDIX

APPENDIX

The following photomicrographs were taken with a Zeiss microscope and 35 mm camera attachment. A 5x eye piece was inserted immediately infront of the camera shutter and the microscope's variable magnification lens (1-5x) was adjusted with a given brain section for optimal exposure area. The microscope light source consisted of a Wotan 12V, 60w bulb powered by a Zeiss Regel-Transformer (type 39 25 33; 3-15V) set at 3.5 volts. Yellow and red filters were placed between the light source and the microscope stage. Exposure times varied from 1/15 to 1/60 second depending upon the stain characteristics of the brain sections. The condenser was not used.

High contrast copy film was employed, developed for seven minutes in D-76 and prints made on Kodak F-5 print paper.

EXPERIMENT 2 ANIMALS

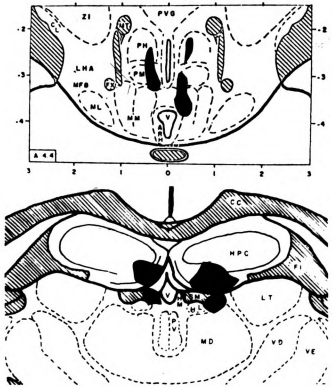
Rat #1: The de Groot insert is at A 4.4. The photomicrograph is of a frozen section stained by the Fink-Heimer method. Magnification x 30.

Rat #3: The de Groot section is at A 4.6. The photomicrograph is of a frozen section stained by the Fink-Heimer method. Magnification x 30.

Rat #5: The de Groot insert is at A 4.8. The photomicrograph is of a frozen section stained by the Fink-Heimer method. Magnification x 32.

Rat #6: The de Groot insert is at A 3.4. The photomicrograph is of a frozen section stained by cresyl violet acetate. Magnification x 30.

Fig. 17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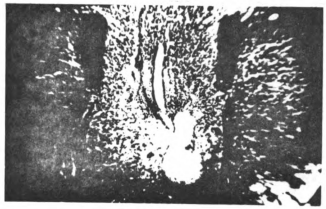
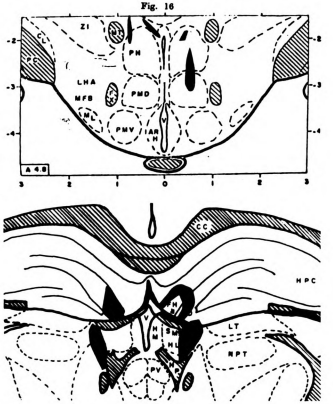


Fig. 16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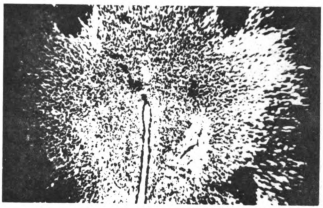
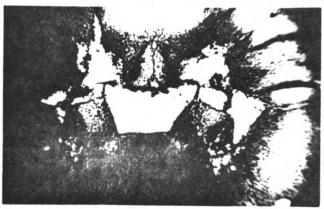


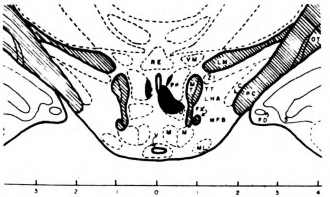


Rat #7: The de Groot insert is at A 4.2. The photomicrograph is of a frozen section stained by the Fink-Heimer method. Magnification x 30.

Rat #8: The de Groot insert is at A 4.6. The photomicrograph is of a celloidin embedded section thionin stained. Magnification x 28.

Rat #9: The de Groot insert is at A 4.6 The photomicrograph is of a celloidin embedded section thionin stained. Magnification x 28.

Rat #10: The de Groot insert is at A 4.2. The photomicrograph is of a frozen brain section stained by cresyl violet acetate. Magnification x 30.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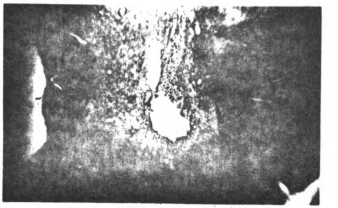

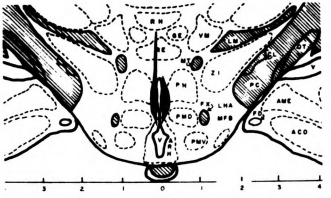
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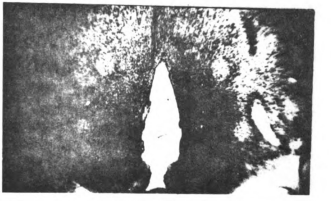

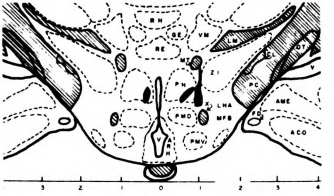
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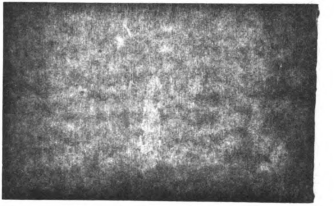

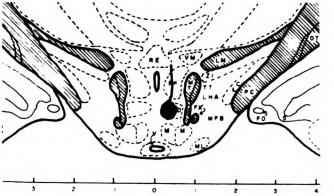
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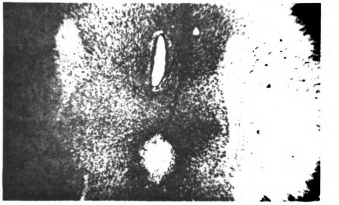


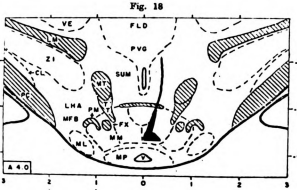
Rat #11: The de Groot insert is at A 4.0. The photomicrograph is of a frozen section stained by cresyl violet acetate. Magnification x 30.

Rat #12: The de Groot insert is at 4.8. The photomicrograph is of a celloidin embedded section thionin stained. Magnification x 28.

Rat #14: The de Groot insert is at A 4.6. The photomicrograph is of a frozen section cresyl violet acetate stained. Magnification x 30.

Rat #15. The de Groot insert is at A 3.8. The photomicrograph is of a celloidin embedded section thionin stainded. Magnification x 28.

Fig. 15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A 4.0

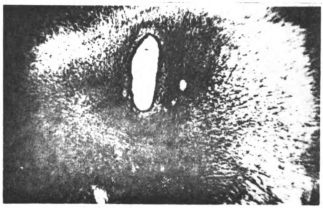
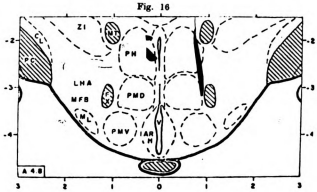


Fig. 16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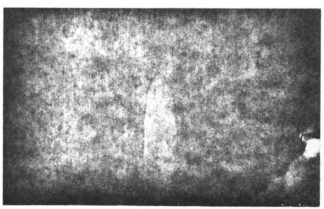

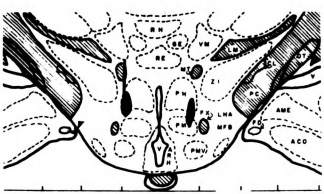
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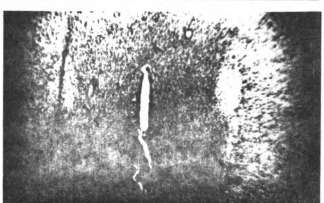

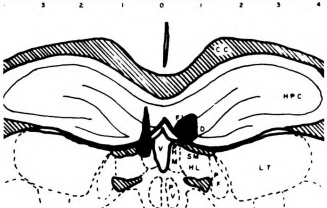
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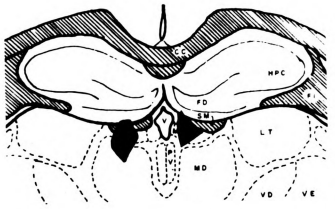
Rat #16: The de Groot insert is at A 4.2. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

Rat #17: The de Groot insert is at A 4.6. The photomicrograph is of a frozen section stained in cresyl violet acetate.

Rat #18: The de Groot insert is at A 4.6. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

Rat #19: The de Groot insert is at A 4.2. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.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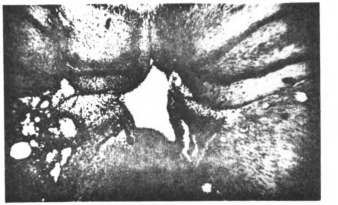

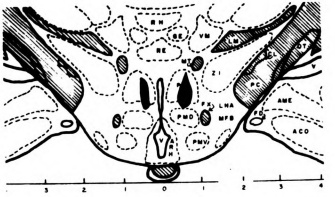
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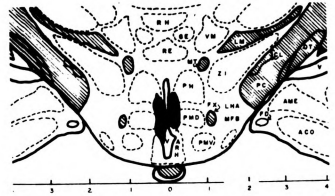
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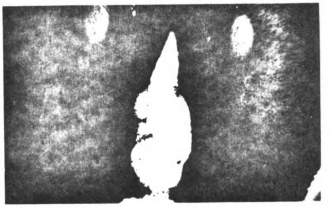

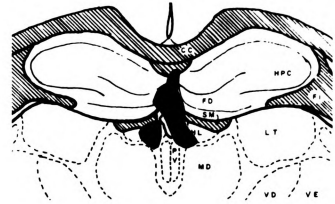
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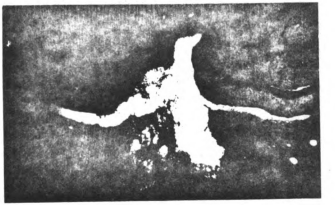


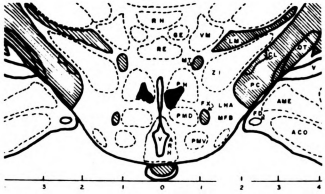
Rat #20: The de Groot insert is at A 4.6. The photomicrograph is of a celloidin embedded section thionin stained. Magnification x 28.

EXPERIMENT 3 ANIMALS

Rat #A: The de Groot insert is at A 4.6. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

Rat #2: The de Groot insert is at A 5.0. The photomicrograph is of a frozen section stained incresyl violet acetate. Magnification x 30.

Rat #3: The de Groot insert is at A 5.0. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.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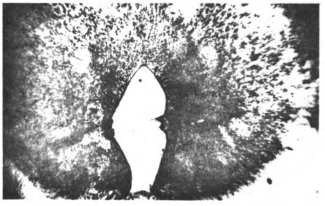

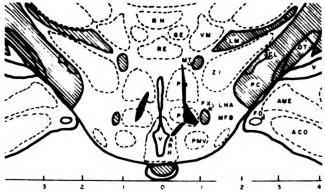
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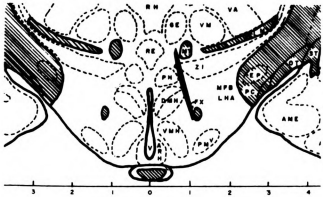
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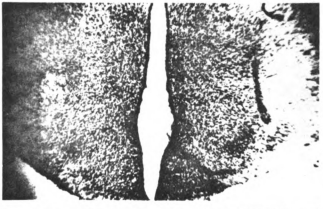

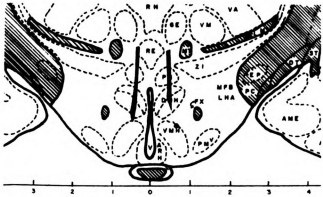
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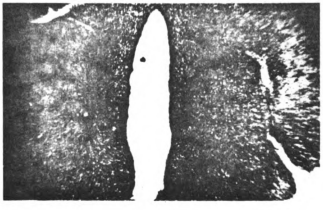


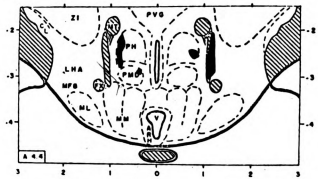
Rat #4: The de Groot insert is at A 4.4. The photomicrograph is of a frozen section stained by cresyl violet acetate. Magnification x 30.

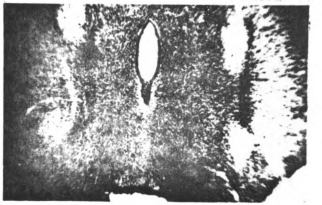
Rat #5: The de Groot insert is at A 4.2. The photomicrograph is of a frozen section stained by cresyl violet acetate. Magnification x 30.

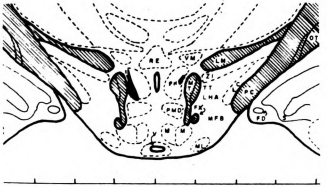
Rat #14b: The de Groot insert is at A 4.4. The photmicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

Rat #15b: The de Groot insert is at A 4.8. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

Fig. 17







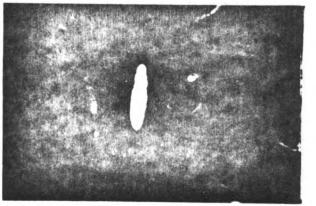


Fig. 17

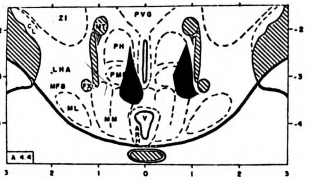
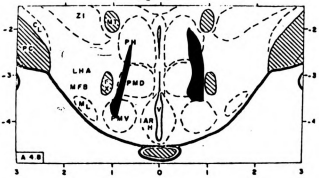
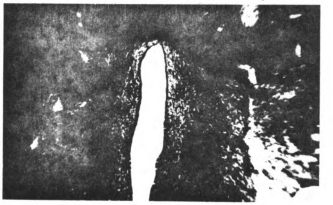


Fig. 16





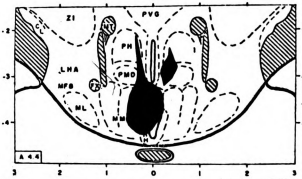
Rat #16b: The de Groot insert is at A 4.4. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

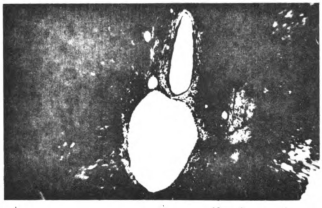
Rat #25: The de Groot insert is at A 4.2. The photomicrograph is of a frozen section stained by cresyl violet acetate. Magnification x 30.

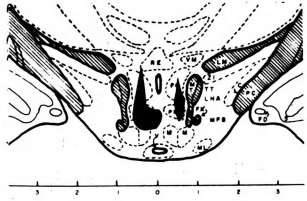
Rat #29: The de Groot insert is at A 3.8. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

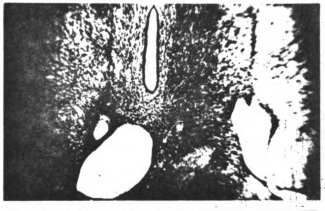
Rat #30: The de Groot insert is at A 3.8. The photomicrograph is of a frozen section stained in cresyl violet acetate. Magnification x 30.

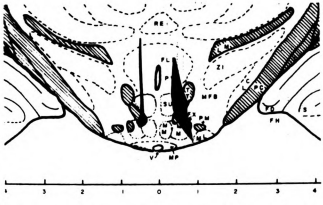
Fig. 17

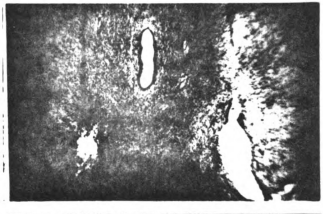

ZI
PVG
PH
LHA
FMD
MFB
ML
MP
A 4·4

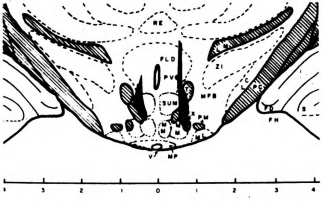


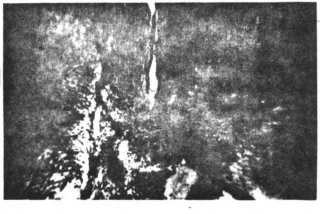

RE
VM
VL
HA
ZI
MFB
FC
ML



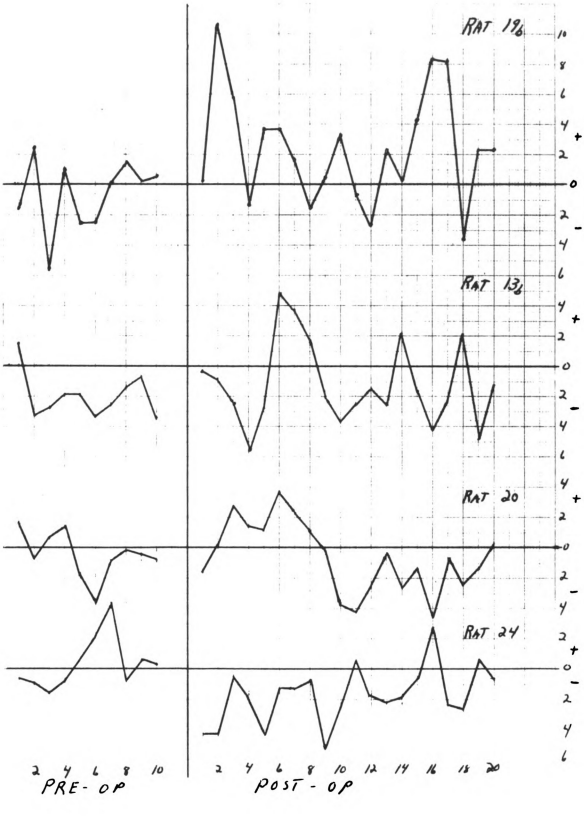

RE
ZI
MFB
FC
FH
MP




RE
FLD
PVG
ZI
SUN
MFB
FC
FH
MP



Total sodium intake as represented by food and 0.87% saline solution consumption minus total urinary sodium output presented in + or - mEq. of Na^+. Rats #19b and 13b were experimental animals utilized in Experiment 3 and maintained on ad libitum Wayne Breeder Blox (Approximately 1% NaCl content), tap water and isotonic saline solution. Rats #20 and 24 were control subjects provided the same diet as described for the experimental animals.


RAT 19
RAT 13
RAT 20
RAT 24
+
-
2 4 6 8 10
PRE-OP
2 4 6 8 10 12 14 16 18 20
POST-OP