BODY FLUID BALANCE REGULATION AFTER ACUTE SODIUM DEFICIENCY

THE INTERACTION OF BEHAVIORAL AND PHYSIOLOGICAL MECHANISMS IN THE RESTORATION OF BODY FLUID BALANCE FOLLOWING ACUTE SODIUM DEFICIENCY

by

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SCOPE AND CONTENTS: Subcutaneous injection of formalin produced acute sodium deficiency in rats, characterized by marked hypovolemia and hyponatremia, due to an extravascular leakage of plasma and destruction of cells at the injection site. This reduction in intravascular fluid volume elicited both behavioral and physiological mechanisms of fluid restoration: sodium appetite and thirst as well as renal retention of sodium and water. Appetite and retention evolved together but intakes continued well after retention ceased and plasma volume and sodium concentration were restored to normal. These results indicate that appetite alone is not a true indicator of need, and that sodium and water balances (intake - excretion) must be considered in defining the deficient state.

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I would like to express my sincere gratitude to Dr. E. M. Stricker for his invaluable assistance and critical guidance throughout this research.

My wife, Mary, deserves a special kind of recognition for her patience, understanding and enthusiasm.

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CHAPTER ONE

Introduction

The homeostatic regulation of body fluid balance depends to a great extent on the proper maintenance of the sodium concentration of the extracellular fluid. Sodium is the major extracellular electrolyte and increases in its concentration will lead to cellular dehydration. Under conditions of hyponatremia when the level of extracellular sodium is decreased, cell volume increases at the expense of extracellular fluid volume to maintain osmotic balance; this results in hypovolemia (plasma deficit). In order to insure the steady state of the internal environment both body fluid balance and sodium balance must be regulated.

In the rat and most other mammals, sodium balance can be regulated physiologically by sodium retention in the kidney and behaviorally by the ingestion of sodium. Renal retention of sodium occurs in the distal tubules and is mediated by the mineralocorticoid, aldosterone. This adrenal hormone enhances the exchange of potassium for sodium, resulting in the retention of sodium and the excretion of potassium. The circulating level of aldosterone has been shown to vary and to increase with increasing need for sodium (Cade & Perenich, 1965). Total absence of aldosterone, as follows bilateral adrenalectomy, results in a continuous loss of body sodium in the urine and will cause death if

large amounts of sodium are not available in the animal's diet to balance the animal's losses (Richter, 1936).

Whereas the role of aldosterone is to minimize sodium loss during times of need, the function of appetite in maintaining sodium balance is to replenish sodium loss in times of need. Since adrenalectomy deprives the animal of the physiological mechanism that is normally responsible for maintaining sodium balance, regulation for these animals depends solely on the increased ingestion of sodium. In fact, it is well documented that adrenalectomized rats increase their intake of sodium chloride solutions (Richter, 1936; Bare, 1949; Epstein & Stellar, 1955). In addition, adrenalectomized rats that have been deprived of sodium will bar press for sodium chloride solutions at rates that are proportional to deprivation, demonstrating that sodium need has potent motivating properties (Lewis, 1960). These rats will also ingest greater volumes of unpalatable, highly concentrated NaCl solutions that normal rats will not approach (Nachman, 1962).

Evidence that a net loss of body sodium elicits an increased appetite for sodium solutions also comes from the work of Denton and Sabine (1961, 1963). They have demonstrated that sheep experiencing a loss of sodium from a chronic parotid fistula manifest an increased ingestion of sodium solutions. Rats fed a sodium deficient diet over extended periods seem to prefer sodium chloride solutions to water as well (Wagman, 1963). Intraperitoneal dialysis (IPD) against glucose also results in a net loss of extracellular sodium when the volume of the dialysing fluid is removed a few hours

after injection by paracentesis (Darrow & Yannet, 1935), and this treatment has similarly been shown to result in an increased appetite for sodium chloride solutions (Falk, 1965).

The physiological basis for sodium appetite is as yet unclear. Rice and Richter (1943) found that normal rats would increase their intake of sodium chloride in response to treatment with the mineralocorticoid, desoxycorticosterone, an intermediary in the bio-synthesis of aldosterone. More recent work by Wolf and Handal (1966) seems to support the hypothesis that endogenous aldosterone does function in the stimulation of sodium appetite in sodium deficient rats, perhaps by potentiating the effects of sodium deficiency. Although increased circulating levels of aldosterone may stimulate sodium appetite in normal and sodium deficient rats, it obviously cannot account for the increased sodium intake of the adrenalectomized animal (Richter, 1936).

Sodium deficiency results in certain changes in body fluids, specifically hyponatremia and hypovolemia, that are potent stimuli for mineralocorticoid secretion (Denton, 1965) and may be more directly involved in the elicitation of sodium appetite than actual body sodium loss or increases in aldosterone levels. For example, Wolf and Steinbaum (1965) have demonstrated that formalin injection, which causes a transient hyponatremia and a marked hypovolemia (Stricker, 1966), can elicit a sodium appetite in both normal and adrenalectomized rats. Since this effect proceeds from a mere redistribution of body fluids and electrolytes, it is evident that neither a net loss of body sodium nor an increased mineralocorticoid

level is necessary for sodium appetite. In this regard, Richter (1956) had postulated that hyponatremia is the important stimulus for sodium appetite. However, although prolonged dilutional hyponatremia may elicit increases in sodium appetite (Tosteson, DeFriez, Abrams, Gottschalk & Landis, 1951), acute hyponatremia induced by gastric water loading has no effect on sodium intake (Stricker & Wolf, 1966). In addition, evidence exists relating increased sodium intake to simple decreases in blood volume in the absence of any alteration of serum sodium concentration. For example, Stricker and Wolf (1966) have recently shown that subcutaneous injection of polythylene glycol, which decreases plasma volume isosmotically, elicits an increased salt intake 24 hours after treatment. In order to account for these seemingly disparate effects, and the natrorexigenic (sodium appetite inducing) effects of aldosterone, hyponatremia, and hypovolemia, Stricker and Wolf (1966) have developed the concept of a sodium reservoir receptor system. The postulated receptor is supposed to initiate sodium appetite in response to reservoir depletion, with hyponatremia and hypovolemia acting upon this reservoir via increased aldosterone secretion (aldosterone potentiates active sodium transport). Although more recent evidence (Wolf & Stricker, 1967) has suggested some modifications of this model, it seems the most parsimonious way of dealing with all of the data at present.

It is interesting to note the relationship between sodium appetite and thirst as behavioral regulators of intravascular fluid volume (IVF). Falk (1965) has noted that IPD treated rats not only

increase their intake of hypertonic saline but also exhibit increased water drinking. Formalin treated animals also substantially increase their water intake 8 hours after injection (Stricker, 1966), as do rats injected with polyethylene glycol, with drinking proportional to the plasma deficit (Stricker, 1968). Hypovolemia has been shown to result in increased circulating levels of aldosterone (Farrell, Rosnagle & Rauschkolb, 1956) and anti-diuretic hormone (Share, 1962). Thus, hypovolemia is not only an effective stimulus for both sodium appetite and thirst but also for salt and water retention. This relationship between water and sodium balances might be expected, since correction of hypovolemia requires the addition of both sodium and water (Stricker & Wolf, 1967).

In summary, the regulation of body fluid balance can be mediated both behaviorally, by sodium appetite and thirst, and physiologically, by the selective renal retention or excretion of water or sodium. In view of this fact, a complete understanding of the regulatory system for sodium balance requires some knowledge of the parameters of both types of responses and their relation to need. The purpose of the work presented here is to examine the interaction of the behavioral and physiological mechanisms involved in the animals' total response to acute sodium deficiency.

CHAPTER TWO

Method

Briefly, sodium balance refers to sodium intake minus sodium excretion, and sodium need develops when the balance is negative. Thus, the sodium need of an adrenalectomized animal would depend on the amount of sodium lost through excretion. However, sodium need also develops when fluid is "lost" internally. For example, the sodium need created by formalin injection is not the result of negative balance. The subcutaneous injection of formalin destroys local capillary walls resulting in the sequestration of protein-rich fluid in an edema at the injection site. The widespread destruction of cells at the injection site results in the exchange of intracellular potassium for extracellular sodium causing a transient decrease in serum sodium concentration (hyponatremia), and the decreased levels of protein and sodium in the extracellular fluid cause cellular over-hydration and decreased plasma volume (hypovolemia) (Stricker, 1966). Hence, the sodium need of formalin treated rats cannot be assessed by monitoring urine sodium loss. The increased sodium appetite of formalin treated rats cannot serve as a true indicator of sodium need either, since factors such as palatability, availability, and familiarity can bias intake and result in ingestion in excess of need. Consequently, in the present experiment, sodium need resulting

from acute sodium deficiency was determined by measuring how much of the ingested sodium was actually retained, and when retention ceased, need was no longer considered to be present.

Subjects and Maintenance: One hundred and nine male, albino rats of the Wistar and Sprague-Dawley strains weighing approx. 350 gms. at the start of the experiment were housed in individual metabolism cages. Drinking fluids were available from two inverted graduated tubes with metal drinking nozzles attached to the fronts of the cages approximately 3 cm. apart. The rats were allowed ad lib access to Purina Lab Chow except on test days.

Procedure:

Balance: The experimental schedule for the twelve animals used in obtaining water and sodium balance information is summarized in Table 1. During the course of the experiment the animals were subjected to five separate drinking test days with food absent. Each test was preceded by at least three days of ad lib access to food and distilled water. The animals were given access to a 3% NaCl solution (513 mEq. Na/l.) beginning on Day 5 and this was continuously available until the end of the experiment. For the first drinking test on Day 4 only water was available. During the second test on Day 8 only 3% NaCl was available. Both water and 3% NaCl were available during the drinking tests on Day 12 (pre-control), Day 16 (Formalin) and Day 21 (post-control). At the beginning of each of the five test days all of the rats were lightly anesthetized with ether. Only on Day 16 were they injected subcutaneously with 2.5 cc. of 1.5% formalin (phosphate buffered) in order to induce acute sodium

TABLE 1

Summary of Experimental Schedule And

Treatment Variations

Days	Water	Lab Chow	3% NaCl
1 - 3	ad lib	ad lib	
4	ad lib		
5 - 7	ad lib	ad lib	ad lib
8			ad lib
9 - 11	ad lib	ad lib	ad lib
12	ad lib		ad lib
13 - 15	ad lib	ad lib	ad lib
16 formalin injection	ad lib		ad lib
17 - 20	ad lib	ad lib	ad lib
21	ad lib		ad lib

deficiency. During each of the drinking tests, fluid intakes and urine volumes were recorded hourly for 24 hours. Graduated urine collection tubes were changed each hour and the contents analysed for Na^+ concentration by flame photometry (Coleman Instruments). Between test days urine samples were not collected and fluid intakes were recorded every 24 hours. The rats were weighed prior to and after each of the three final drinking tests.

Blood Analyses: Ninety-seven rats were used to determine the intravascular changes resulting from acute sodium deficiency. Under ether anesthesia, blood was withdrawn from the abdominal aorta of formalin treated rats after varying periods of deprivation (n=48) or ad lib access to water and 3% NaCl (n=44). Water and sodium balance information was collected as before by monitoring intakes and excretions. Blood samples were also obtained from 5 untreated rats for baseline values.

Generally, 5-6 cc. of blood were withdrawn into heparinized vessels. Hematocrits were determined with micro-capillary tubes, plasma protein concentration by a refractometer, plasma water content by oven-drying to constant weight, plasma osmolalities by freezing point depression (Advanced Osmometer) and plasma sodium and potassium concentrations by flame photometry (Instrumentation Laboratories).

CHAPTER THREE

Results

Water and Sodium Balances: The drinking tests on Day 4 (Water only) and Day 8 (3% NaCl only) were conducted to accustom the animals to the deprivation condition and to determine the effects of food deprivation on sodium and water balances. As would be expected, water intake decreased substantially when food was not present. The mean water intake of the three preceding days was 31.6 ml., whereas on Day 4 the mean intake was only 16.7 ml. Urine volume exceeded intake throughout the 24 hours resulting in a continuous negative fluid balance (Fig. 1). Since sodium was not available on Day 4, sodium balance was also continuously negative (Fig. 1) although a decrease in urine sodium concentrations after 12 hours (Fig. 2) demonstrated some renal conservation of sodium. Saline intake was increased on Day 8 as compared to the preceding days when food, water and saline were available ($\overline{X}$ = 3.0 ml. vs. $\overline{X}$ = 7.6 ml.) but again both fluid and sodium balances were always negative (Fig. 3). Urine sodium concentrations were much higher than those of the previous drinking test and remained high for the duration of the drinking period (Fig. 2). Since none of the sodium ingested was retained it is evident that the increased saline intake was not the result of sodium need. It was obvious

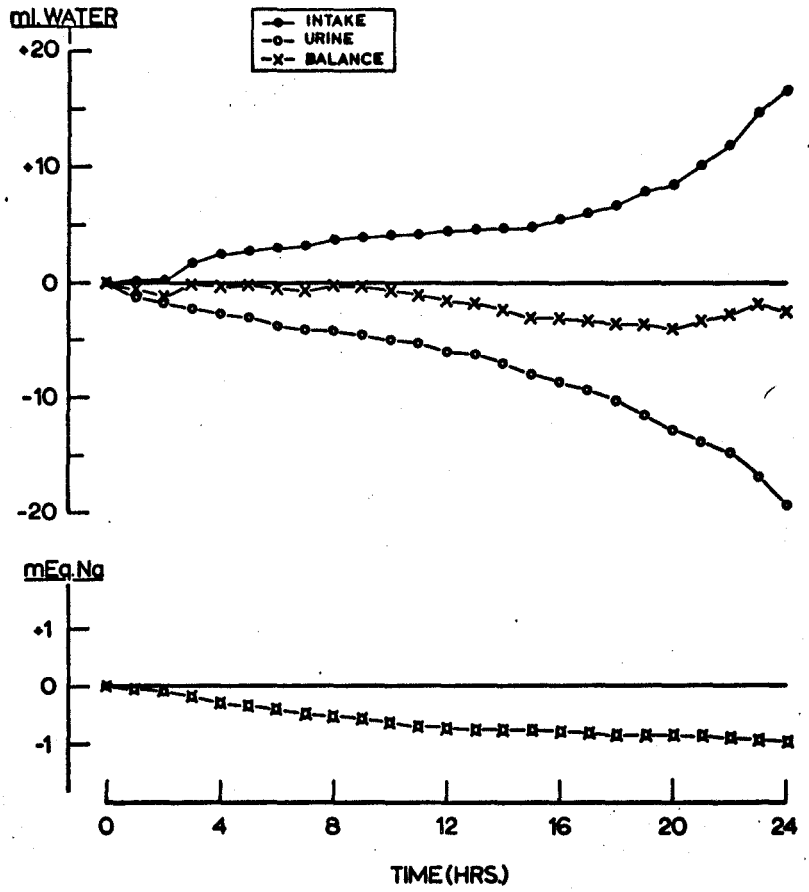

Fig. 1. Water and sodium balances during access to only water (Day 4).

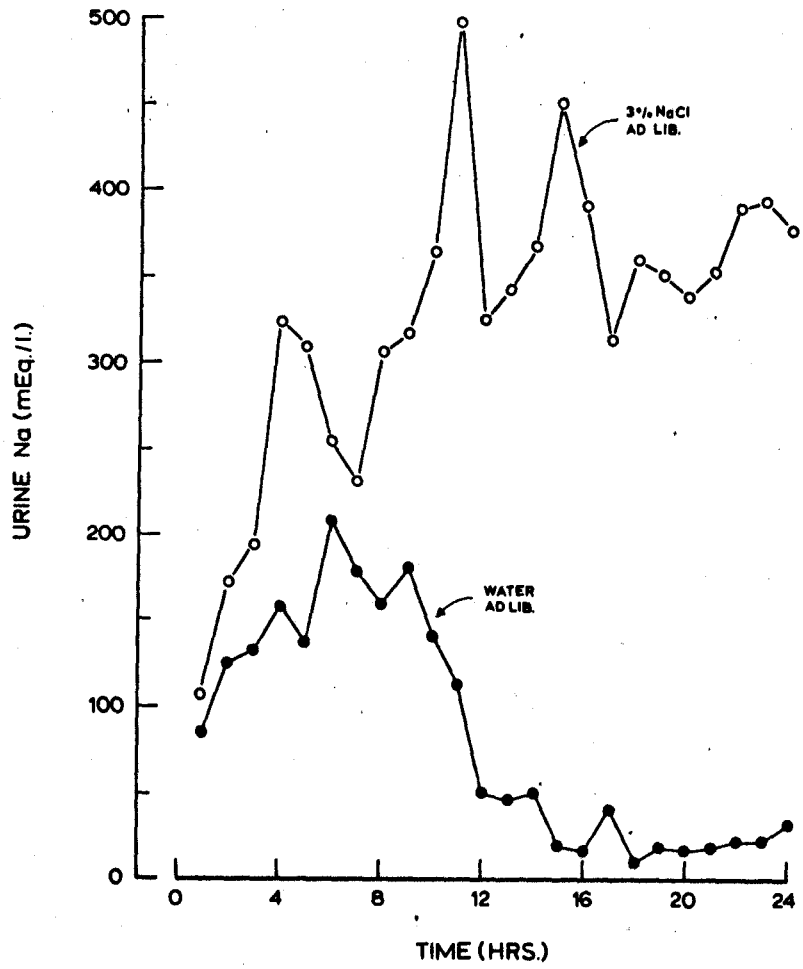

Fig. 2. Urine sodium concentrations during access to water or 3% NaCl (Days 4 and 8).

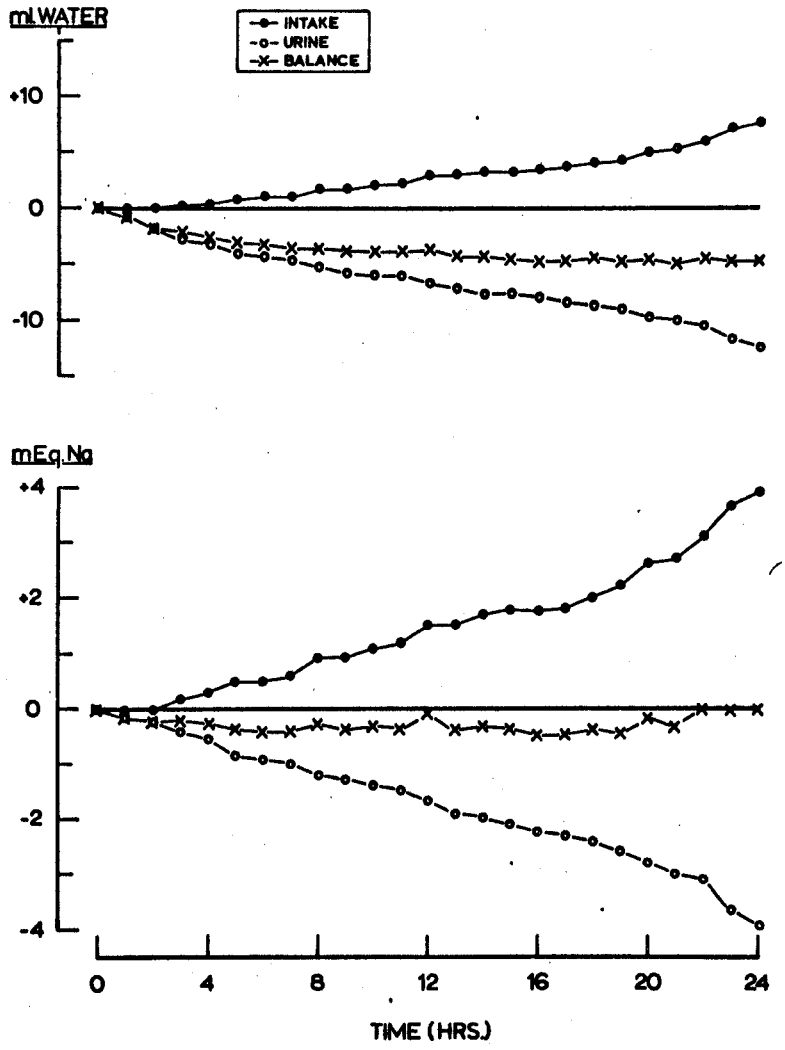

Fig. 3. Water and sodium balances during access to only 3% NaCl (Day 8).

from these initial tests that food deprivation did not increase need, retention or appetite for either sodium or water.

A comparison of the animals' responses during the pre control drinking test on Day 12 and the formalin test on Day 16 demonstrates the striking effects of formalin injection. Intakes of both water and 3% NaCl were significantly greater after formalin treatment by the 4th hour (p's $<.01$ and $<.001$ respectively; 2-tailed *t* test for differences between means) and became increasingly greater up to the end of the test period (Fig. 4). Net sodium intake (mEq) was also greater after formalin injection (4 hr $p<.001$; Fig. 6). Urine volume and sodium excretion were substantially decreased after formalin despite the augmented intakes (4 hr p's $<.001$ and $<.01$) and remained lower than the pre-control excretions until the 21st hour for volume and the 18th hour for sodium (Fig. 5 and 6). The increased intake and retention of water and sodium after formalin injection resulted in the maintenance of a high positive water and sodium balance throughout the test period (both 4 hr p's $<.001$; Fig. 5 and 6) whereas both balances were approximately zero during the pre-control test. The increased fluid intake and retention after formalin treatment (Day 16) caused most of the animals to experience a substantial weight gain despite the absence of food ($\bar{X}$=+15.8 gms; range 0 gm. to 27 gms.), whereas on Day 12 (pre-control) all rats lost weight ($\bar{X}$=24.5 gms; range -14 gms. to -33 gms; $p<.01$). Actual urine sodium concentrations (mEq/l.) did not change over time during the pre-control test but after the formalin injection concentrations decreased almost immediately and

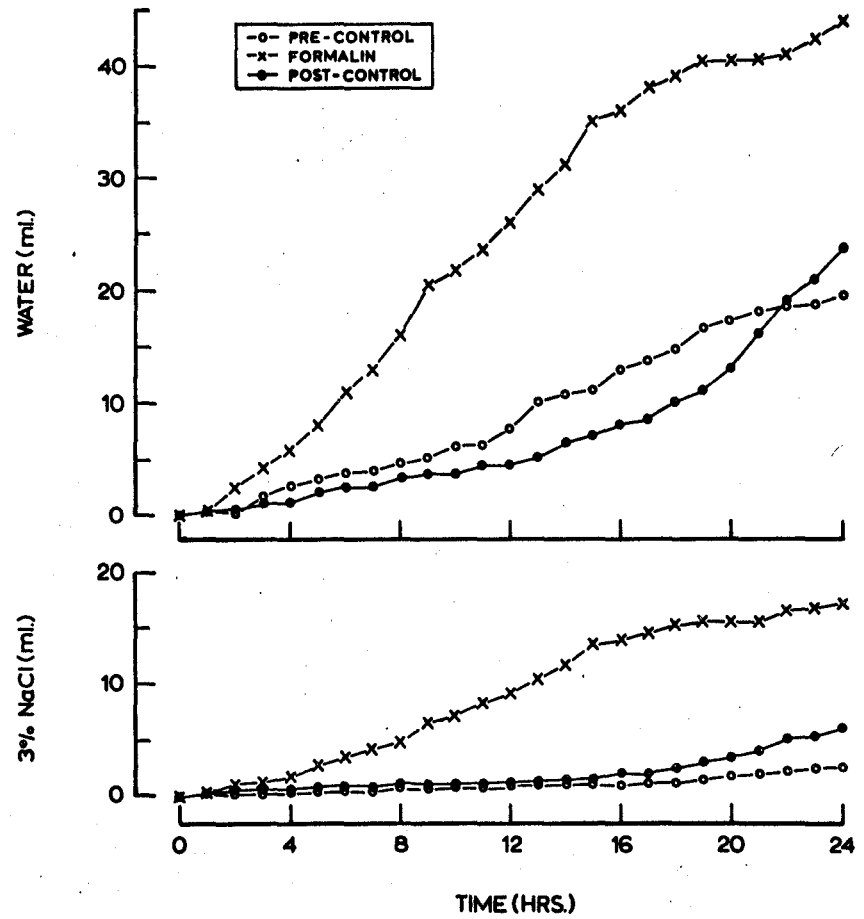

Fig. 4. Water and 3% NaCl intake during pre-control (Day 12), formalin (Day 16), and post-control (Day 21) tests.

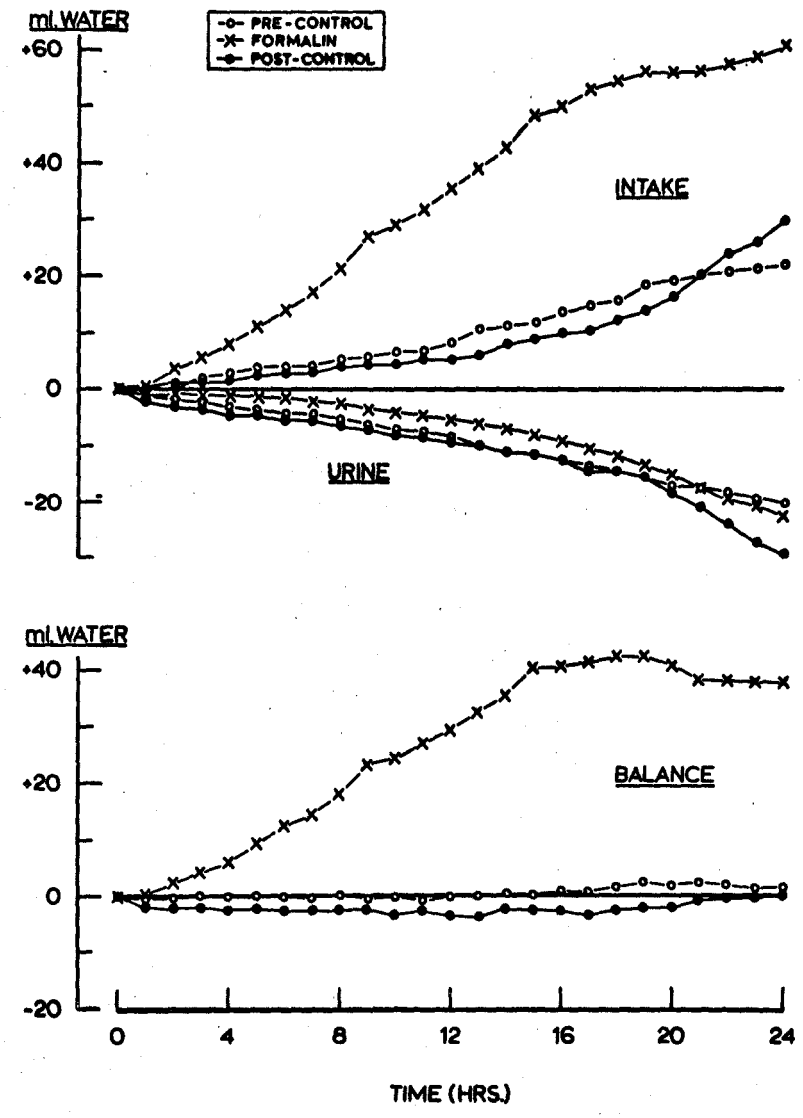

Fig. 5. Water balances during the pre-control, post-control and formalin tests.

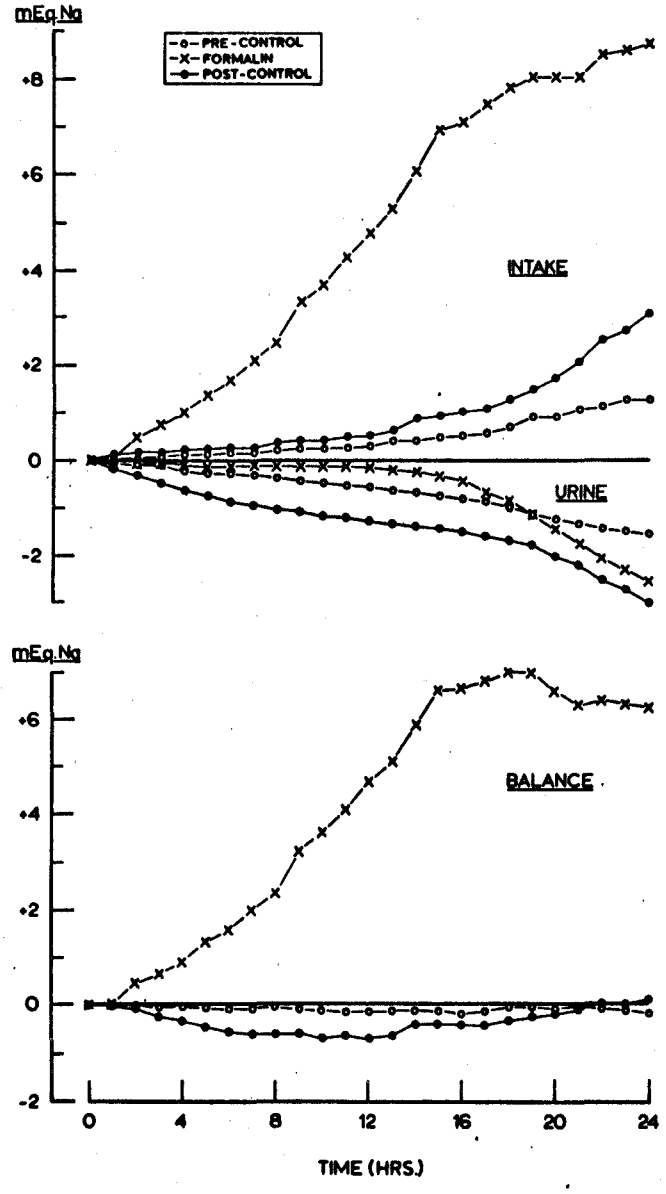

Fig. 6. Sodium balances during the pre-control, post-control and formalin tests.

remained low until approximately the 15th hour (Fig. 7).

In order to assess the possible long term effects of formalin induced sodium deficiency, the animals were subjected to the post-control drinking test on Day 21. The differences between the pre-control test and the post-control test in terms of water intake and urine excretion were not significant although fluid balance was slightly more negative during the latter test (Fig. 4 and 5). Saline solution intake and net sodium intake were somewhat greater during the post-control drinking test (both 24 hr p's $<.05$; Fig. 4 and 6). However, sodium excretion was also significantly greater during the post-control test (24 hr p $<.05$; Fig. 6 and 7), resulting in a greater negative sodium balance throughout most of the post-control test. As on the pre-control day, all of the rats lost weight on Day 21 ($\bar{X}$ = -28.8 gms; range -22 gms. to -38 gms.).

Blood Analyses: The progressive changes in the intravascular fluid of animals deprived of water and salt (Table 2) indicated that hyponatremia and hypovolemia occurred rapidly after formalin injection and were seemingly long lasting. Hematocrits were well above normal one hour after injection and did not return to normal even after 24 hours, indicating a pronounced reduction in blood volume. Plasma water was generally elevated and plasma protein decreased as would be expected from the protein leakage at the injection site. Serum sodium concentrations were lower than normal whereas serum potassium concentrations increased due to cell destruction at the injection site.

A second group of animals was allowed access to water and

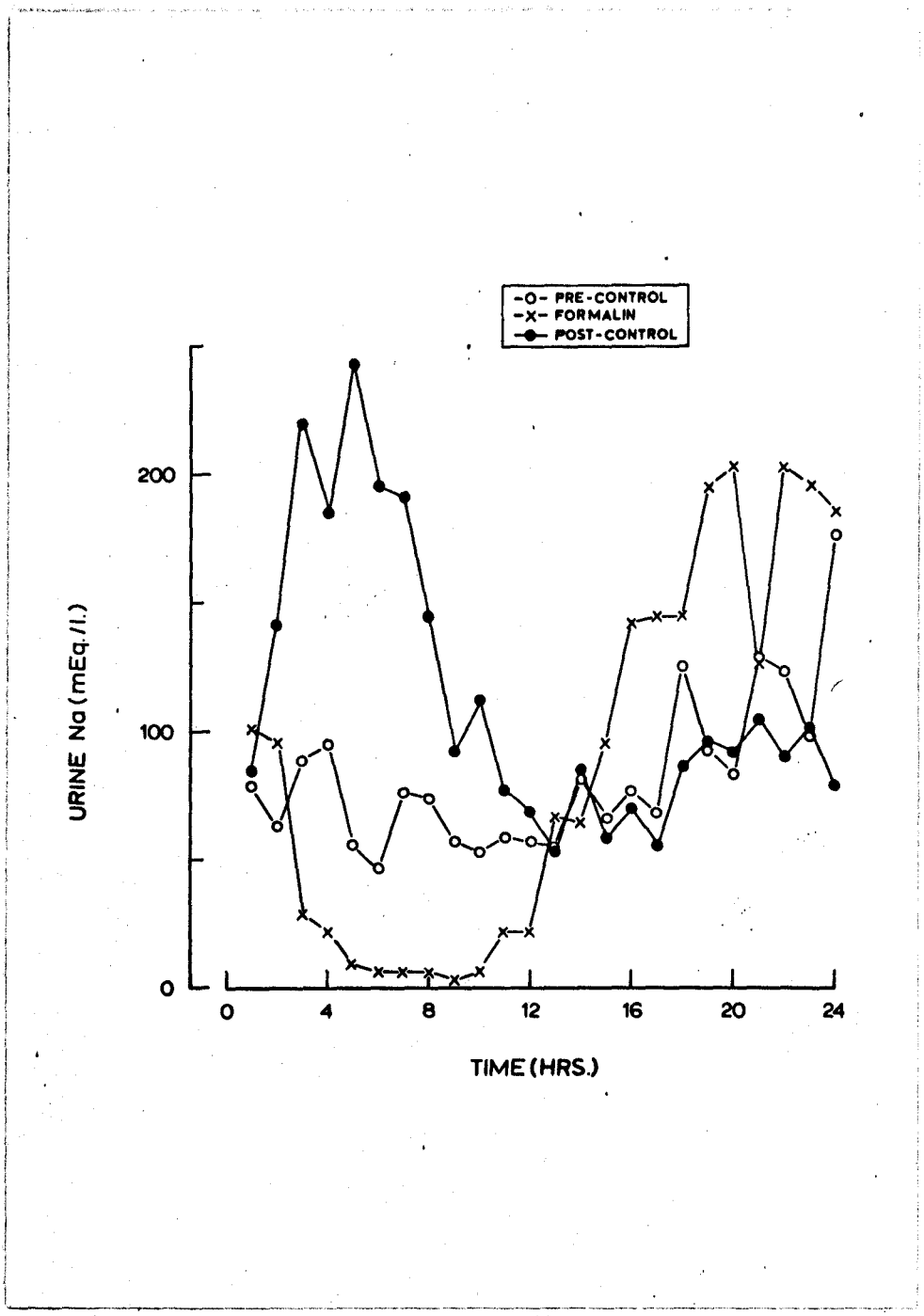

Fig. 7. Urine sodium concentrations during the pre-control, post-control and formalin tests.

TABLE 2

Progressive Changes In Some Blood Parameters In Deprived Rats After Formalin Injection

Time	Hematocrit	g(HOH)kg Serum HOH	g/100 ml. Serum Protein	mEq/l. Serum HOH Serum Na+	Serum K+
Normal	44.9 ± 0.4**	918.5 ± 1.5	5.9 ± 0.4	151.9 ± 1.6	5.1 ± 0.2
	(5)*	(5)	(5)	(5)	(5)
1 hour	50.0 ± 1.2	925.8 ± 1.6	5.7 ± 0.1	144.5 ± 1.9	5.8 ± 0.3
	(8)	(8)	(8)	(4)	(4)
4 hour	59.2 ± 1.1	932.5 ± 1.6	5.1 ± 0.1	143.3 ± 3.0	5.9 ± 0.5
	(8)	(8)	(8)	(4)	(4)
8 hour	58.9 ± 0.1	936.2 ± 1.3	4.8 ± 0.3	144.4 ± 0.9	6.5 ± 0.5
	(8)	(8)	(8)	(4)	(4)
12 hour	54.2 ± 1.8	936.0 ± 1.6	4.9 ± 0.4	147.4 ± 2.4	6.4 ± 0.4
	(4)	(4)	(4)	(4)	(4)
16 hour	54.7 ± 1.2	934.8 ± 1.1	4.9 ± 0.2	146.7 ± 1.7	5.8 ± 0.6
	(7)	(7)	(7)	(4)	(4)
20 hour	52.4 ± 1.0	934.8 ± 2.0	4.7 ± 0.2	147.8 ± 0.3	5.3 ± 0.6
	(7)	(7)	(7)	(4)	(4)
24 hour	47.1 ± 0.9	933.9 ± 2.3	5.1 ± 0.2	148.7 ± 1.4	5.0 ± 0.3
	(6)	(6)	(6)	(6)	(6)

* Number of determinations

** Mean ± SEM

3% NaCl after formalin injection to determine the effect of increased intake of these fluids on various blood parameters (Table 3a). A comparison of these rats with the deprived group indicated that water and salt drinking tended to lessen the effect of formalin treatment on blood volume and constitution. It was clear from individual data that those animals which ingested large amounts of both water and salt yielded near-normal hematocrits and plasma sodium concentrations. In contrast, rats ingesting little salt yielded blood samples which were similar to the deprived animals, regardless of water intakes. This is especially evident in the early periods (1, 4 and 8 hour) when most animals drank water almost exclusively (Table 3b).

The hourly urine sodium concentrations of formalin treated rats having ad lib access to water and 3% NaCl decreased shortly after injection to less than 10 mEq./l but later abruptly increased to more than 40 mEq./l when retention ceased (Formalin group, Fig. 7). This "break" in urine sodium concentration occurred at different times and after different intakes and retentions. To determine whether plasma volume and sodium concentration had been restored by fluid ingestion and retention at the time of the "break", urine samples of a third group of rats (n=14) allowed water and 3% NaCl were analyzed after each excretion and blood was withdrawn within 20 min. of the break (Table 3a). As before, the time elapsed before the "break" varied (from 14.5 hrs to 20.0 hrs) and did not seem related to sodium intake (2.3 - 6.2 mEq. Na) or total water intake (19.5 - 53.0 ml.). Hematocrits were somewhat lower than normal,

TABLE 3a

Progressive Changes in Some Blood Parameters In Formalin Treated Rats Allowed Access To Water and 3% NaCl

Time	Hematocrit	g(HOH)/kg Serum HOH	g/100 ml. Serum Protein	mEq/l Serum Na+	Serum HOH Serum K+
1 hour	48.6 ± 1.6**	929.2 ± 4.3	5.6 ± 0.4	146.0 ± 2.4	5.5 ± 0.5
	(4)*	(4)	(4)	(4)	(4)
4 hour	53.2 ± 2.1	939.4 ± 1.3	4.5 ± 0.3	146.6 ± 2.9	5.4 ± 0.4
	(4)	(4)	(4)	(4)	(4)
8 hour	53.7 ± 1.5	942.6 ± 2.1	4.1 ± 0.3	141.7 ± 1.6	6.5 ± 0.3
	(6)	(6)	(6)	(6)	(6)
12 hour	50.6 ± 1.6	942.0 ± 3.7	4.0 ± 0.1	145.7 ± 1.9	6.1 ± 0.4
	(4)	(4)	(4)	(4)	(4)
16 hour	45.2 ± 2.7	945.1 ± 5.8	3.6 ± 0.1	149.3 ± 1.9	4.7 ± 0.4
	(4)	(4)	(4)	(4)	(4)
20 hour	43.4 ± 1.0	939.9 ± 0.6	4.3 ± 0.2	150.7 ± 0.1	4.3 ± 0.5
	(4)	(4)	(4)	(4)	(4)
24 hour	43.4 ± 0.5	939.3 ± 4.1	4.2 ± 0.4	149.2 ± 1.2	4.3 ± 0.3
	(4)	(4)	(4)	(4)	(4)
Break	41.3 ± 0.2	946.5 ± 2.3	3.5 ± 0.2	152.4 ± 0.4	4.7 ± 0.1
	(14)	(14)	(14)	(14)	(14)

* Number of determinations

**. Mean ± SEM

TABLE 3b

Water and Sodium Balances In Formalin Treated Rats Allowed Access to Water and 3% NaCl

Time	Total H_2O (ml)	Total Na (mEq.)	Urine Vol. (ml)	Urine Na (mEq.)	H_2O Balance (ml)	Na Balance (mEq.)
1 hour	0.4 ± 0.2** (4)*	0.11 ± 0.1 (4)	0.4 ± 0.2 (4)	0.04 ± 0.0 (4)	0.1 ± 0.2 (4)	0.7 ± 0.1 (4)
4 hour	8.0 ± 2.7 (4)	1.26 ± 0.5 (4)	1.9 ± 1.3 (4)	0.09 ± 0.0 (4)	6.0 ± 2.0 (4)	1.2 ± 0.5 (4)
8 hour	17.4 ± 3.0 (6)	1.23 ± 0.6 (6)	3.0 ± 0.5 (6)	0.07 ± 0.0 (6)	14.4 ± 2.7 (6)	1.2 ± 0.5 (6)
12 hour	22.0 ± 2.0 (4)	2.24 ± 0.9 (4)	6.3 ± 1.3 (4)	0.16 ± 0.1 (4)	15.7 ± 3.2 (4)	2.1 ± 0.8 (4)
16 hour	42.1 ± 5.2 (4)	5.66 ± 1.2 (4)	11.5 ± 1.1 (4)	0.63 ± 0.3 (4)	30.6 ± 5.8 (4)	5.0 ± 0.9 (4)
20 hour	37.0 ± 2.0 (4)	5.60 ± 0.7 (4)	14.2 ± 1.8 (4)	0.90 ± 0.3 (4)	22.8 ± 3.6 (4)	4.7 ± 0.4 (4)
24 hour	47.7 ± 5.2 (4)	7.25 ± 0.7 (4)	26.6 ± 7.8 (4)	2.82 ± 0.6 (4)	24.1 ± 3.2 (4)	4.4 ± 0.6 (4)
Break	38.8 ± 2.4 (14)	4.68 ± 0.3 (14)	14.8 ± 3.2 (14)	0.21 ± 0.1 (14)	24.0 ± 2.0 (14)	4.5 ± 0.3 (14)

* Number of determinations

** Mean ± SEM

indicating not only plasma volume restoration but hemo-dilution. Plasma water contents were greatly increased and serum protein levels were extremely low (2.9 - 4.2 gms./100 ml.) as a consequence of this dilution and the protein leakage at the edema. Serum sodium levels were normal as were serum potassium levels.

CHAPTER FOUR

Discussion

The results clearly show the marked increases in sodium intake and renal conservation which follow formalin treatment (Fig. 6 & 7). Low urine sodium concentrations were evident within a few hours after injection, suggesting an increased aldosterone secretion. Sodium appetite appeared slowly in these animals and, although statistically significant four hours after injection, the greatest intakes occurred between 8 and 14 hours (Fig. 4). The high intakes and low sodium losses resulted in an increasing positive sodium balance and ultimately in plasma restoration. With plasma volume and sodium concentration replete, need was no longer present and consequently renal conservation ceased. However, intake of 3% NaCl continued to increase. Post retention appetite was not related to a sodium deficit, not only because Ss bled at the "break" were replete (Table 3a) but also because virtually all of the sodium ingested was rapidly excreted as sodium balance stabilized. Thus sodium appetite seemed not as closely related to need as sodium retention. This was also seen in the pre-control and post-control situations when sodium was not needed

and all of the ingested sodium was excreted (Fig. 6).

Analysis of the results with reference to total water balance (Fig. 5) showed that the hypovolemia induced by formalin injection also caused substantial increases in water intake and retention. Water retention was not as complete as retention of the sodium ingested (83% compared to 97% at 12 hours) probably because of obligatory excretion of cellular debris (urine potassium levels were extremely high, up to 275 - 325 mEQ K+/l) and other metabolic waste products. A word of caution is necessary for the interpretation of the balance information reported here, since the true water balance over an interval of time must also take into account the small amounts of fecal and evaporative water losses. The fact that hypovolemia is a potent stimulus for thirst and renal water conservation has been previously reported (Stricker, 1966).

Water intake and anti-diuresis began shortly after injection (1 - 4 hours) and it was clear that thirst and sodium appetite evolved together. In simple hypovolemia where plasma is withdrawn isosmotically leaving external sodium balance unchanged, thirst always precedes sodium appetite (Stricker & Wolf, 1966; and unpublished observations on PG injected rats). In general, hypovolemic rats (PG treated) exhibit a sodium appetite only after ingesting appreciable quantities of water, whereas formalin treated animals which are concurrently hyponatremic and hypovolemic show sodium appetite and thirst at the same time. This suggests that

hyponatremia potentiates the hypovolemic stimulus for sodium appetite. Furthermore, although hypovolemia induces thirst, large water intakes in PG treated rats are inhibited until sodium appetite begins, suggesting that sodium appetite relieves this inhibition (Stricker, in press).

The magnitude of the need produced by acute sodium deficiency was unexpectedly large. Since the extracellular fluid accounts for 70 to 88 ml. (20 to 25% body weight) in a 350 gm. rat, the positive fluid balance (mean peak = +42.7 ml.) represents an expansion of the extracellular fluid volume of some 45 to 55%. Indeed, the accumulated fluid was quite visibly located in a huge edema at the injection site. In the present experiment the rats retained a slightly hypertonic volume of fluid during the 24 hours following injection (net sodium balance/net water balance = approx. 160 mEq K+/l) which would be expected since these animals were hyponatremic as well as hypovolemic. Water intake was slightly greater than twice the intake of 3% NaCl during the course of drinking, demonstrating that the rats maintained isotonicity in their drinking. Close co-ordination between water and sodium balances might be expected, since the correction of hypovolemia requires the addition of a substantially isotonic NaCl fluid. Water alone would be ineffective since it would not remain extracellular but would also permeate the cells. This was evidenced by the minimal repletion in the early hours after injection despite substantial water intakes. However, some

repletion becomes noticeable with the ingestion of sodium (Tables 3a & 3b). This agrees with reports that isotonic preloads are more effective than water preloads in restoring plasma volume and reducing hypovolemic thirst (Stricker & Wolf, 1967).

Wolf and Steinbaum (1965; also Stricker & Wolf, 1966) reported that in deprived rats serum sodium concentrations were normal 24 hours after formalin injection. In the present experiments the rats also showed some "recovery" in blood parameters (Table 2) despite sodium deprivation, although hypovolemia and hyponatremia were present to some degree throughout the 24 hours after injection. This recovery might be the result of some endogenous mechanism for recovering internal electrolyte balance such as a labile sodium reservoir in bone. Consistent with the "reservoir" hypothesis of Stricker and Wolf (1966; 1968), a slow repletion of this sodium reservoir might also explain the sodium appetite that continued after renal retention ceased. In brief, renal sodium retention would thus be related to deficits in intravascular volume and sodium concentration and would cease when the plasma was replete, whereas sodium appetite would be stimulated by reservoir depletion and inhibited only by restoration of reservoir sodium.

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