SODIUM APPETITE INDUCED BY DIETARY DEPRIVATION: THE PHYSIOLOGY OF ITS DEVELOPMENT AND THE BEHAVIORAL RESPONSES LEADING TO ITS SATIATION

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ABSTRACT

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TO ITS SATIATION

By

Robert John Contreras

The specific hunger for salt is an inherited motivation of the land dwelling animal that is activated in conditions of sodium need. Sodium is the mainstay of the extracellular fluid compartment. Its concentration has an effect on body water distribution and therefore on the proper functioning of the cells of the body. When a nutritional deficit has been incurred, the animal responds in two ways: (1) physiologically through the adrenal-renal axis via aldosterone to promote the reabsorption of sodium, and (2) behaviorally to ingest greater quantities of salt.

In Experiments I and II, research was directed at determining the urinary and blood chemical changes associated with sodium deficiency. The results show that urinary sodium excretion is greatly reduced after one day of sodium deprivation and minimized thereafter. The fall in urinary sodium paralleled the rise in plasma aldosterone concentration. Plasma sodium and plasma protein levels were not associated with sodium appetite. However, a significant increase in

plasma potassium levels was detected after twenty days of sodium deprivation. It was suggested that sodium appetite might be important to combat against hyperkaelemia (high plasma potassium) although the appetite develops long before plasma potassium increases.

The temporal characteristics of drinking saline and distilled water by sodium deprived rats were analyzed in Experiment III. These rats were observed to montonically decrease the proportion of time spent drinking saline as they approached satiation. The dynamic aspects of the behavior underlying this proportional decrease were that drinking bursts remained constant in length and pauses between bursts became longer. It was pointed out that satiation of sodium appetite through saline ingestion was not a stepwise but rather a continuous process, and arguments were made to explain salt intake in terms of an adaptation mechanism.

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By

Robert John Contreras

A THESIS

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To My Parents

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INTRODUCTION

It would be adaptive for fluctuations in the internal milieu to have a direct effect upon the gustatory system. In this case, information about the physiological condition of the organism is transmitted to the sensing system where it is used to determine the relative desirability of various substances in the external environment. Halpern (1967) has summarized the ways that internal changes may influence taste. He divided them into neural, salivary, and vascular factors. The specific appetite for salt is a phenomenon that lends itself quite readily to this problem of internal-external environment interaction. The role of blood in information transfer has not been uncovered, yet.

Several methods have been used to produce the specific hunger for salt in the laboratory. Bilateral adrenalectomy results in the loss of aldosterone, the mineralocorticoid hormone which normally maintains sodium balance by promoting reabsorption of sodium by the kidney. Bare (1949) showed that an adrenalectomized rat ingests significantly greater quantities of salt solution compared to normal controls and to its own preoperative levels. This increase in salt intake occurs across a wide range of concentrations. In need-free rats there is an increasing preference (over water) for the salt

solution as its concentration approaches isotonicity (.9%), with a sharp decline at greater concentrations. Increasing the animal's salt need elevates total NaCl solution intake and shifts the maximal preference point out toward the higher concentration. By implanting a unilateral fistula into the parotid gland of sheep results in a rapid depletion through salivary loss. Denton and his associates (1965, 1969) demonstrated that the amount of saline consumed is quantitatively related to the extent of the animal's deficit. By the method of intraperitoneal dialysis using a glucose solution large amounts of sodium are removed from the body (Falk and Herman, 1961). Subcutaneous injections of formalin (Jalowiec & Stricker, 1970) produced salt appetite in rats by sequestering body fluids at the injection site. A less widely used method, is to place animals on a low sodium diet (Nachman, 1962; Wagman, 1963; Jalowiec and Stricker, 1973).

Studies of sodium appetite have generally found that sodium deficient rats increase their sodium solution intake until the internal environment reaches hydromineral balance. Two types of satiation take place during sodium repletion; one type is mediated through oral and stomach receptors, and the other through a long term metabolic satiation. An immediate or short term satiation refers to the neural changes that occur in response to orogastric stimulation. Long term control arises when the ingestants are absorped into the blood.

Metabolic Control of Salt Appetite

By employing long-term tests, Fregly (1958) found that adrenalectomy in rats led to an increased preference for sodium salts (chloride, sulfate, bicarbonate, and nitrate) but did not produce an increased preference for non-sodium salts such as potassium, lithium, or calcium chloride. Denton (1965) was able to show the same thing in sodium-deficient sheep which developed preferences for NaCl or $NaHCO_3$ but not for potassium, calcium, or magnesium chloride.

Need-free rats prefer to drink a dilute saline rather than water. However, Davenport (1973) was able to eliminate palatability factors in his experiments and demonstrated that need-free rats prefer to drink water rather than isotonic saline. The ingestion of sodium salts is more susceptible to learned preferences based on the beneficial or toxic consequences of metabolism in long term rather than in short term tests.

Taste Control of Salt Appetite

C. P. Richter (1939) proposed that sodium deficiency altered the taste bud membrane in a way that increased its sensitivity to external stimulation. He proposed a peripheral "on" mechanism that would enhance taste receptor acuity in salt need. In a free choice situation, where subjects were allowed to ingest tap water and/or NaCl solution, preference thresholds were measured (Richter, 1939; Bare, 1949). Both experiments demonstrated that the preference threshold for NaCl solution to be significantly lower among adrenalectomized than among normal controls. This result is due to the

increased motivation of sodium deficiency rather than to changes in absolute sensitivity. When adrenalectomized and normal controls are highly motivated to discriminate between water and weak concentrations of saline, psychophysical thresholds for salt detection were the same for both groups of animals (Carr, 1952; Harriman & Macleod, 1953).

Behaviorally, at least, receptor acuity has not been increased by physiological deprivation. Electrophysiological evidence substantiated the idea that absolute thresholds are not altered by sodium deficiency. Pfaffmann and Bare (1950) report that the minimum concentration necessary to evoke increased discharge of the chorda tympani nerve to different suprathreshold concentrations of NaCl solutions. They confirmed that sodium deficiency did not alter the afferent neural response. The response profile of single units may exhibit threshold and suprathreshold differences that would not show up in a whole nerve recording, however. The results from these behavioral and electrophysiological experiments are consistent with the notion that salt preferences are centrally rather than peripherally mediated.

It has been shown that physiological deprivation does not alter the sensitivity of the taste receptors. However, physiological deprivation may alter the adaptive state of the taste system and this can be used to explain salt appetite. This "off" or "stop" mechanism proposes that sodium deficient and normal controls adapt to a salt stimulus at different rates. This mechanism could be peripheral or central in nature. In the first case, sodium deficiency would alter the sodium receptor membrane in a way that would prolong its reactivity to a sodium stimulus. In turn, the peripheral

nerve taste response would adapt less readily in a sodium deficient rat than in a nondeficient one.

A central "stop" mechanism to account for salt appetite would depend upon the hedonic qualities of sensory stimulation. In this instance, all concentrations of NaCl solution would initially taste pleasant to both a normal and sodium deficient rat, but this hedonic quality would persist longer for the deficient animal. In order that gustatory stimulation have an affective component, this input must reach the hypothalamus and/or limbic structures (Pfaffmann, 1960; 1961). Recent evidence (Bernard and Nord, 1971; Norgren and Leonard (1971) suggested that the central tegmental pathways, which emanate from the gustatory pontine nucleus, provide a potential route whereby gustatory inputs, could reach the hypothalamus.

It is believed that information concerning the internal state of sodium balance is transmitted to the gustatory system to modify the response during external sodium stimulation. There is evidence (Bradley, 1973) to show that alterations in the chemistry of the blood perfusing the tongue can produce an identifiable effect on the multi-fiber response of the gustatory nerve (chorda tympani). It would seem that the blood may be an important means of communicating the state of the internal environment to the sensory system of taste. Through this mechanism, preferences for certain food elements may be enhanced or attenuated depending upon physiological need. It is hypothesized, for sodium appetite, that plasma sodium levels perfusing the tongue region affect the gustatory neural response, a decrease in plasma sodium resulting in an increase in the response strength,

as measured by degree of adaptation. to external sodium chloride stimulation.

Before the question could be attacked directly, it was felt that more basic questions needed to be answered. First, it was important to know what blood chemical changes were associated with sodium deficiency. If the blood does indeed alter gustatory neural responses, what causes these effects and how do they arise? Secondly, it was important to find some behavioral support for the adaptation hypothesis. Does the temporal pattern of saline ingestion in a sodium deficient rat suggest an adaptation-like process?

With these thoughts in mind experiments were designed to investigate the relationship between dietary sodium deprivation and salt appetite. Experiment I determined the physiological and behavioral responses of water replete and water deprived rats who were given a dietary sodium depleted diet ad libitum. The relationship between blood electrolyte composition and dietary sodium deprivation was examined in Experiment II. The purpose of experiment III was to study changes in the temporal characteristics of salt preference during a one hour, three bottle test.

In summary, the purpose of these studies was to answer the following questions:

1. What are the effects on those variables, known to be crucial in body water and electrolyte balance, of two diets that are nutritionally balanced, but differ in sodium, potassium, and chlorine content?

2. What changes in urinary and blood electrolyte composition occur in water replete and water deprived rats that are maintained on a sodium deficient diet?
3. Can a preference for salt be induced in rats that are dietary sodium deficient but are not water deprived?
4. What are the temporal characteristics of sodium chloride solution intake as a previously dietary sodium deprived rat drinks to satiation?

EXPERIMENT I

The specific appetite for salt has been produced experimentally by placing animals on a low sodium diet for an extended period of time (Nachman, 1962; Nachman & Pfaffmann, 1963). A preference test, a measure of salt appetite, usually follows this deprivation period. These preference tests are always given to water deprived rats. It was the interest of this experiment to compare changes in variables, known to be crucial in body water and electrolyte balance, in water replete and water deprived rats that were maintained on a sodium deficient diet. The major variables were the levels of sodium and potassium that remained in the blood or were excreted in the urine.

Procedure

Subjects. Animals were 24 male albino rats of the Holtzman strain, 90-100 days old at the start of the experiment. They were individually housed and had Wayne Mouse Breeder Blox and demineralized water continually present. They were situated in a windowless room which was maintained at 22-25 C. The fluorescent lighting of the room was on a 14-10 hour (lights on--500 hour; lights off--1900 hour) light-dark cycle for the duration of the experiment.

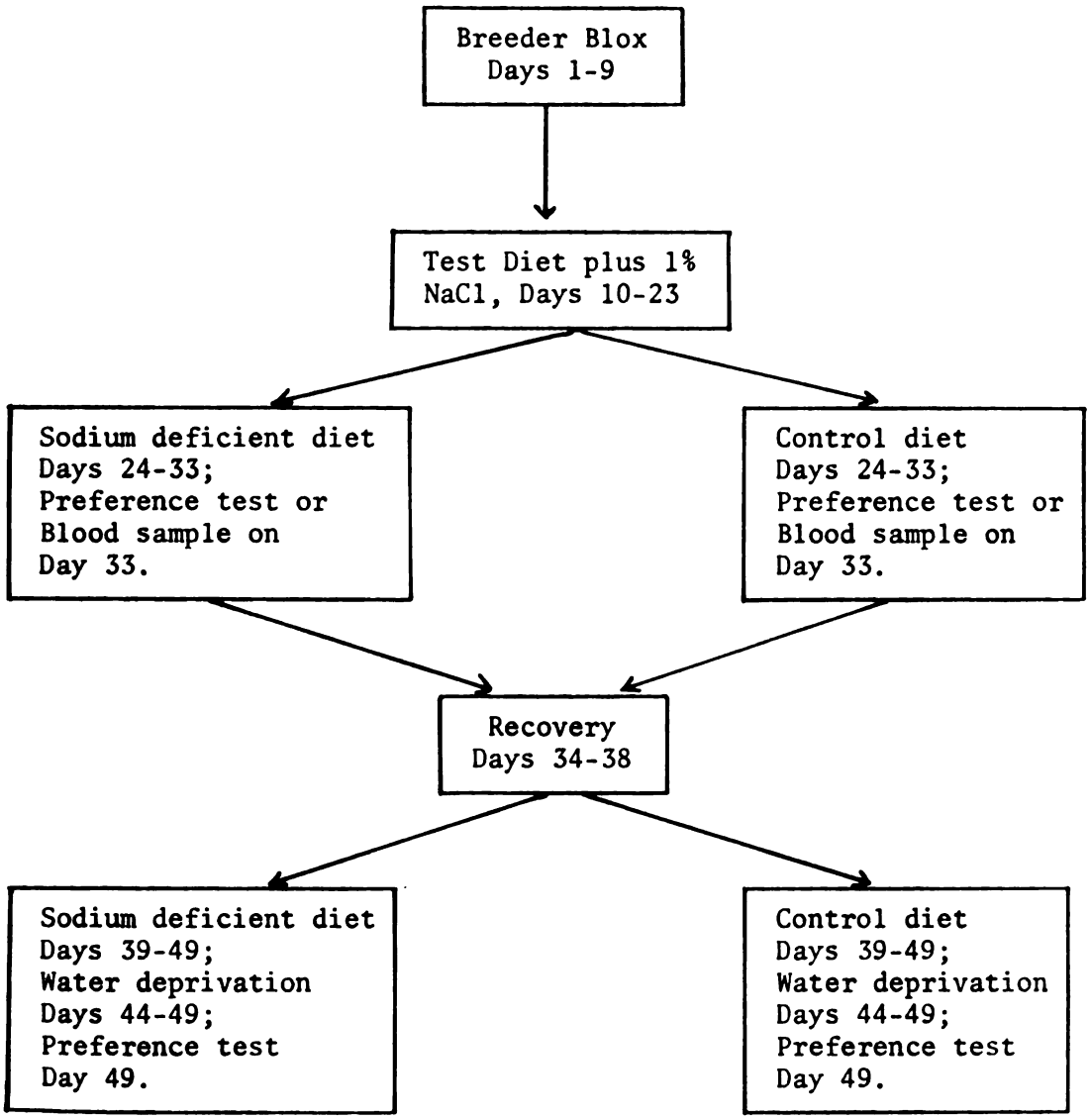
Metabolism cages. Rats were housed in standard Acme Metal Products metabolism cages. A 50 cm. tall base supported each cage.

A total of 12 metabolism cages were arranged on two tables, six per table. The surfaces of the tables were elevated 94 cm. above the floor.

The week before the experiment began, each rat was handled for two minutes a day. These rats were tested in squads of size 12; they were run in the summer and fall of 1972. Depicted by flow chart in Figure 1 and proceeding from the top to the bottom are the different stages of the experiment. For the entire experiment, 24 hour measures of body weight, food and water intake, and urine volume were recorded for each animal. These measures were always recorded between 1000 and 1100 hours. A daily urine specimen from each animal was saved for future analysis of sodium and potassium concentration by flame photometry and total solids by use of a refractometer. Two 100 ml. calibrated drinking tubes were always fixed to the metabolism cage, one cylinder containing de-mineralized water and the other empty. These two cylinders were randomly placed between drinking outlets to discourage position preferences.

Diets. Two powdered laboratory diets, Wayne Mouse Breeder Blox (BB) and a sodium deficient Nutritional Biochemicals Test Diet (TD), were used. A superficial comparison of the diets indicated an odor and texture dissimilarity. When a 1 percent sodium chloride mixture was added to the TD it contained 1.4% potassium, 1.53% chlorine, and .42% sodium. On the other hand, BB contained .77% potassium, .66% chlorine, and .42% sodium. The first nine days (days 1-9) served as a baseline period for input and output variables under BB. The following 14 days (days 10-23) served the same

Figure 1. This is a flow chart depicting the various stages of Experiment I.


Breeder Blox
Days 1-9
Test Diet plus 1%
NaCl, Days 10-23
Sodium deficient diet
Days 24-33;
Preference test or
Blood sample on
Day 33.
Control diet
Days 24-33;
Preference test or
Blood sample on
Day 33.
Recovery
Days 34-38
Sodium deficient diet
Days 39-49;
Water deprivation
Days 44-49;
Preference test
Day 49.
Control diet
Days 39-49;
Water deprivation
Days 44-49;
Preference test
Day 49.

function for the TD which was supplemented with a 1% NaCl mixture. An average body weight was computed for each animal for this 14 day period. From this average, animals were divided equally in number and by weight into four conditions by the following method.

Rats were rank ordered from heaviest to lightest and paired on the basis of body weight similarity. Within each animal pair one animal was randomly designated as either sodium deprived (D=TD, no sodium) or normal (N=TD plus 1% NaCl), and as either preference test (PT) or blood sample (BS). The other member of the pair was automatically designated conditions opposite to those of the first. If the first rat was designated D-PT (sodium deprived--preference test), then the second rat was designated as N-BS (Normal--Blood Sample). Therefore, subjects were divided into two diet groups, 10 days later half of each group were to receive a preference test and the other half a blood sample.

Although Figure 1 shows that all subjects had a 14 day TD baseline, some subjects had more. Only the last 14 days were included in the data. To avoid having preference tests and blood samples fell on the same day one animal pair per day was started on its diet condition. From day 24 till the end of the experiment, the first animal pair was one day ahead of the second pair, two days ahead of the third, and so on.

Blood samples and preference tests were given on day 33. The blood sample was acquired after metabolic data collection, and the preference test immediately followed the blood sample.

Blood sample. The rat was removed from his home cage and taken to a separate room. The subject was anesthetized with ether and a 1.5 to 2 ml blood sample was withdrawn from the left ventricle by inserting a needle of a 2 cc. glass syringe through the rat's rib cage. All samples were centrifuged; a sample of the serum was analyzed for protein concentration in a refractometer, and the remainder was frozen for subsequent analysis by flame photometry.

Solutions. All saline solutions were made as molar concentrations with anhydrous NaCl and distilled water and were kept at room temperature.

Preference tests. A one hour, two-bottle preference test was given to the second rat in his home cage. He was given a choice between .4M NaCl solution and distilled water to drink. The metabolism cage was fixed with two 100 ml gas collecting tubes that were calibrated to a .2 ml and contained drinking solutions. The experimenter sat on a stool behind the metabolism cage so that he could record the amount of fluid intake on a minute to minute basis. Both bottle spouts were introduced into the cage simultaneously. A five second taste sample was allowed from each solution; thereafter, the drinking spout was quickly withdrawn. After both solutions were tasted the drinking spouts were concurrently inserted into the cage and the rat was allowed a one hour access.

A five day recovery period (days 34-38) was instituted such that a 1% NaCl mixture was added to the TD of the experimental group. The control group's diet remained unchanged. Following recovery,

experimental subjects were again deprived of salt. On day 43, both control and experimental subjects were adapted to a 23.0 hour water deprivation schedule. At 1100 hour subjects were removed from their metabolism cage and put into drinking boxes for one hour. Each box was fixed with two 100 ml gas measuring tubes, graduated in 0.2 ml, had Plexiglas covers for observations of drinking, and had a moveable guillotine door which separated the drinking spouts from the rest of the box (for detailed description of apparatus, see Appendix A). Adaptation to this schedule lasted 6 days. The position of each drinking cylinder was randomized, one bottle contained distilled water and the other remained empty. A two bottle preference test was given on day 49. The same method for presenting drinking spouts was used for this preference test as in the previous one. The amount of fluid intake from each tube was recorded for the first 15 sec, 30 sec, and every minute during the 60 min drinking period. Those subjects who were tested in the summer were given a choice between .4M NaCl solution and distilled water; however, a weaker concentration (.3M) of NaCl solution was used to test the second set of animals.

Results

Body metabolism data were grouped into blocks of days in the following manner: 7-9, 10-13, 14-18, 19-23, 24-28, 29-33, 35-38, 39-43, and days 44-49. To eliminate any carry over effects from the treatments day 34 was excluded from statistical analysis. Every metabolic variable was averaged within each block of days for experimental and control groups. A student *t*-test was used to make between

group comparisons and the results of the analysis are located in Appendix B.

The effects that BB and TD had on the metabolism of the albino rat are documented in Table 1. The numerical figures in each cell are the means plus or minus the standard error. These numbers represent an average measure of 24 animals over a three-day period. Days 7 through 9 and days 21-23 were chosen to represent baseline metabolism under BB and TD respectively. As indicated by Table 1, the following variables were significantly different: Water intake (t = 3.541, df = 142, $p < .001$), urine sodium concentration (t = 6.568, df = 142, $p < .001$), total urinary sodium excretion (t = 8.750, df = 142, $p < .001$), and urinary total solids (t = 3.453, df = 142, $p < .001$).

In Figure 2 total urinary sodium excretion is graphed across days. Data points of every third day are plotted through day 39. Thereafter daily measurements are plotted. These third day data points are representative of the overall trend of the data as determined by t-test analysis (see Appendix B). Minor differences that existed between the two groups during dietary baseline or recovery phases of the experiment were nonsignificant (see Appendix B). However, highly significant results were obtained when sodium was removed from the experimental group's diet (see Appendix B). There was no overlap in the distribution of the total amount of sodium excreted in the urine between groups. Every experimental subject excreted less sodium than any control subject each day of both sodium deprivation periods. On the first day of each sodium deprivation period the average urinary sodium loss was .209 mEq. on day 24 and .446 mEq. on

TABLE 1

Effects of Diet on Metabolic Exchange Variables in Holtzman Rats

Metabolic Variables	Mouse Breeder Blox	Nutritional Biochem Test diet + 1% NaCl
Food intake (g)	19.777 ± .268	20.319 ± .311
*Water intake (ml.)	32.708 ± .699	37.736 ± 1.229
Urine volume (ml.)	17.208 ± .528	19.152 ± .965
*Urine Na Conc. (mEq./L.)	190.180 ± 6.280	132.263 ± 6.140
Urine K Conc. (mEq./L.)	168.625 ± 6.126	169.875 ± 8.093
*Urine Na (mEq./Day)	3.105 ± .073	2.230 ± .069
Urine K (mEq./Day)	2.719 ± .063	2.823 ± .076
*Urine-total solids (Refractive Index)	455.375 ± 3.965	434.513 ± 4.527

Note: Values are $\bar{X}$ ± S.E., N = 24 in each cell.

*$p < .05$ in comparison between laboratory diets.

Figure 2. The mean total urinary sodium excreted as a function of time under conditions of: (A) Mouse Breeder Blox food diet baseline; (B) Nutritional Biochemicals Test Diet (plus 1% NaCl) baseline; (C) sodium deprivation; (D) recovery; (E) sodium deprivation and water deprivation in sodium deprived (Exptl.) and normal (Control) rats.

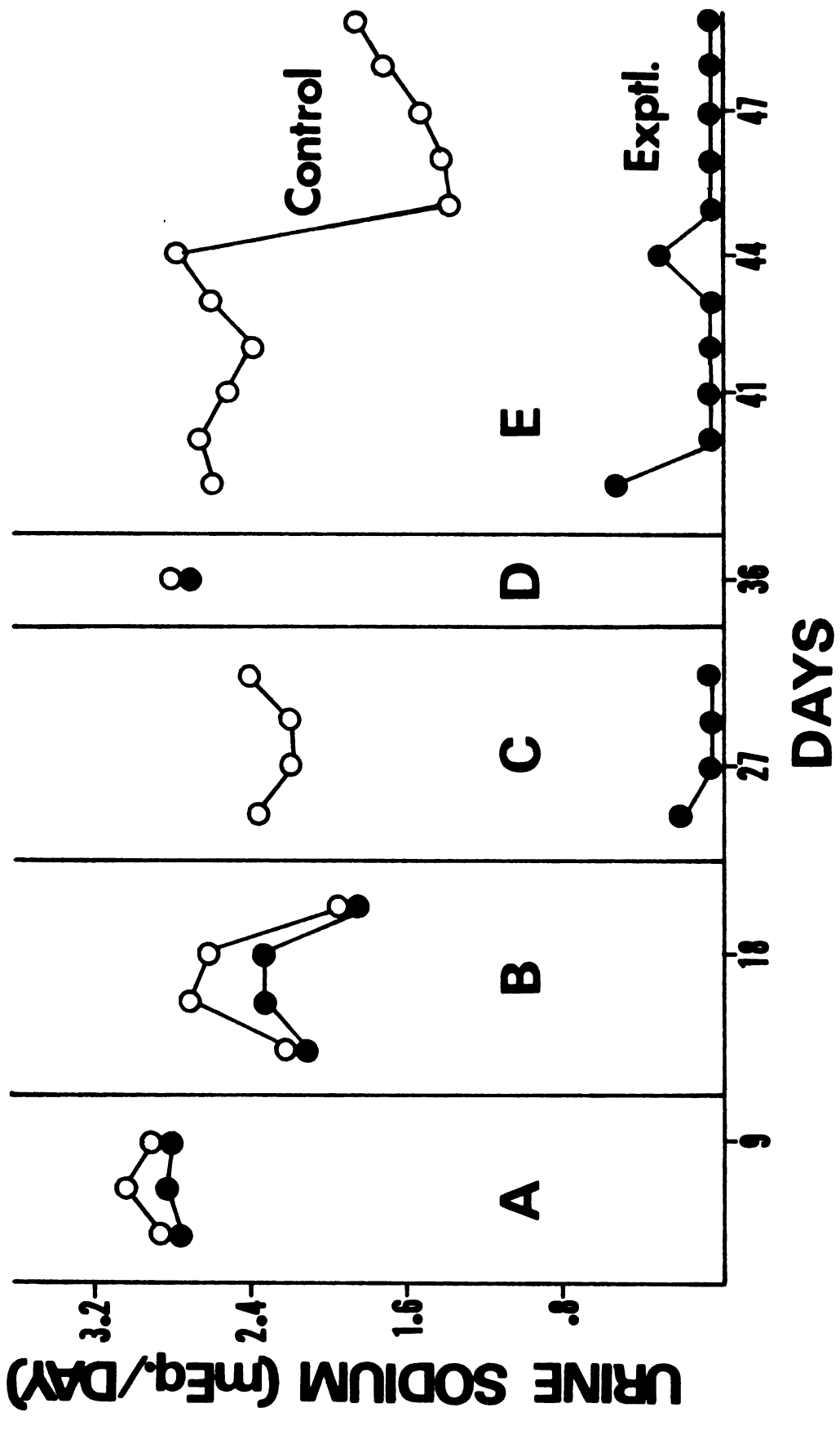

URINE SODIUM (meq./DAY)
3.2
2.4
1.6
.8
A
B
C
D
E
9
18
27
36
41
44
47
DAYS
Control
Exptl.

Figure 2

day 39. On subsequent days except on the first day of water deprivation, the average sodium less was .021 mEq. per day. Each subject's urine sodium output was higher on the first day of water deprivation when compared with its previous day's output (t = 4.600, df = 22, p <.001). Control subjects underwent a similar increase but was nonsignificant (t = .457, df = 22, p > .50). When water deprivation days (45-49) were compared with pre-water deprivation days (39-43), control animals decreased the amount of sodium eliminated in the urine (t = 9.650, df = 118, p < .001). This drop in sodium excretion neither reached the low levels of the experimental group nor did it recover to the level that existed prior to water deprivation.

Total urinary sodium excretion (mEq./day) is a derived measure whose value is determined by the multiplicative relationship: Urine volume (ml.) X urine sodium concentration (mEq./L.). Urine volume and urine sodium concentration measures basic to Figure 1 are summarized in Table 2. A perusal of this table shows that the experimental group urinated more per day than the control group. The experimental group should have lower urinary sodium concentrations to parallel their higher urine volumes. This result is verified in Table 2. When the urine volume factor is eliminated in the total urinary sodium excretion measure, differences between groups during TD baseline and recovery phases of this study were nonsignificant (see Appendix B).

The average amount of sodium excreted in the urine of experimental animals following 10 days of sodium deficiency was .373 mEq., 56 percent of that total was eliminated on the first day. As expected,

TABLE 2

Urine Volume and Urine Sodium Concentration Values
From Sodium Deprived and Control Rats

Days	Urine volume ml.		Urine sodium concentration (meq./L.)	
	Experimental	Control	Experimental	Control
3	19.8	15.2	155.7	193.8
6	20.3	15.3	154.8	200.8
9	16.4	15.2	181.7	205.0
12	25.3	17.8	116.1	137.2
15	27.1	18.8	102.3	158.8
18	22.7	15.0	117.6	178.5
21	21.0	16.0	114.7	160.5
24	19.3	15.7	16.0	164.8
27	22.5	16.9	1.1	139.8
30	22.6	17.1	.6	142.3
33	23.4	18.8	.8	143.2
36	27.7	23.3	113.4	125.4
39	25.5	20.1	16.6	144.8
40	29.6	22.1	1.1	133.3
41	26.0	19.7	1.1	147.0
42	24.0	19.3	1.1	141.9
43	28.0	18.3	.8	168.9
44	7.5	9.1	38.8	298.2
45	5.0	6.1	9.4	220.7
46	5.3	6.0	3.2	236.3
47	6.1	6.4	3.2	235.4
48	5.8	7.3	3.6	247.0
49	5.5	7.5	4.0	247.3

there were no significant differences between experimental and control subjects' serum sodium levels (t = .930, df = 9, p > .10). In Table 3 the values of serum sodium, potassium, and protein are reported for experimental and control groups. The numbers in each cell represent the mean plus or minus the standard error. Sodium deficient animals did not have significantly different serum potassium (t = .130, df = 9, p > .50) and serum protein (t = 1.70, df = 9, p > .10) levels than controls. An insufficient amount of blood was extracted from one control subject to be analyzed and included in the data.

Although the experimental groups' serum sodium level was not appreciably different from that of the controls, sodium deprived subjects preferred to drink a .4 M NaCl solution rather than distilled water in a two-bottle preference test situation. Figure 3 shows the cumulative mean intake of both solutions by water replete subjects. As soon as they tasted the salt solution, the activity level of most subjects increased. Experimental subjects drank significantly more saline than controls (t = 7.67, df = 10, p < .001). In fact every subject drank more NaCl solution than any one of the controls. A preference ratio is the amount of NaCl solution intake divided by the total amount of fluid intake times 100. The preference ratio for experimental subjects was 60% whereas it was 38.4% for controls. Sodium deprived rats drank 12.13 ml. of saline which is equal to 4.35 mEq. of sodium. This amount surpassed their urinary sodium less by almost four milliequivalents. The supplementary intake of water diluted the ingested saline to 1.4% (about .25 M NaCl). The control

TABLE 3

The Values of Serum Sodium, Potassium, and Protein in Sodium Deprived and Control Subjects

	Experimental group N = 6	Control group N = 5
Serum Na	131.16 ± 1.35	133.40 ± 2.20
Serum K	4.83 ± 0.31	4.80 ± .37
Serum protein	6.16 ± 0.13	6.33 ± .10

Note: Values are the $\bar{X}$ ± S.E.

Figure 3. The cumulative mean intake of distilled water and .4 molar saline as a function of time. There were two groups of water replete subjects, those that were sodium deficient (E-NaCl; E-H_2O) and those that were normal controls (C-NaCl; C-H_2O).

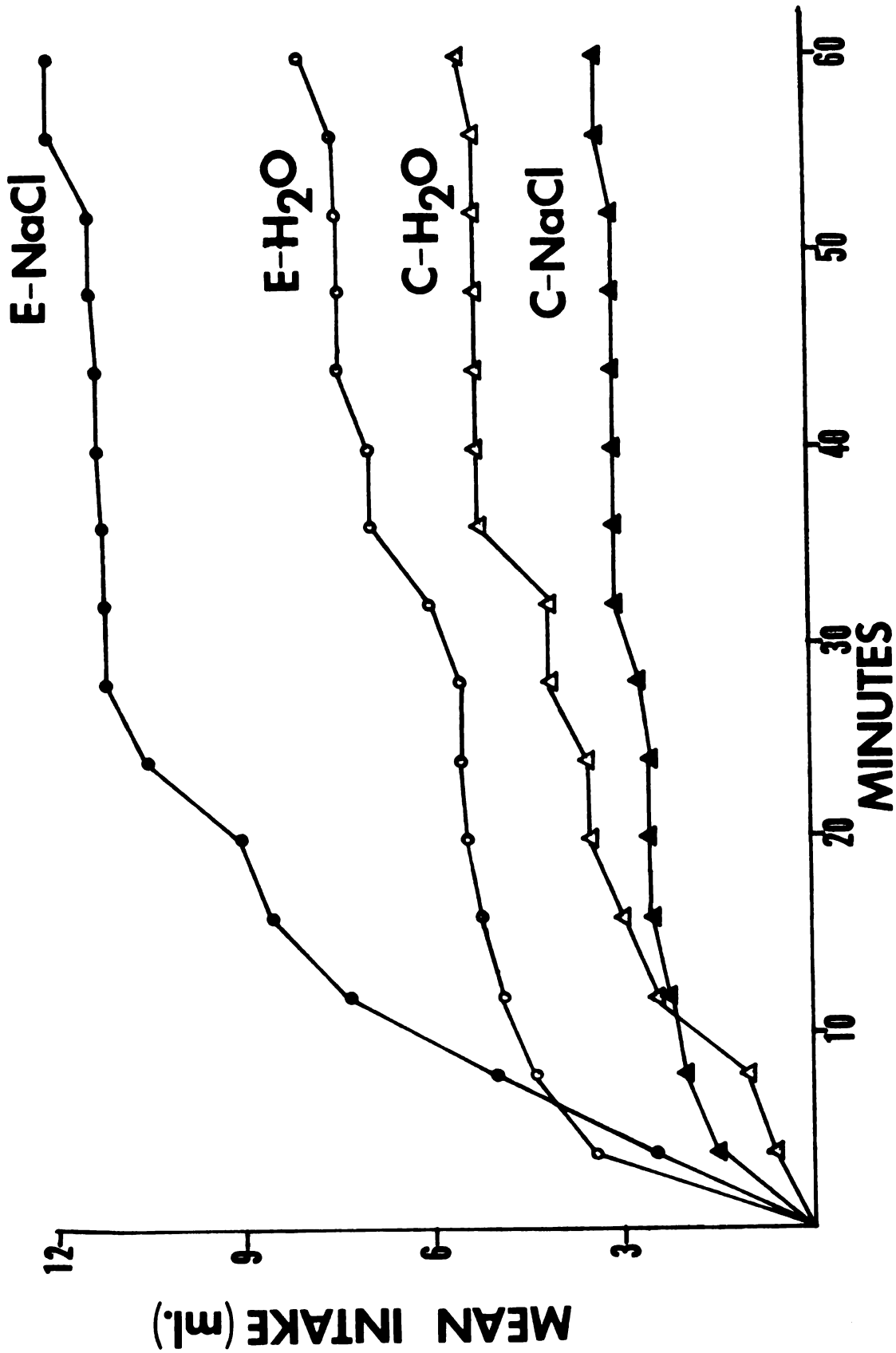

E-NaCl
E-H2O
C-H2O
C-NaCl
MEAN INTAKE (ml.)
12
9
6
3
10
20
30
40
50
60
MINUTES

Figure 3

group, however, drank 1.25 mEq. of sodium and diluted their ingested saline to .89% which is isotonic with plasma.

The preference test data from water deprived rats are graphed in Figures 4 and 5. The cumulative fluid intake of .4M (t = 5.37, df = 8, $p < .001$) and .3 M (t = 3.49, df = 10, $p < .001$) NaCl solution was significantly greater for experimentals than for controls. Their corresponding preference ratios were 45.1% and 39.4% for the experimental group and 16.5% and 24.2% for the control group. Sodium deprived subjects drank 36% more .3 M saline (13.06 ml.) than .4 M saline (9.63 ml.) but drank 56% more distilled water (33.06 ml. vs. 21.33 ml.) as well. In contrast with water replete subjects water deprived rats mix their intakes between saline and water to lower concentrations. The experimental group ingested substantial amounts of sodium (3.828 mEq. of .4 M NaCl; 3.898 m Eq. of .3 M NaCl) but their combined intakes of saline and water were near isotonic levels (1.05% for .4 M NaCl, .69% for .3 M NaCl). Controls, on the other hand, ingested less sodium (1.349 mEq. of .4 M NaCl; 1.871 mEq. of .3 M NaCl) at lower dilutional levels (.38% for .4 M NaCl, .42% for .3 M NaCl).

Figure 4. The cumulative mean intake of distilled water and .4 molar saline as a function of time. There were two groups of water deprived subjects, those that were sodium deficient (E-NaCl; E-H_2O) and those that were normal controls (C-NaCl; C-H_2O).

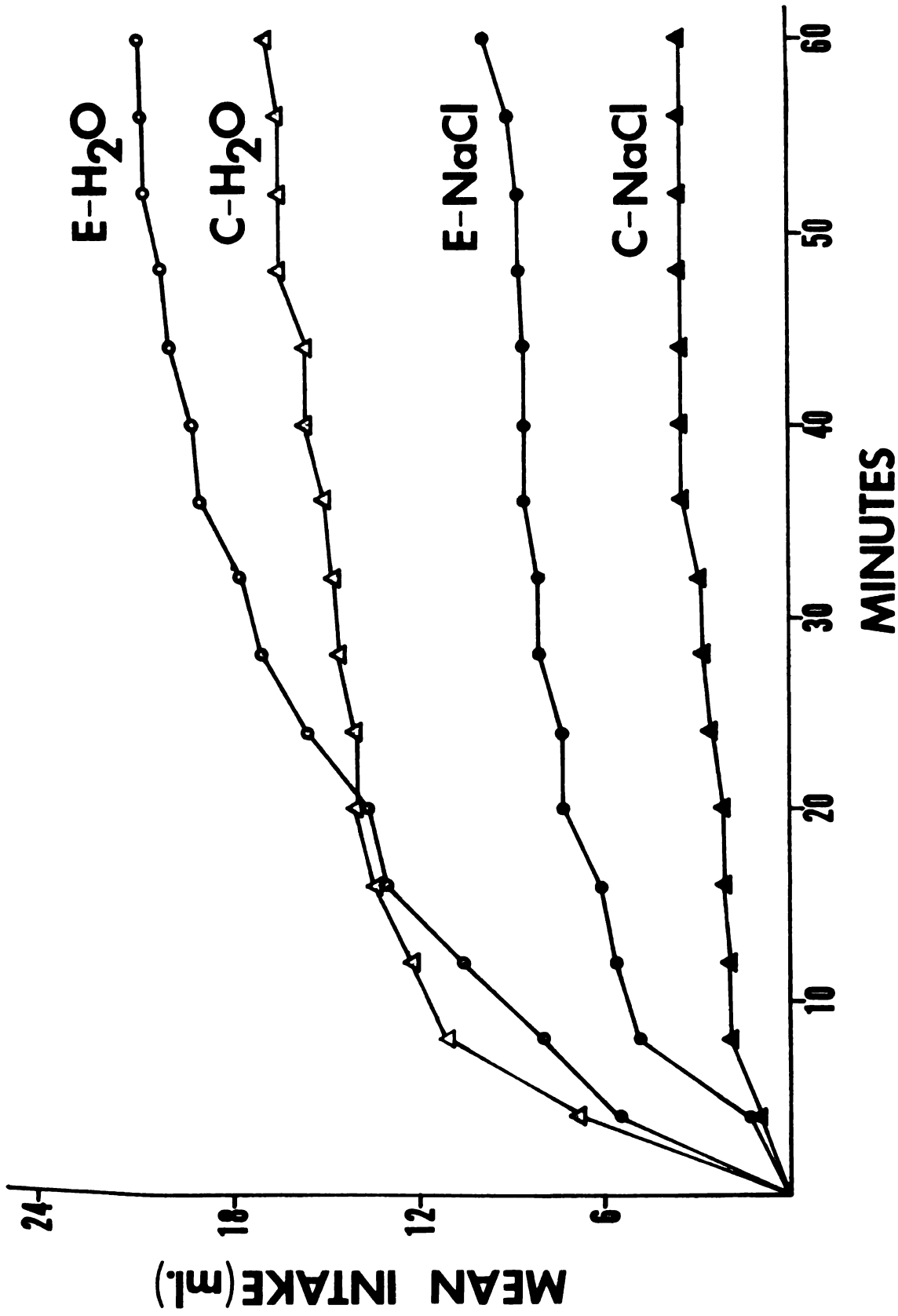

E-H2O
C-H2O
E-NaCl
C-NaCl
MEAN INTAKE(mL)
24
18
12
6
10
20
30
40
50
60
MINUTES

Figure 4

Figure 5. The cumulative mean intake of distilled water and .3 molar saline as a function of time. There were two groups of water deprived subjects, those that were sodium deficient (E-NaCl; E-H_2O) and those that were normal controls (C-NaCl; C-H_2O).

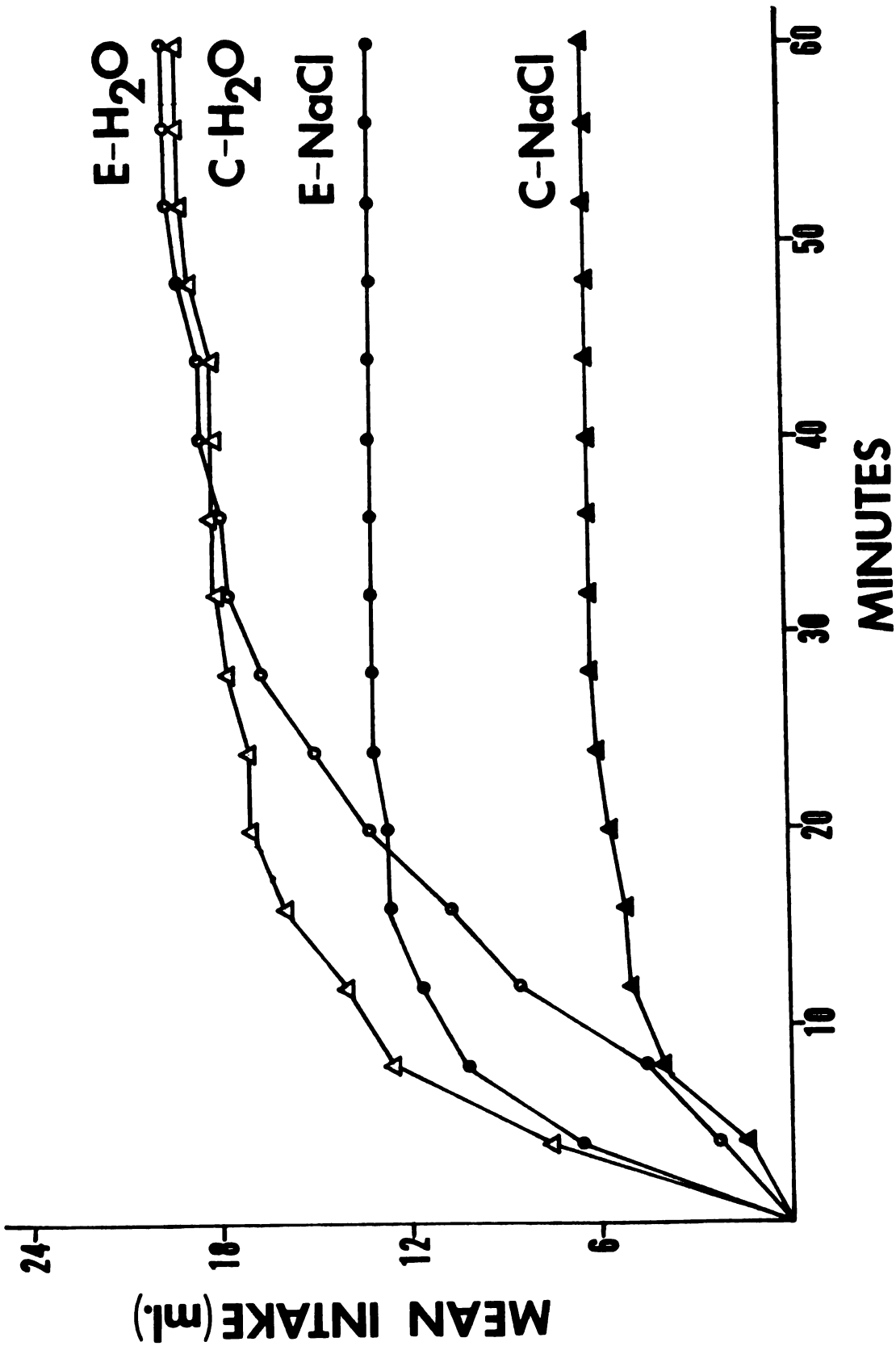

E-H2O
C-H2O
E-NaCl
C-NaCl
MEAN INTAKE (ml.)
24
18
12
6
10
20
30
40
50
60
MINUTES

Figure 5

EXPERIMENT II

The abnormally low serum sodium values obtained in Experiment I were suspect. This experiment was carried out to reassess the role that blood factors have in salt appetite. Blood samples were obtained from rats after 10 and 20 days of sodium deprivation.

Procedure

Subjects. Animals were 12 male albino rats of the Holtzman strain, 90-100 days old at the start of the experiment. They were housed and maintained under the same conditions as described for the animals of Experiment I, with one exception. They were given TD supplemented with a 1 percent NaCl mixture ad libitum instead of BB.

Rats were adapted to the TD for two weeks, during which time individual body weights were recorded daily at 0900 hour. An average body weight was computed over these 14 days for each rat. From this average animals were divided equally in number and by weight into experimental and control groups. The experimental group did not have their diet supplemented with NaCl, but the diet for the control group remained unchanged. Two blood samples were obtained from each animal by heart puncture 10 and 20 days after subjects were divided into groups.

Blood sample. The same blood sampling technique employed in Experiment I was used here, except that the experimenter used heparinized syringes.

Results

Table 4 summarizes the results of Experiment II, and indicates that the absolute levels of blood sodium were higher than those obtained in Experiment I (see Table 3). The experimental group's plasma sodium and plasma protein values were not significantly different from controls after 10 (t = .010, df = 10, p > .50; t = .323, df = 10, p >.50) and 20 (t = .344, df = 8, p > .50; t = .239, df df = 8, p > .50) days of sodium deficiency, respectively. Unexpectedly plasma sodium increased for both groups; however, when the data from the two groups were combined no significant increases were found (t = 1.590, df = 21, p > .10). The overall plasma protein level marginally decreased from 6.59 to 6.37 when the data from both groups were combined at 10 and at 20 days, respectively (t = 1.880, df = 20, .10 < p < .05). Findings from a between-group comparison of plasma potassium show insignificant differences after 10 days (t = .843, df = 10, p < .10), but a significant increase was obtained in 20 day sodium deficient rats over controls (t = 2.904, df = 9, .05 < p < .02).

Discussion of Experiments I and II

Body metabolism. Although it was not a crucial aspect of this thesis, baseline comparisons were made between an unfamiliar diet with one that had been used extensively. This meant comparing

TABLE 4

The Values of Plasma Sodium, Potassium, and Protein in Sodium Deprived and Control Subjects

	Experimental group	
	10 day	20 day
Plasma Na	143.00 ± 1.417	148.20 ± 4.771
*Plasma K	4.46 ± .077	5.33 ± .285
Plasma protein	6.53 ± .120	6.350 ± .051
	Control group	
Plasma Na	142.90 ± .945	146.40 ± 2.616
Plasma K	4.53 ± .059	4.45 ± .146
Plasma protein	6.58 ± .109	6.38 ± .108

*Significant difference, $p < .05$.

changes in the metabolic variables crucial for maintaining hydromineral balance in rats maintained on a standard diet (BB) with those maintained on the experimental test diet (TD + 1% NaCl). Baseline data revealed elevated water intakes for those rats maintained on TD over those with BB, although equal volumes of urine were eliminated. Ostensibly, the data imply that blood volume was higher in TD fed rats. This is reasonable since TD had a higher mineral content (1.4% potassium, 1.53% chlorine versus .77% potassium, .66% chlorine) so more water was needed to dilute the metabolites to isotonicity. The flexibility of urinary sodium loss should be underscored because the urinary levels of potassium were not raised but that of sodium was lowered to satisfy homeostasis (Table 1). This drop in urinary sodium excretion was enough to be reflected in the urinary total solids measurement, too. Potassium and sodium excretion levels are complementary processes since renal potassium absorption coincides with sodium reabsorption (Ganong, 1971, p. 525). Therefore, an increased concentration of potassium might be expected to accompany a decreased sodium output. Although urinary potassium levels were not significantly greater in TD fed rats, the importance of reduced sodium loss may rest with the need to excrete potassium (Michell, 1972).

The dynamic picture of sodium exchange is understood best by examining urinary excretion levels under different conditions of nutrition. Urinary sodium levels are sensitive to subtle changes in intake since excretion levels fluctuate in order to maintain internal constancy. Variations of sorts in the amount of sodium excreted per day are conveyed in Figure 2. As previously mentioned, baseline

levels of sodium were different under diets that differed in their mineral content.

A most important deviation occurs when sodium is subtracted from the TD. The rat responds quickly by decreasing its sodium output to "obligatory" (Chew, 1965) amounts. This minute sodium loss is a consequence of the physical environment and energy metabolism of the rat. These losses are unavoidable and are responsible for the eventual deficiency. After ten days of sodium deprivation approximately .3 to .6 mEq. of sodium loss were associated with increased saline preference. The major portion of these losses occurred within the first couple of days, thereafter mineralocorticoid levels evidently increased enough to facilitate virtually complete renal sodium retention (Bojesen, 1966; Marusic & Mulrow, 1967; Jalowiec & Stricker, 1973). When sodium was returned to the deficient diet sodium excretion levels were quick to return to normal. The urinary sodium excretion pattern that was characteristic of the first sodium deprivation period was also characteristic of the second. These rats re-experienced a rapid reduction in sodium excretion, although the first day's loss was larger this time. By the third day of deprivation sodium losses were again minimal.

A peculiarity of the second sodium deficiency period was the introduction of water deprivation that offset the ordinarily low quantities of urinary sodium loss. Water deprivation was signaled by an increase in the amount of sodium excreted. This finding is compatible with the results obtained with Walters' (1973) investigation. This occurrence was probably induced by an increase in osmotic stress

as a consequence of an increased food to water intake ratio. The quantity of food ingested was reduced to approximately two-thirds of their normal amount, but that of water was reduced to about 50%. In the interest of preserving intracellular fluid volume the concentration of the extracellular fluid was reduced. On subsequent days of the water deprivation regimen, sodium elimination was reduced to pre-water deprivation levels. These urine sodium losses remained low although food intake progressively increased but water intake and urine volume remained constant. In this instance, osmotic stress was relieved by an increased potassium output. Increased plasma aldosterone concentrations probably prevented sodium elimination (Marusic & Mulrow, 1967).

Water deprivation only slightly increased the control group's urinary sodium output. A comparable description of the regulatory determinants that underlie the increase has already been stated for sodium deficient rats. Following this increase in sodium output, urinary sodium dropped to its lowest level and then began to gradually increase as the animals adapted to their water deprivation schedule. This increase is due primarily to the fact that they eat more food. This increased osmotic stress did not result in higher water intakes as Hatton and Almli (1967) had found. Water intake remained relatively stable, but the concentrations of sodium and potassium increased to appease the osmotic load. Apparently, the animals were already ingesting asymptotic amounts of water during the hourly drinking period. Hatton and Almli (1967) gave their subjects a .5 hour free access to water.

The experimental group's urine output was significantly higher than control values throughout the study and was attributed to population differences in water exchange and not to experimental manipulation. Water intake was also higher in the experimental group. Although the two groups were equated on the basis of body weight, this did not insure equivalence in fluid exchange. However, these differences were overlooked because similar baseline values of total urinary sodium excretion were found between the two groups.

Preference tests. Short-term preference tests are usually given only to water deprived rats. This procedure makes it difficult to attribute preference behavior to taste factors because of the added motivational dimension of thirst confuses the situation. However, in this study, a short term preference for salt was established in sodium deficient rats that were water replete (Figure 3). As soon as these animals tasted the salt solution their general activity level increased substantially. They drank saline quite readily. They drank sparingly from the distilled water cylinder to supplement their salt intakes. Control subjects initially showed interest in the salt solution, but it subsided after three milliliters had been ingested. Clearly, sodium deficiency altered the acceptability of saline, as control animals drank more water than saline whereas experimental subjects did the converse. The combined intake of water and saline was hypertonic in sodium deficient rats and isotonic in control rats. This result is in conflict with Jalowiec's and Stricker's (1973) results as they observed their adrenalectomized rats to supplement their saline intakes with enough water to dilute their intake to

isotonic levels. This difference may be attributable to the elevated plasma aldosterone concentrations in dietary sodium depleted rats potentiating their sodium appetite. As in the case with adrenalectomized rats, sodium deficient rats overcompensate their sodium losses by ingesting much more than they excreted. The reason for this overshoot may be caused by learning factors which result from association of sodium taste with recovery from sodium deficiency as McCutcheon and Levy (1972) submit.

Water deprivation tended to reverse the emphasis in drinking. On a percentage basis, experimental animals drank more water than saline. The cumulative volumetric intake curves (Figure 4) for saline intake and water intake correspond with those of Nachman and Pfaffmann (1963). The sodium deficient subjects readily ingested .4 M saline whereas control subjects drank less. For both concentrations (.4 M and .3 M) of saline, ingestion increased for approximately the first twenty minutes, thereafter it tapered off. Decreasing the concentration of salt solution to .3 M (Figure 5) increased the rate of saline ingestion. Since the animal is motivated by a salt need and a water need, he initially drinks more saline to satisfy both needs. As he drinks saline, his salt need becomes satisfied, but if he continues this course his body fluids will become hypertonic to the plasma. Therefore, his preference for water remains steadfast. Osmotic stress and sodium satiation are induced more slowly while drinking a less concentrated salt solution.

These curves of cumulative volumetric intake produced a negatively accelerating curve. If the assumption is made that

volumetric intake is a linear function of the time spent drinking, then a cumulative curve will show negative acceleration if either burst duration decreased and/or interdrink interval increased (Allison & Castellan, 1970).

EXPERIMENT III

The standard measurement in a two-bottle preference test has been the amount of solution taken in over a given period of time. The emphasis in most of these experiments has been on the manipulation of the bodily state and how this affects intake. The purpose of this experiment is to analyze the immediate intake pattern of rats deprived of sodium and that of control rats, in particular their preference intake of distilled water and saline. The purpose of this approach is to elucidate taste factors in the restorative process of sodium replenishment.

Procedure

Subjects. Animals were 24 male albino rats of the Holtzman strain, 90-100 days old at the start of the experiment. They were housed and maintained under the same conditions as described for the animals of Experiment I, except that they were given TD supplemented with 1 percent NaCl mixture ad libitum instead of BB.

Apparatus. Three 100 ml graduated cylinders, fitted with rubber stoppers and glass drinking spouts, were attached to the front of each cage with broom clamps. The drinking cylinders were randomly interchanged among the broom clamps each day. Two cylinders

were filled with demineralized water and the third remained empty. A drinkometer was connected to each fluid containing cylinder. Licks on the drinking cylinders were recorded on cumulative recorders (moved at a speed of .33 mm/sec).

Two independent groups of rats were run in squads of size six because there were only 12 drinkometers available. Subjects were adapted to TD and divided into two groups by the same manner as described for the animals of Experiment II. All animals were given two preference tests under two conditions of water balance following the same sequence of dietary regimens as described for the animals of Experiment I (see Table I). Individual body weights and fluid intakes were the only metabolic variables recorded each day for the duration of the experiment. Body weight was recorded at 0800 hour and a one hour measurement of fluid intake was recorded over the ensuing hour with drinkometer-recorder circuit turned on. Although the animals had 24 hour access to the drinking spouts except during water deprivation, fluid intake was only measured during this hour.

Solutions. NaCl solutions were made the same way as in Experiment I.

Preference tests. Each rat was given a five second taste sample from a drinking cylinder that contained a .4M NaCl solution and from one containing distilled water; thereafter, the drinking spouts were withdrawn. A moveable masonite door, one quarter inch thick, was placed on the inner front surface of each cage which separated the three drinking spouts from the rest of the cage. The drinking cylinders were reattached to the cage, the

drinkometer-recorder circuit was turned on, and the masonite doors were removed to give the animals a one hour access. In the water replete condition, subjects were not given taste samples prior to preference testing.

The basic datum that was collected was whether the subject was drinking or not drinking. Drinking was indicated by an upward deflection of the pen of an event marker. Licking characteristics of drinking were beyond the resolution of the cumulative recorder. The criterion for identifying successive drinking periods was the shortest distance X traveled by the pen of the event marker such that two successive contacts with a drinking spout can reasonably be regarded as members of two different bursts. This distance was approximately .33 mm.

Results

Interdrink intervals (offset to onset, IDI), and burst duration (onset to offset, BD) are recorded in Table 5 for salt solution drinking only. Both IDI and BD were analyzed across the first 10 minutes and the last 50 minutes of the one hour preference test for both water replete (WR) and water deprived (WD) subjects. As can be seen, the average interval between drinking bursts increased in the last 50 minutes when compared to the initial 10 minutes of the test period. This result occurred under both conditions of water hydration.

Water replete. All but one subject's average IDI was longer during the last 50 minutes of the drinking period compared to the

TABLE 5

Characteristics of Drinking .4 M Saline by Water Replete and Water Deprived Rats

IDI = interdrink interval

BD = burst duration

DD = drinking duration, 1st encounter

PR = preference ratio

	Water replete			
	Control		Experimental	
	10 minutes	50 minutes	10 minutes	50 minutes
IDI	.575 min.	1.790 min.	.218 min.	1.128 min.
BD	.335 min.	.219 min.	.364 min.	.251 min.
	Water deprived			
IDI	1.087 min.	11.399 min.	.125 min.	2.483 min.
BD	.395 min.	.373 min.	.492 min.	.304 min.

	Water replete		Water deprived	
	Control	Exptl.	Control	Exptl.
DD	1.200 min.	5.992 min.	.900 min.	5.656 min.
PR	51.42%	73.95%	28.07%	54.33%

first 10 minutes for both control (sodium deficient plus 1% NaCl) and experimental (sodium deficient diet) animals. The experimental group exhibited a significantly lower IDI for the first 10 minutes of drinking than the corresponding value for the control group (t = 2.418, df = 10, $p < .05$). There were no differences between groups in IDI for the last 50 minutes, however. Because of these differences in IDI, a cumulative frequency distribution of IDI is graphed in Figs. 6 through 8. There were proportionately more short IDIs and fewer long IDIs for the experimental group than for the control group. Furthermore, the number of long IDI increased proportionately for both groups as the drinking period progressed. Statistically, BD remained constant across the entire drinking session for both groups (F = .421, df = 3/9, $p > .20$). However, it appears to decrease during the last 50 minutes.

The distribution of time spent drinking salt solution and distilled water is graphed in Figs. 9 through 12. It is evident that experimental subjects spend more time drinking salt solution than control subjects. Two distributions of the time spent drinking were graphed for each group and were based upon whether the subject started drinking salt solution or distilled water first. Out of 12 rats, 9 started with the salt solution.

Other characteristics of drinking behavior are described in Table 6. Drinking duration (DD) was defined as the amount of successive drinking time from one solution before switching to another solution. Successive drinking bursts were combined as long as the rat continued drinking from the same solution. Pauses between bursts were not included in the computation. When the DD for the first

Figure 6. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the first 10 minutes of the preference test. There were two groups of water replete rats, one group was fed a sodium deficient diet (Exptl.) and the other was a normal control (Control) group.

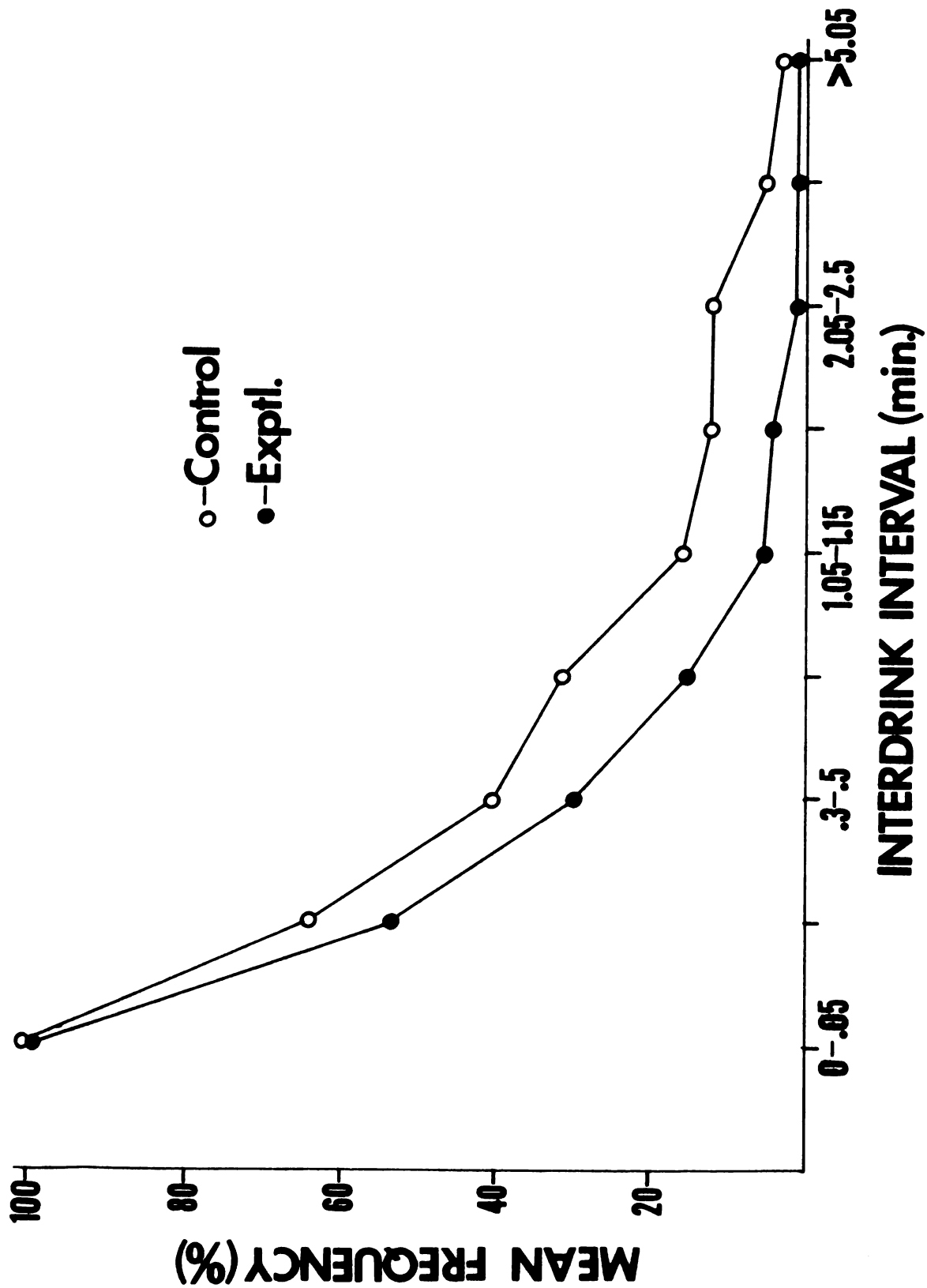

MEAN FREQUENCY (%)
100
80
60
40
20
o–Control
•–Expt.
0–.05
.3–.5
1.05–1.15
2.05–2.5
>5.05
INTERDRINK INTERVAL (min.)

Figure 6

Figure 7. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the second 10 minute period of the preference test. There were two groups of water replete rats, one group was fed a sodium deficient diet (Exptl.) and the other was a normal control (Control) group.

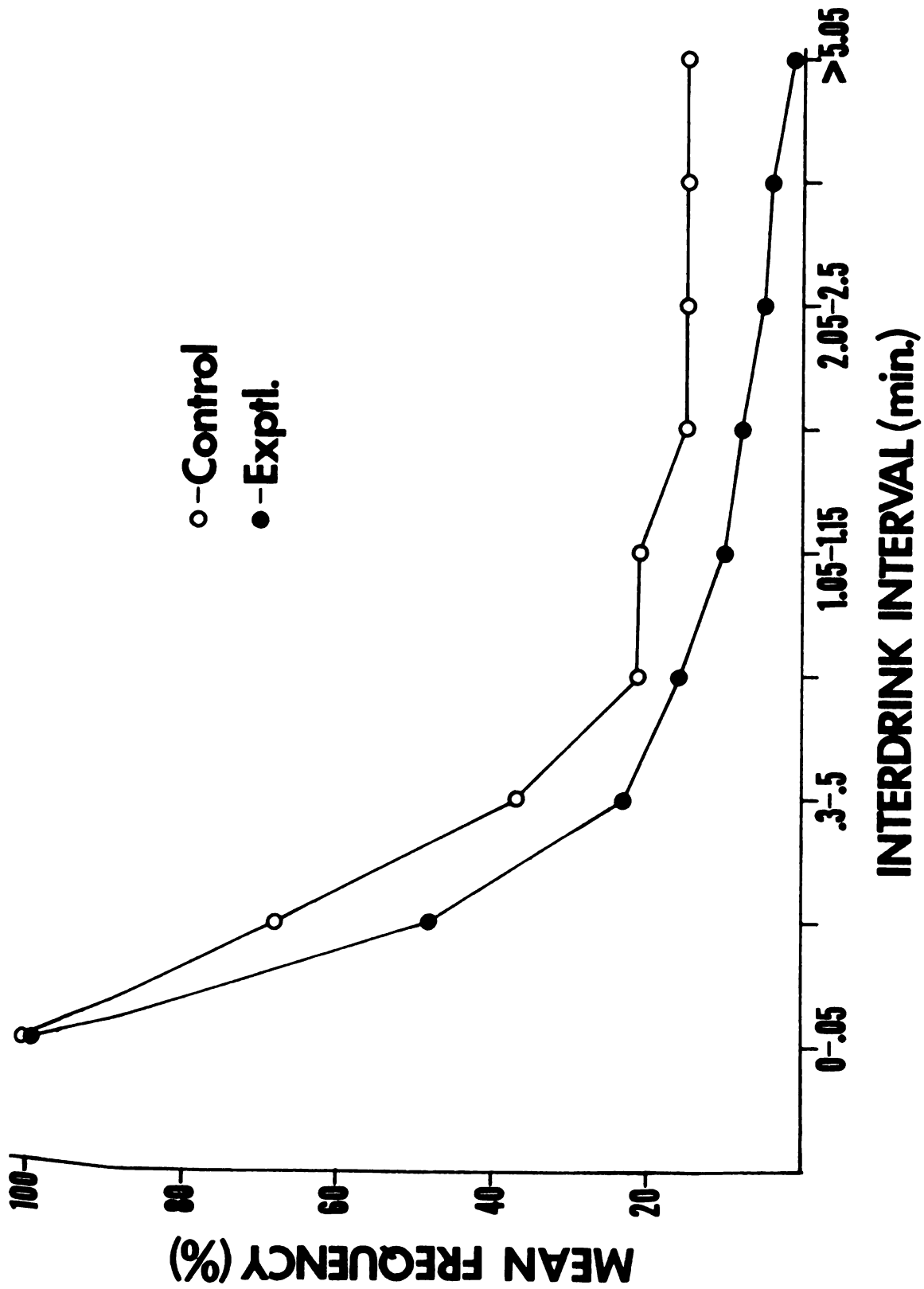

MEAN FREQUENCY (%)
100
80
60
40
20
0-.05
.3-.5
1.05-1.15
2.05-2.5
>5.05
INTERDRINK INTERVAL (min.)
o-Control
•-Exptl.

Figure 7

Figure 8. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the last 40 minutes of the preference test. There were two groups of water replete rats, one group was fed a sodium deficient diet (Exptl.) and the other was a normal control (Control) group.

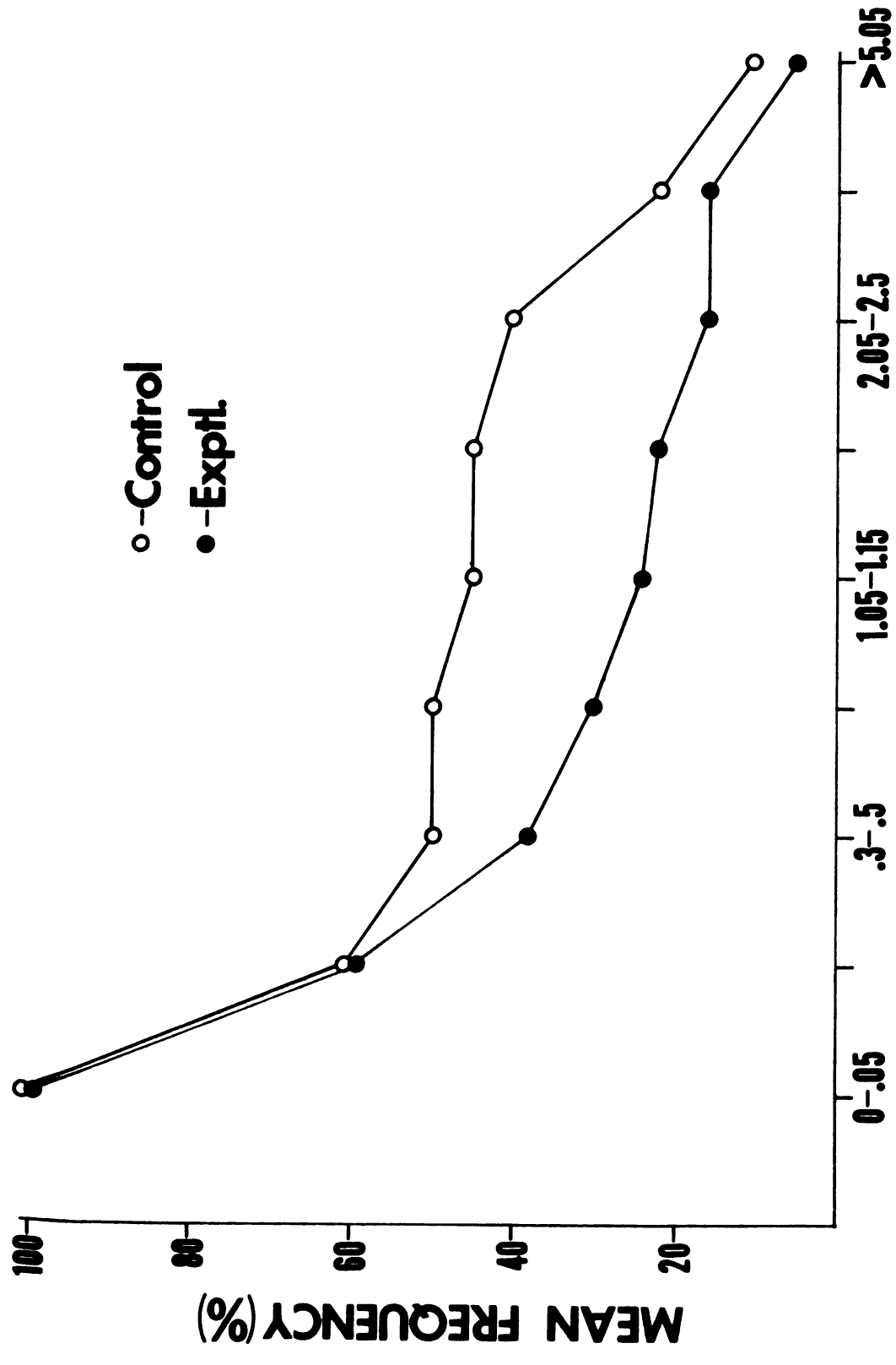

MEAN FREQUENCY (%)
100
80
60
40
20
0-.05
.3-.5
1.05-1.15
2.05-2.5
>5.05
INTERDRINK INTERVAL (min.)
o-Control
●-Expt.

Figure 8

Figure 9. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water replete, normal controls, and drank from the distilled water bottle first.

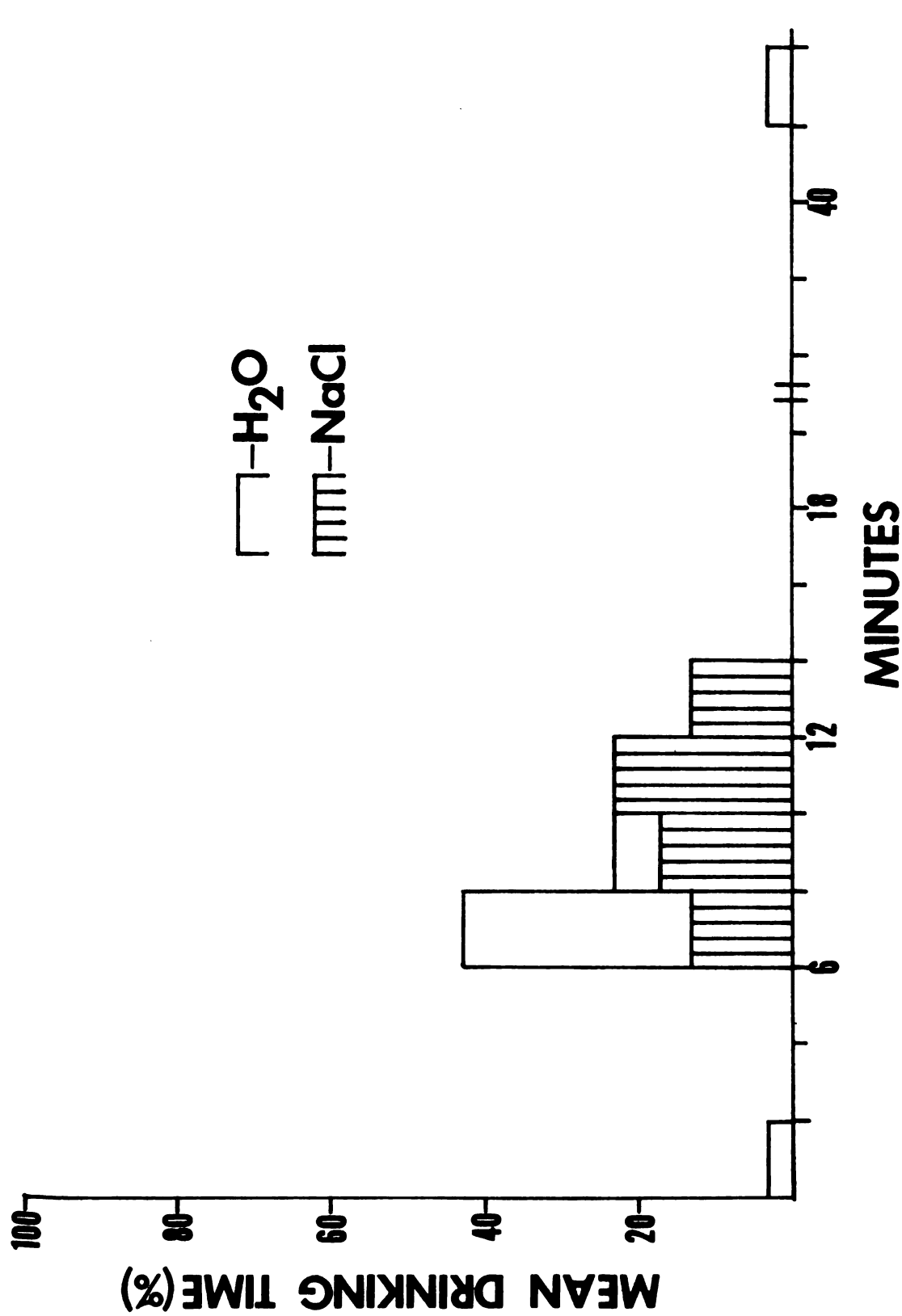


Figure 9

Figure 10. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water replete, sodium deficient, and drank from the distilled water bottle first.

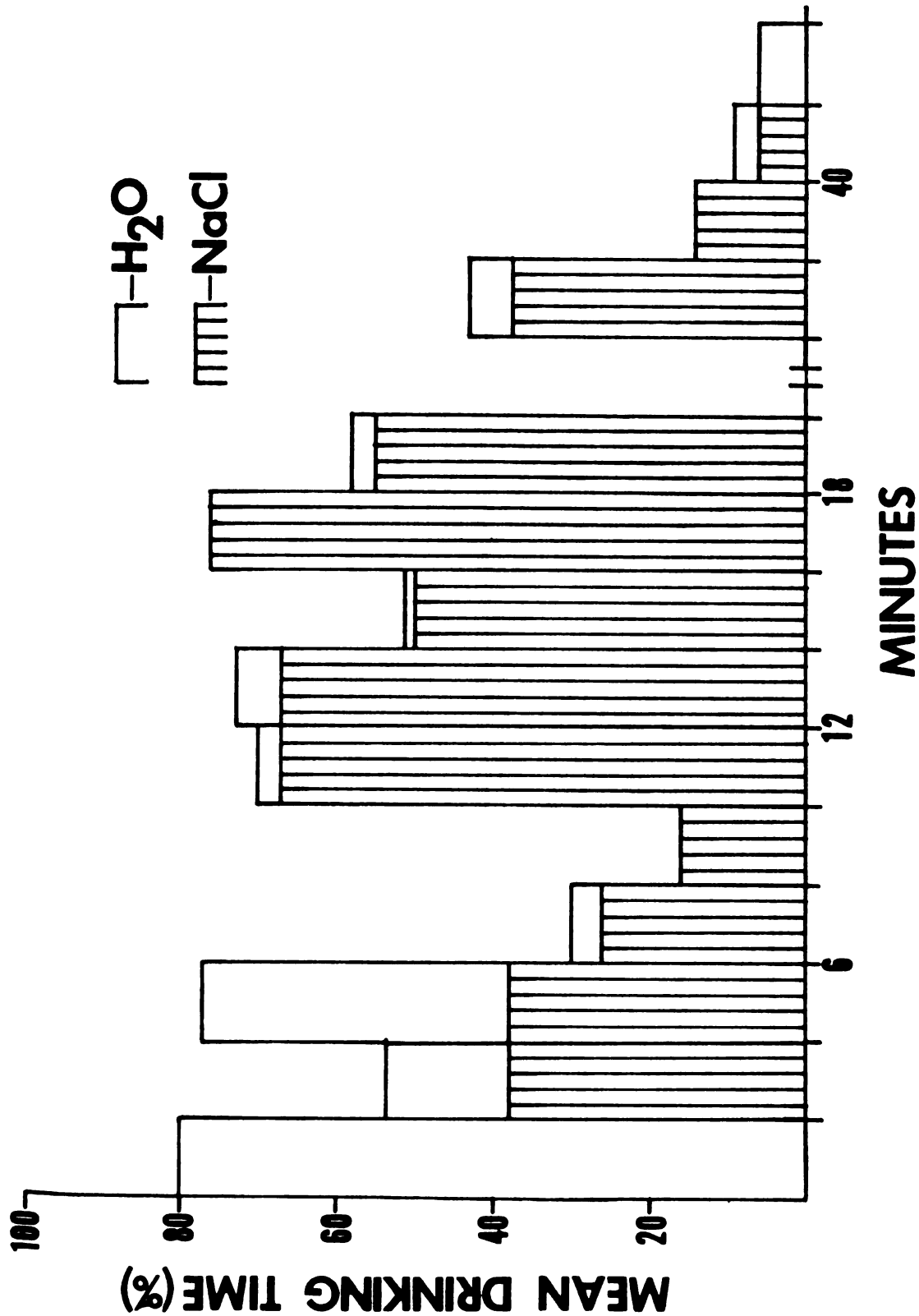


Figure 10

Figure 11. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water replete, sodium deficient, and drank from the saline bottle first.

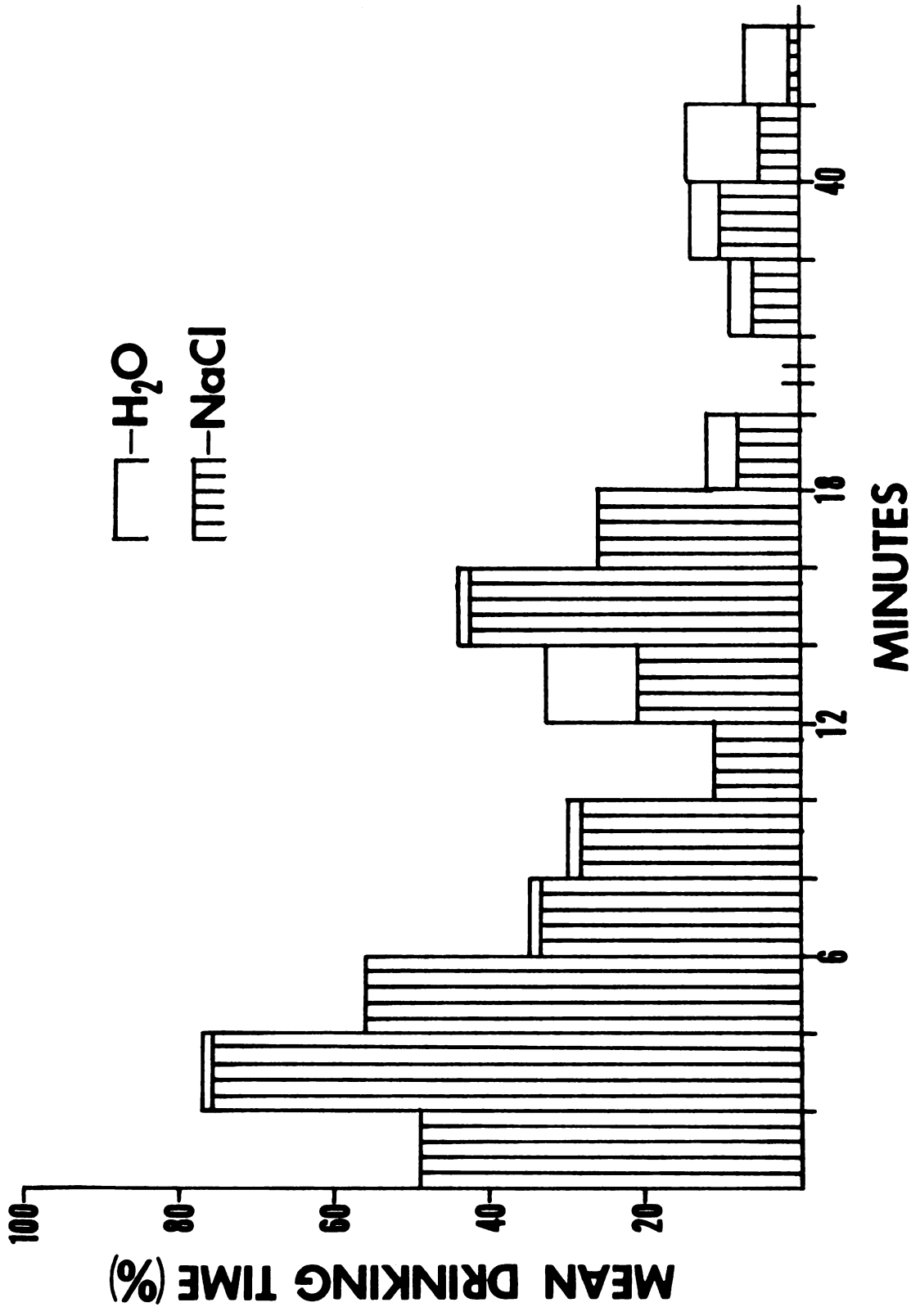


Figure 11

Figure 12. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water replete, normal controls, and drank from the saline bottle first.

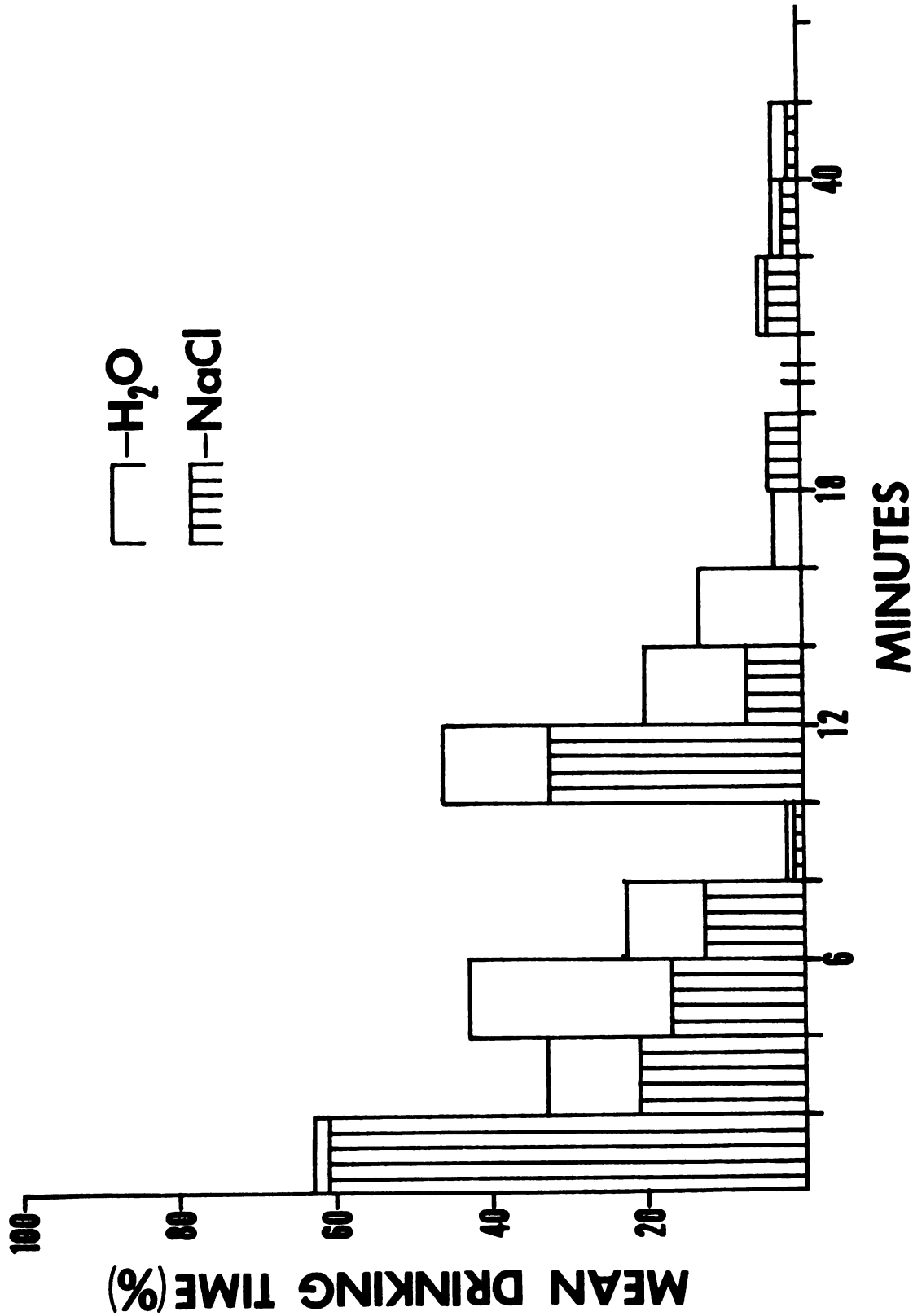


Figure 12

TABLE 6

Characteristics of Drinking by Water Replete and Water Deprived Rats

	Water replete			
	Control		Experimental	
	H_2O	NaCl	H_2O	NaCl
A.	2.008	3.492	2.933	11.158
B.	.794	1.046	1.157	4.598
C.	3.000	2.833	4.167	11.833
D.	1.936	1.115	1.455	1.113
	Water deprived			
A.	9.344	3.969	8.163	10.188
B.	2.137	.878	2.343	3.182
C.	15.375	6.000	12.500	14.875
D.	1.645	1.512	1.584	1.518

A = Total time spent drinking (min.).

B = Continuous drinking time (min.).

C = Total fluid intake (ml.).

D = Intake to time spent drinking ratio (ml./min.).

encounter of salt solution was compared, it was found that it was significantly longer for the experimental group over the control group (t = 2.454, df = 10, $p < .05$). The average DD across the entire drinking session for distilled water and salt solution did not differ significantly between groups. The total amount of time spent drinking (t = 4.177, df = 10, $p < .01$) salt solution and the total amount of solution intake (t = 4.989, df = 10, $p < .001$) was significantly greater for experimentals than for controls.

Water deprived. The behavioral features of drinking peculiar to water deprived animals in contrast with those of water replete animals are a matter of degree and not of substance. That is, IDI increased proportionately for both groups as the drinking period progressed. For the first 10 minutes of drinking IDI was significantly shorter for the experimental group (t = 3.666, df = 14, $p < .01$) but not during the last 50 minutes of the drinking session. The cumulative frequency distributions of IDI as shown in Figs. 13 through 15 indicate the same pattern of proportional differences described for water replete subjects, although differences between the groups were larger. Similarly, BD remained constant throughout the drinking period for both groups.

A perusal of the distribution of the time spent drinking both solutions (Figs. 16-19) but focusing on the time spent with saline, both groups experienced an initial heightened response followed by a steady decline in the time that was spent drinking. The experimental group was characterized by having a larger initial response and a slower decline than the control group. Proportionally,

Figure 13. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the first 10 minutes of the preference test. There were two groups of water deprived rats, one group was fed a sodium deficient diet (Exptl.) and the other was a normal control (Control) group.

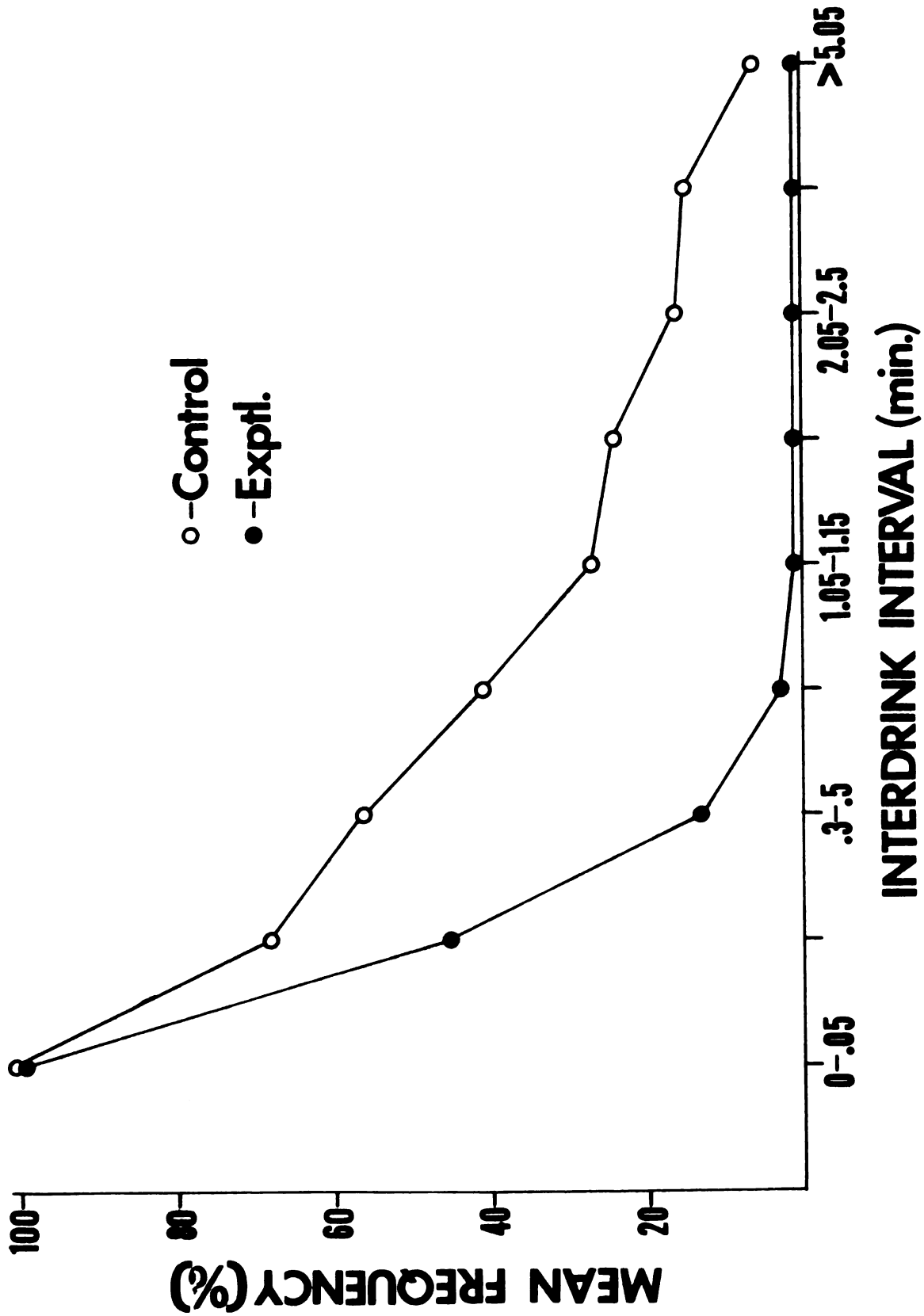

MEAN FREQUENCY (%)
100
80
60
40
20
o-Control
•-Expt.
0-.05
.3-.5
1.05-1.15
2.05-2.5
>5.05
INTERDRINK INTERVAL (min.)

Figure 13

Figure 14. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the second 10 minutes of the preference test. There were two groups of water deprived rats, one group was fed a sodium deficient diet (Exptl.) and the other was a normal control (Control) group.

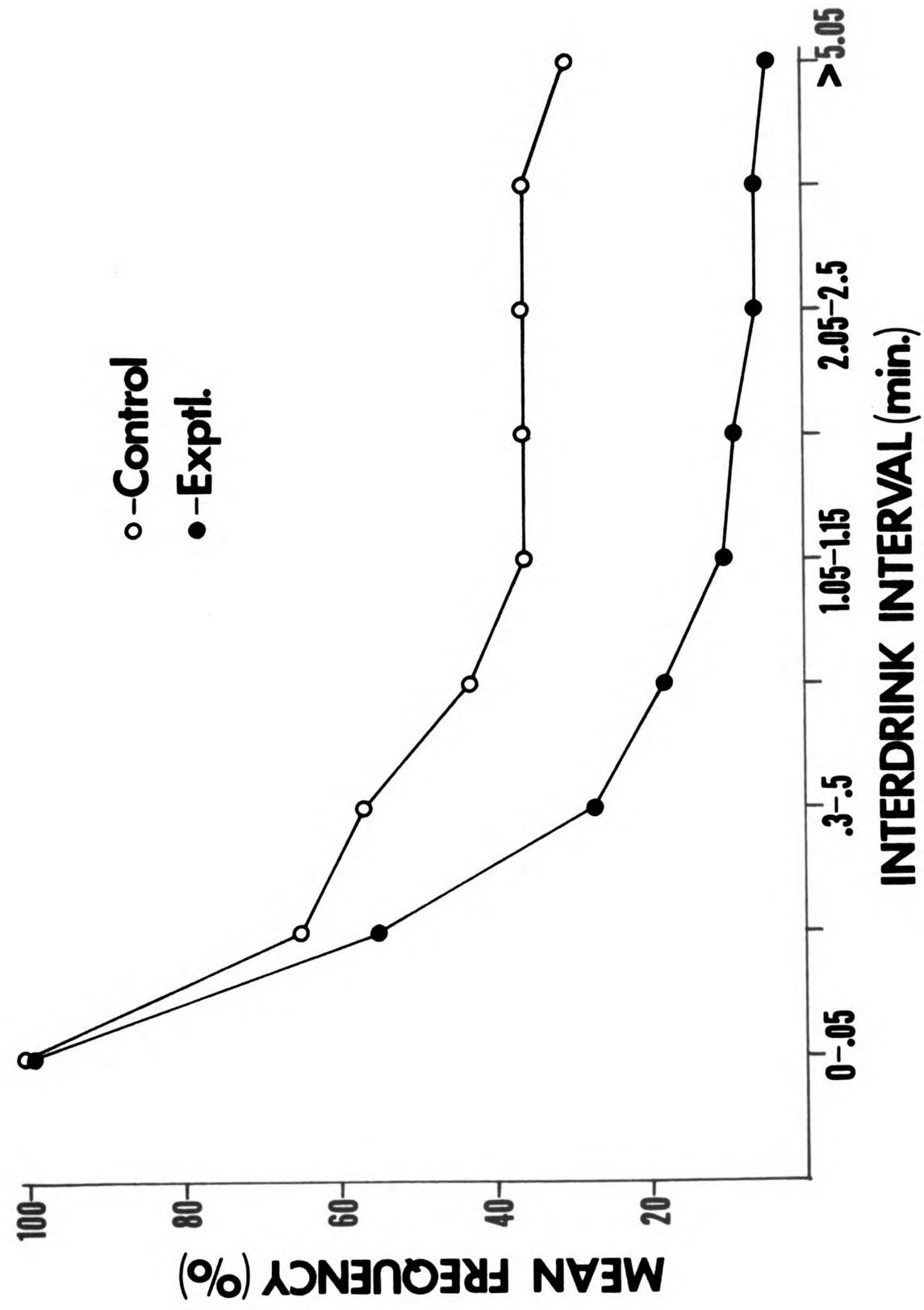

o-Control
•-Expt.
MEAN FREQUENCY (%)
100
80
60
40
20
0-.05
.3-.5
1.05-1.15
2.05-2.5
>5.05
INTERDRINK INTERVAL (min.)

Figure 14

Figure 15. The cumulative (from right to left) mean frequency of interdrink intervals occurring within the last 40 minutes of the preference test. There were two groups of water deprived rats, one group was fed a sodium deficient test (Exptl.) and the other was a normal control (Control) group.

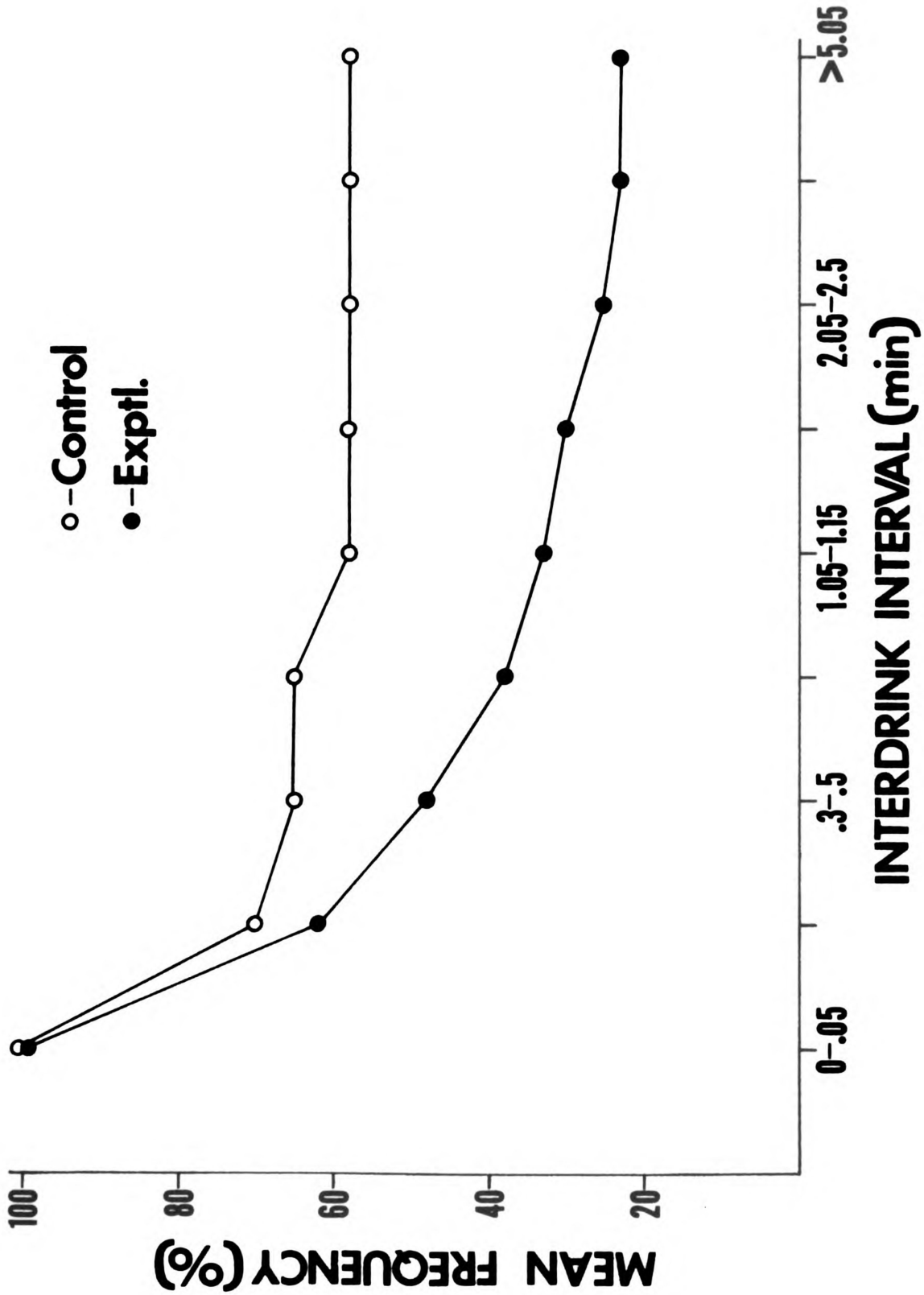

o-Control
●-Expt.
MEAN FREQUENCY (%)
100
80
60
40
20
0-.05
.3-.5
1.05-1.15
2.05-2.5
>5.05
INTERDRINK INTERVAL (min)

Figure 15

Figure 16. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water deprived, sodium deficient, and drank from the saline bottle first.

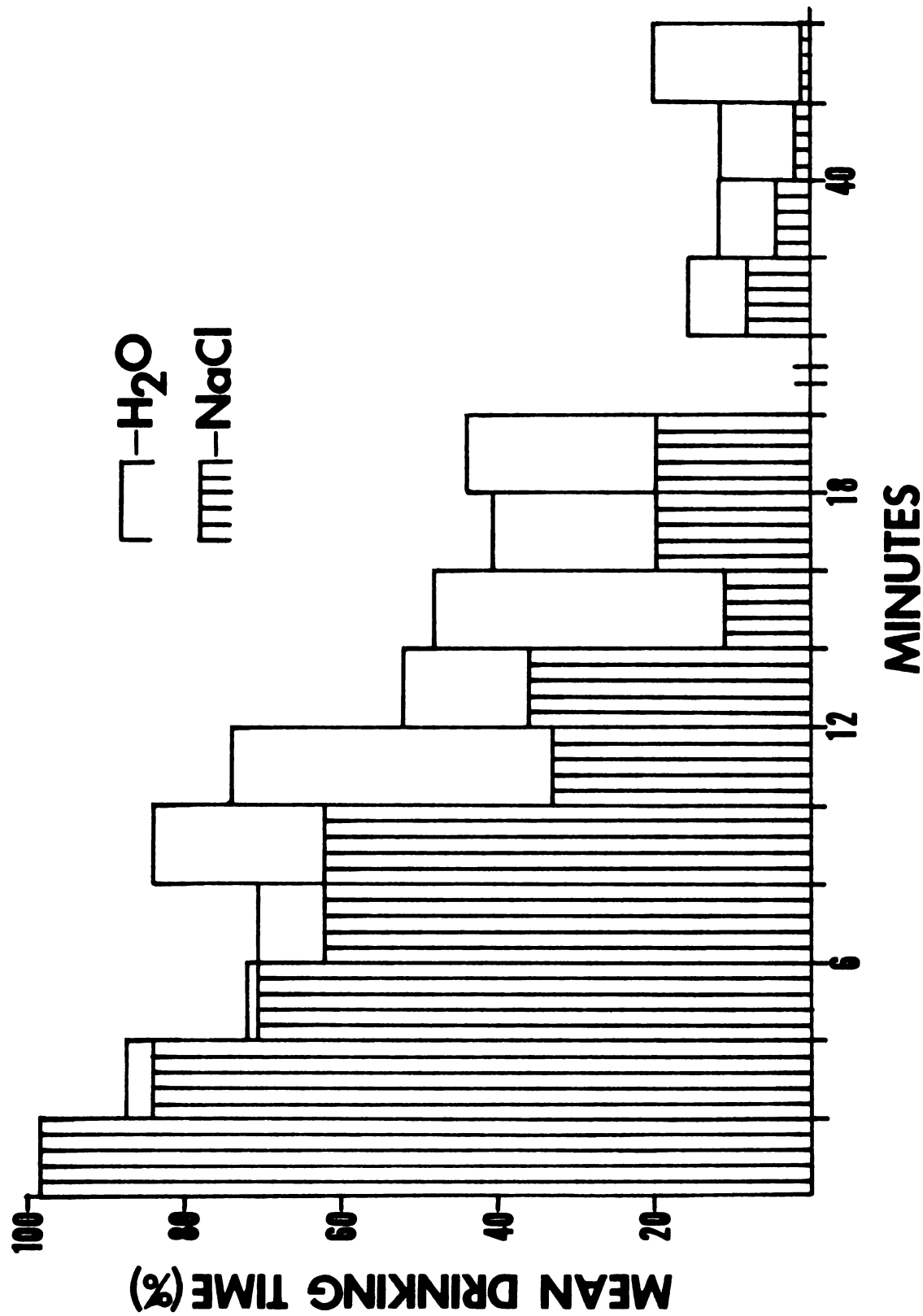


Figure 16

Figure 17. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water deprived, normal controls, and drank from the saline bottle first.

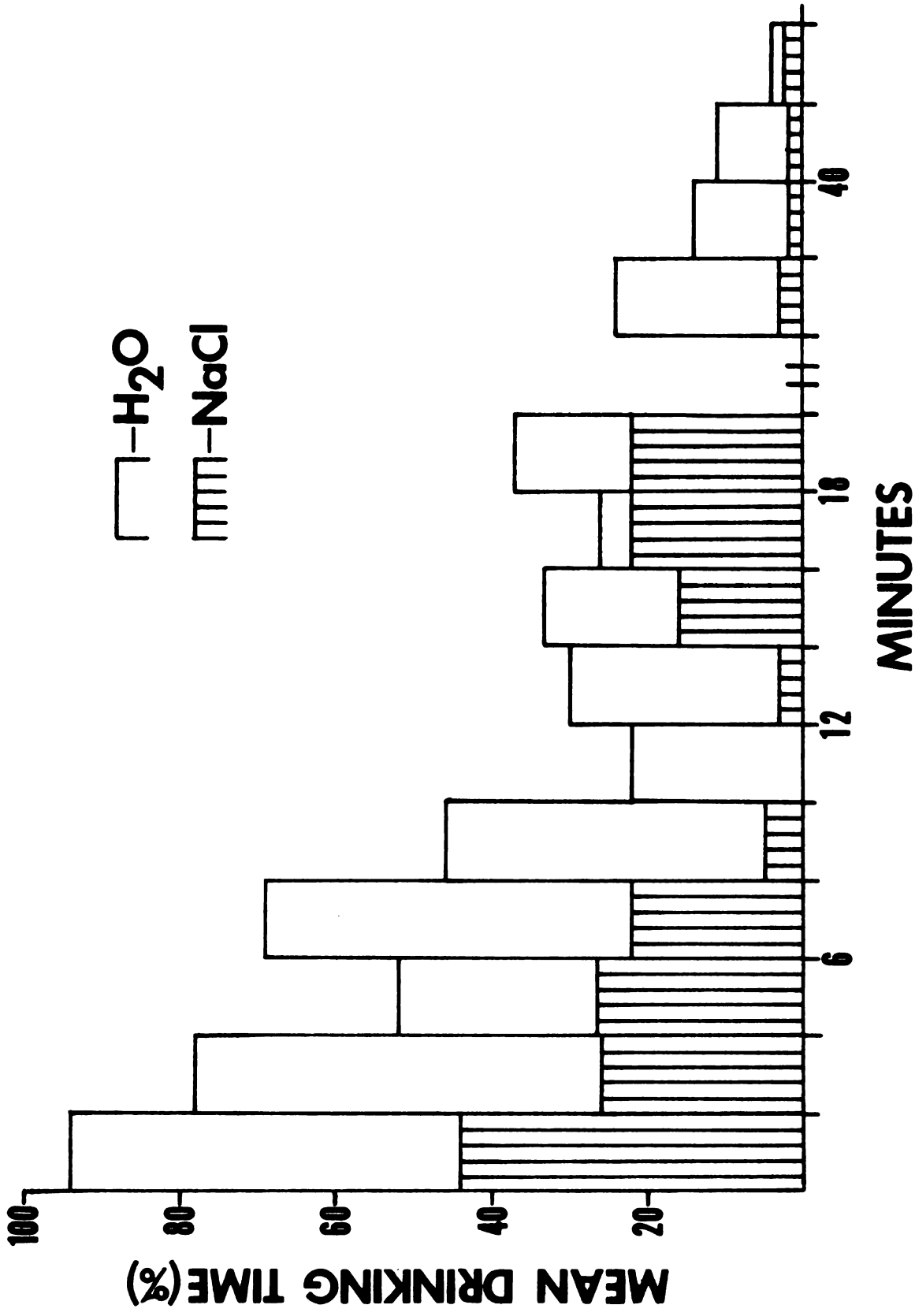


Figure 17

Figure 18. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water deprived, normal controls, and drank from the distilled water bottle first.

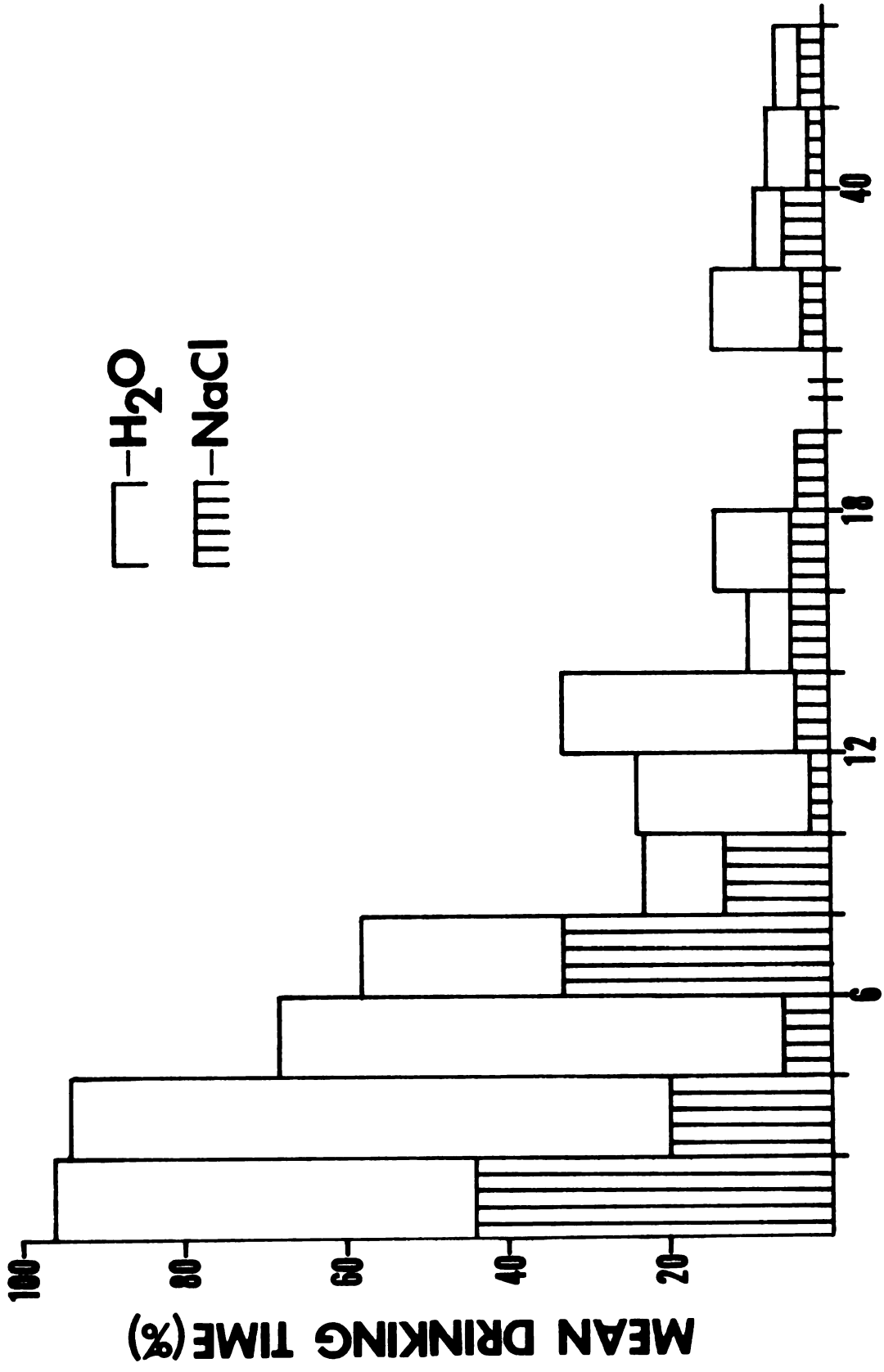


Figure 18

Figure 19. The mean proportion of time spent drinking distilled water and .4 molar saline as a function of time. These animals were water deprived, sodium deficient, and drank from the distilled water bottle first.

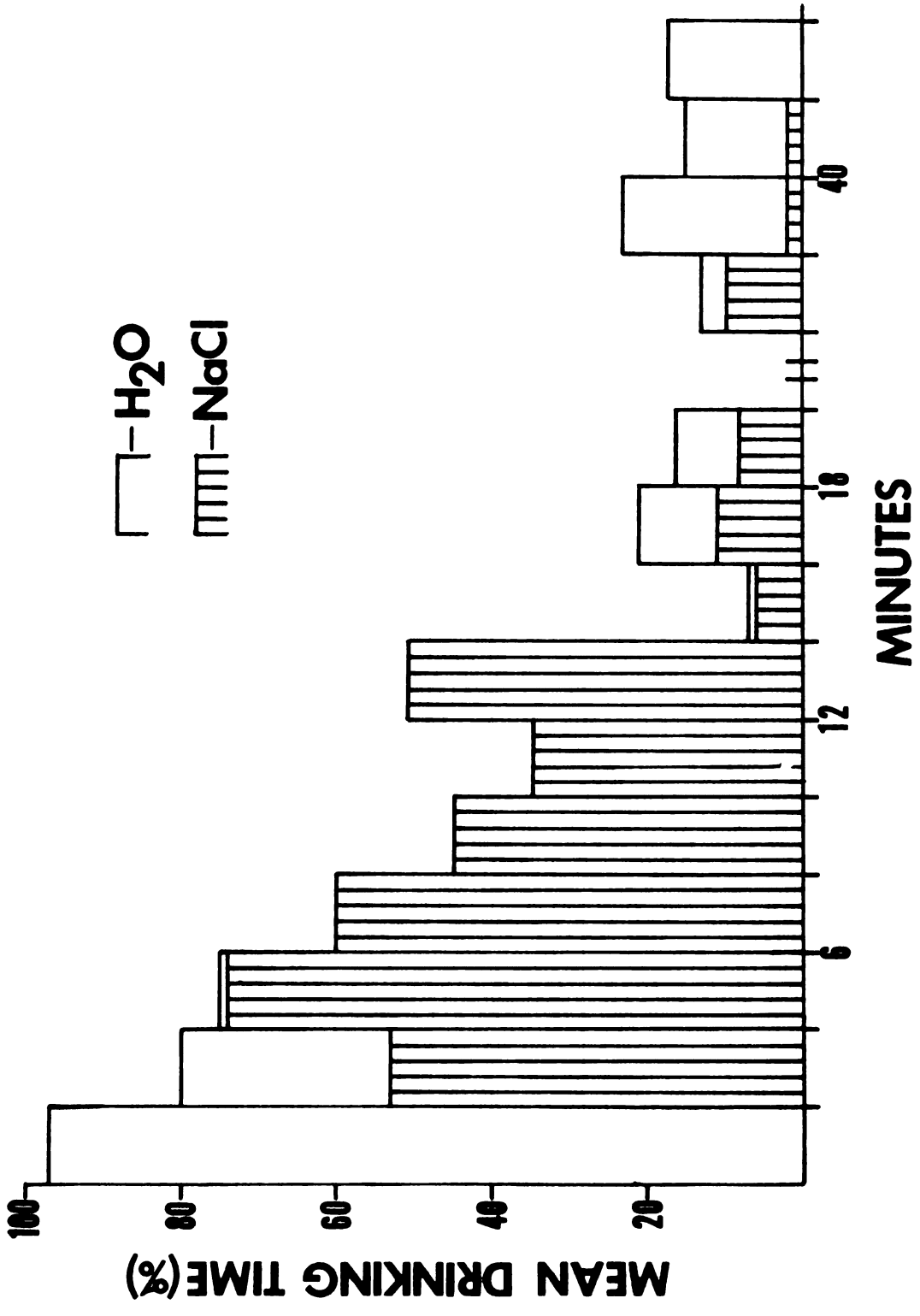


Figure 19

the experimental group spent about 95% of its time drinking during the initial two minutes of saline ingestion. It was after 14 minutes of drinking before it declined to less than 10%. The corresponding percentages for the control group were 44% and declined to less than 10% after six minutes of saline ingestion. This aforementioned description was less apparent for water replete subjects.

The total amount of time spent drinking (t = 4.332, df = 14, $p < .001$) salt solution and the total amount of salt solution intake (t = 4.710, df = 14, $p < .001$) were significantly greater for sodium deprived animals than for controls. Unlike the water replete condition, the average DD for the first encounter (t = 4.261, df = 14, $p < .001$) and for the entire drinking session (t = 2.381, df = 14, $p < .05$) for saline were both significantly longer for experimental animals than controls.

GENERAL DISCUSSION

Blood. With an average of only .373 mEq. of sodium eliminated over a ten day period of sodium deficiency, a significant decrease in serum sodium was unlikely to be obtained. Serum measurements of sodium, potassium, and protein were nonsignificant and therefore implied that they were not major conditions eliciting sodium appetite due to diet deficiency. These results are consistent with those of Denton (1965) and Jalowiec and Stricker (1973) which suggest that variations in the concentration of sodium in the plasma does not determine the appetite for sodium. However, plasma aldesterone concentrations were significantly above baseline values (Marusic & Mulrow, 1967). It is possible that mineralocorticoids have a two-fold function: (1) to prevent severe sodium losses through renal reabsorption; and perhaps (2) to potentiate the specific hunger for salt in conditions of sodium deficiency (Jalowiec & Stricker, 1973). This last premise must be guarded with skepticism, since adrenalectomized rats can have a sodium appetite without mineralocorticoids. On the other hand, Wolf and Handal (1966) demonstrated that normal rats administered desexycorticosterone (DOC) or aldosterone develops a salt appetite. Jalowiec and Stricker (1973) demonstrated that

despite smaller sodium deficits dietary sodium deprived rats showed larger NaCl intakes than adrenalectomized rats.

These serum values were generally lower than those reported in previous investigations (Jalowiec & Stricker, 1973; Wright, 1973). Secondly, in order to extend the observations of the first experiment blood samples were also acquired from 20 day sodium deficient rats. These were the reasons for doing Experiment II. The results from data taken after ten days of sodium deficiency parallel those in Experiment I, but the absolute levels of blood sodium compare favorably with previous investigations. An extended observation of rats deprived of salt for 20 days show that blood sodium did not depart from control values. Blood potassium levels of sodium deficient animals, on the other hand, was significantly higher compared to normal controls. Potassium levels after 20 days of salt deprivation was higher than that after 10 days of deprivation; but the level after 10 days of deprivation was not different from that of controls. Mitchell (1972) noticed that sheep with higher concentrations of plasma potassium tend to show greater sodium preference. Furthermore, he argues that the augmented sodium intake in sodium depleted sheep might be important to combat against hyperkalaemia.

Patterns of intake. Both water replete and water deprived rats showed a monotonic decrease in the proportion of the time spent drinking saline as they approached satiation. The dynamic aspects of the behavior underlying this proportional decrease were that drinking bursts remained constant and pauses became longer. This is in disagreement with the pattern of intake reported for water drinking

(Stellar & Hill, 1952), saccharin drinking (Hulse, 1967), and nutritive drinking (Allison & Castellan, 1970) as they also found burst duration to decrease. A procedural difference to account for this contradiction is that these aforementioned experiments measured licking characteristics of drinking. Hence, decreasing lick duration and increasing interlick intervals would appreciably lower burst duration and go undetected in the method used here. If volumetric intake was recorded periodically, the ratio of intake to burst duration should decrease progressively during the drinking session.

Scrutinizing the temporal patterns of drinking and the cumulative frequency distribution of interdrink intervals for water replete (Figs. 6-12) and water deprived (Figs. 13-19) rats indicate that satiation through saline intake is not a stepwise process but rather a continuous process. As the rat begins to drink in his sodium deficient condition the probability of drinking saline first is high. If he chooses saline, initially a large proportion of the time is spent drinking but progressively this proportion decreases. The proportion of saline drinking decreased because the probability of initiating a burst decreased.

The drinking behavior in rats is generally intermittent. In the preference test situation of this experiment, the rats were observed to drink in short time intervals. Occasionally they would also alternate in their drinking by switching from saline to distilled water and vice versa. Meiselman and Halpern (1973) using human subjects demonstrated an enhancement of taste intensity by stimulating the tongue with alternating pulses of saline and water.

This was a reverse adaptation affect, since continuous stimulation with a single solution generally causes decrements in both human magnitude estimation and in neural firing rates. The drinking duration data (Table 6) show that sodium deficient rats drank from the salt solution cylinder successively for longer periods of time than normal rats. However, they did not switch to water any more or less frequently than control animals. It seems unlikely that any differential adaptation effects would arise between groups due to alternating fluid drinking. The drinking duration data do imply that sodium deficient rats adapt more slowly to the salt stimulus than normal controls.

Halpern and Marowitz (1973) demonstrated in the rat that short lick-duration stimuli generated a consistent phasic response in the electrical transcript of a multiunit chorda tympani nerve response. Rats drink in a discontinuous fashion because they lick when they drink from a spout. It is possible that the neural response one normally sees when a continuous amount of solution is placed on the tongue, is actually an accumulation of these short serial phasic responses (Meiselman & Halpern, 1973). These responses perhaps summate within drinking bursts and perhaps across bursts if the interval between them is not too long.

The temporal drinking patterns of sodium deficient rats as opposed to those of normal rats reveal differences that may be explained in terms of an adaptation process. Sodium deficient rats drink more saline than normals because it takes longer for their gustatory system to adapt to the salty taste. The neural correlates

of such a process perhaps lie in analyzing the adaptive characteristics of the taste system. Keidel, et al. (1961) concluded that adaptation is a fundamental principle by which the nervous system accumulates information about the external and internal environments. After Bradley's (1973) demonstration of blood chemical changes affecting gustatory neural responses, it seems that the first order neuron is a good place to start looking for adaptation changes.

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APPENDICES

APPENDIX A

APPARATUS

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APPARATUS

Description of the drinking box

The drinking box was made up of six individual drinking compartments. Twelve glass gas collecting tubes were attached to the drinking box. The tubes were calibrated in 0.2 ml. Each compartment was 25.5 cm. long, 14 cm. wide, and 14.5 cm. deep. The entire bottom of the drinking box consisted of hardware cloth and stood 4 cm. from the floor. Two drinking spouts extended into each compartment by 2.54 cm., these holes were six cm. from the bottom and 2.5 cm. from the side of the compartment. A small guillotine door was built into each drinking compartment which could be raised to expose the spouts of the gas-collecting tubes. An aluminum track permitted verticle mobility by the masonite door. Each compartment had a plexiglas top, which was hinged at one end and had a magnetic lock on the other end in which to secure the compartment.

Equipment, suppliers, and unit prices

1. Individual metabolism cage with base, Acme Metal Products, $45.00.
2. Animal balance, Ohaus, $74.75.
3. Flame photometer #143, Instrumentation Laboratories, $2390.00.

4. Centrifuge, International Equipment Co., $270.00.
5. Drinkometer, Grason-Stadler Co., Entire unit - $96.00/ea.
6. Gas measuring tubes, Arthur H. Thomas, $28.12/cs.
7. 100 ml. graduate cylinders, M.S.U. General Stores, $3.28/ea.

APPENDIX B

RAW DATA

TABLE 7

Raw Data--Body Metabolism

		Days				
		7-9	10-13	14-18	19-23	24-28
Food intake (g)	Exptl.	19.7 ± .4	17.1 ± .5	19.8 ± .4	20.4 ± .4	20.5 ± .5
	Control	19.9 ± .4	17.2 ± .5	20.6 ± .3	20.6 ± .3	20.1 ± .3
Water intake (ml.)	Exptl.	31.1 ± 1.0	44.0 ± 2.4	41.2 ± 1.4	41.9 ± 1.5	38.0 ± 1.4
	Control	32.3 ± .9	34.8 ± 1.6	33.1 ± 1.1	32.5 ± .8	32.9 ± .9
Body weight (g)	Exptl.	391.9 ± 2.6	383.8 ± 2.9	393.7 ± 2.5	408.2 ± 2.3	420.5 ± 2.4
	Control	392.8 ± 2.9	381.8 ± 3.0	393.7 ± 2.6	407.4 ± 2.4	420.3 ± 2.5
Urine volume (ml.)	Exptl.	17.8 ± .8	25.8 ± 1.6	23.8 ± 1.1	23.5 ± 1.2	22.0 ± 1.2
	Control	16.7 ± .7	19.6 ± 1.2	17.5 ± .8	115.6 ± .6	16.6 ± .7
Urine Na Concentration (mEq./L)	Exptl.	176.8 ± 7.8	104.5 ± 9.7	112.2 ± 7.0	113.3 ± 6.9	4.10 ± 1.1
	Control	203.6 ± 9.5	128.1 ± 7.9	158.5 ± 7.1	160.2 ± 6.1	150.9 ± 6.2
Urine K Concentration (mEq./L)	Exptl.	157.2 ± 7.7	133.5 ± 10.9	142.4 ± 8.8	143.2 ± 8.8	153.2 ± 10.0
	Control	180.1 ± 9.3	155.6 ± 9.0	201.5 ± 9.4	203.8 ± 7.7	194.1 ± 8.1
Refractive Index (urine total solids)	Exptl.	446.1 ± 5.1	413.7 ± 6.9	417.5 ± 4.9	417.5 ± 4.5	420.0 ± 6.0
	Control	464.6 ± 5.7	444.3 ± 6.5	459.8 ± 5.8	455.6 ± 4.6	455.8 ± 5.4
Urine Na output (mEq./day)	Exptl.	2.98 ± 0.9	2.17 ± .10	2.35 ± .08	2.31 ± .07	.070 ± .02
	Control	3.24 ± .11	2.21 ± .10	2.56 ± .09	2.35 ± .07	2.33 ± .07
Urine K output (mEq./day)	Exptl.	2.62 ± .07	2.73 ± .12	2.99 ± .10	2.88 ± .08	2.78 ± .08
	Control	2.82 ± .10	2.70 ± .11	3.23 ± .11	2.97 ± .09	2.95 ± .08

TABLE 7--Continued.

		Days			
		29-33	35-38	39-43	44-49
Food intake (g)	Exptl.	18.3 ± .3	20.3 ± .5	20.0 ± .4	15.3 ± .3
	Control	20.2 ± .3	20.5 ± .5	19.3 ± .4	14.7 ± .3
Water intake (ml.)	Exptl.	42.4 ± 1.7	43.2 ± 1.6	41.6 ± 1.7	15.1 ± .4
	Control	35.2 ± .9	35.7 ± 1.2	34.5 ± 1.3	15.0 ± .4
Body weight (g)	Exptl.	427.4 ± 2.4	438.7 ± 2.5	444.8 ± 2.3	426.0 ± 1.8
	Control	431.1 ± 2.5	440.5 ± 2.7	446.9 ± 2.7	422.7 ± 2.0
Urine volume (ml.)	Exptl.	23.9 ± 1.4	27.4 ± 1.3	26.7 ± 1.5	5.91 ± .2
	Control	18.0 ± .8	21.2 ± 1.0	19.9 ± 1.0	7.0 ± .2
Urine Na Concentration (mEq./L)	Exptl.	0.8 ± .1	110.8 ± 7.6	4.13 ± 1.5	10.4 ± 2.0
	Control	142.7 ± 6.2	142.6 ± 7.4	145.5 ± 7.4	247.5 ± 5.9
Urine K Concentration (mEq./L)	Exptl.	126.6 ± 8.8	153.3 ± 9.5	154.6 ± 10.9	407.7 ± 7.8
	Control	186.5 ± 8.2	192.3 ± 8.6	207.5 ± 9.7	367.1 ± 4.8
Refractive Index (urine total solids)	Exptl.	406.2 ± 5.1	418.3 ± 4.8	414.0 ± 6.0	586.0 ± 4.0
	Control	447.2 ± 4.8	444.7 ± 5.3	452.3 ± 5.5	580.0 ± 3.6
Urine Na output (mEq./day)	Exptl.	.016 ± .00	2.72 ± .14	.108 ± .05	.065 ± .01
	Control	2.36 ± .08	2.75 ± .11	2.54 ± .08	1.76 ± .08
Urine K output (mEq./day)	Exptl.	2.50 ± .09	3.60 ± .13	3.32 ± .08	2.36 ± .09
	Control	3.10 ± .10	3.67 ± .12	3.47 ± .11	2.55 ± .08

TABLE 8

Blood Data

10 days of sodium deficiency					
Serum Na		Serum K		Serum protein	
Exptl.	Control	Exptl.	Control	Exptl.	Control
134	138	5	4	6.0	6.45
134	134	5	6	6.2	6.3
128	135	5	4	6.0	6.1
127	125	4	5	6.4	6.6
132	135	4	5	6.2	6.2
132		6		6.2	5.9
10 days of sodium deficiency					
Plasma Na		Plasma K		Plasma protein	
Exptl.	Control	Exptl.	Control	Exptl.	Control
137	143	4.5	4.45	6.1	6.1
143	145	4.2	4.5	6.5	6.8
145	140.5	4.3	4.45	6.7	6.7
146	140	4.55	4.8	6.8	6.5
144	143.5	4.5	4.55	6.8	6.7
146.5	145.5	4.7	4.45	6.3	6.7
20 days of sodium deficiency					
Plasma Na		Plasma K		Plasma protein	
Exptl.	Control	Exptl.	Control	Exptl.	Control
146.5	144	5.4	4.55	6.3	5.9
143.5	156	4.6	4.45	6.3	6.5
167.0	151	5.4	4.9	6.5	6.6
142	139	6.3	4.7		6.3
142	147.5	4.95	3.9	6.3	6.5
	141		4.2		6.5

TABLE 9

Raw Data--Patterns of Intake

Water replete--Exptl.					
Total fluid intake (ml)		Total drinking time (min)		Total drinking duration (min)	
H_2O	Saline	H_2O	Salt	H_2O	Saline
5	10	2.05	6.90	1.025	3.45
1	8	.90	6.20	.45	3.10
5	18	3.55	13.65	.50	1.95
7	9	4.90	14.00	.45	1.40
4	15	3.95	14.85	3.95	14.85
3	11	2.25	11.35	.57	2.90
Volumetric intake per minute (ml/min)		Interdrink interval (min)		Burst duration (min)	
H_2O	Saline	10 min	50 min	10 min	50 min
2.44	1.45	.25	1.38	.31	.21
1.11	1.29	.11	1.91	.14	.14
1.41	1.32	.30	..92	.30	.32
1.43	.64	.23	.56	.33	.15
1.01	1.01	.05	.08	.75	.40
1.33	.97	.37	1.92		.31

Drinking Duration First encounter (min)	Water replete--Control Total fluid intake (ml)	
	H_2O	Saline
6.75	2	2
1.70	5	7
4.00	5	3
2.65	2	2
14.85	1	1
6.00	3	2

TABLE 9--Continued.

Total drinking time (min)		Drinking duration (min)		Volumetric intake per minute (ml/min)	
H_2O	Saline	H_2O	Saline	H_2O	Saline
1.90	.60	1.90	.60	1.05	3.33
3.15	7.60	.79	1.90	1.59	.92
.85	3.85	.43	1.93	5.88	.78
3.10	4.80	.78	.96	0.65	.42
1.05	1.30	.21	.33	.95	.77
2.00	2.80	.67	.57	1.50	.71

Interdrink interval (min)		Burst duration (min)		Drinking duration first encounter (min)
10 min	50 min	10 min	50 min	saline
.54	2.30	.10	.05	.60
.15	1.25	.17	.17	1.35
.33	3.09	.68	.27	2.05
.88	2.44	.77	.18	1.90
.50	.23	.09	.09	.05
1.07	1.45	.21	.55	1.25

Total fluid intake (ml)		Water deprived--Exptl. Total drinking time (min)		Drinking duration (min)	
H_2O	Saline	H_2O	Saline	H_2O	Saline
15	8	6.1	4.3	1.22	1.08
15	9	8.2	5.35	1.64	.89
14	17	11.0	9.25	5.50	9.25
14	18	10.2	11.75	3.40	3.92
15	11	8.3	11.05	2.08	3.69
8	23	5.55	16.00	.62	1.78
6	17	4.4	13.15	.44	1.32
13	16	11.55	10.65	3.85	3.55

TABLE 9--Continued.

Volumetric intake per minute (ml/min)		Interdrink interval (min)		Burst duration (min)	
H_2O	Saline	10 min	50 min	10 min	50 min
2.46	1.86	.19	9.90	.23	.26
1.83	1.68	.17	3.88	.35	.15
1.27	1.84	.13	.10	.57	.31
1.37	1.53	.11	2.34	1.11	.57
1.81	1.00	.08	.73	.17	.23
1.44	1.44	.10	.83	.53	.36
1.36	1.29	.14	.90	.60	.34
1.13	1.50	.10	1.19	.37	.22

Drinking duration first encounter (min)	Water-replete-Control Total fluid intake (ml)		Total drinking time (min)	
Saline	H_2O	Saline	H_2O	Saline
1.90	15	4	6.50	2.15
2.20	17	7	7.70	3.70
9.25	14	5	7.30	3.50
9.30	13	4	7.40	2.90
8.05	16	7	10.85	4.25
6.30	19	7	10.85	3.95
3.40	13	8	13.25	5.60
4.85	16	6	10.90	5.70

TABLE 9--Continued.

Water deprived--Control

Drinking duration (min)		Volumetric intake per minute (ml/min)		Interdrink intervals (min)	
H_2O	Saline	H_2O	Saline	10 min	50 min
1.63	.67	2.31	1.86	.95	2.67
1.28	.62	2.21	1.89	2.45	4.98
1.04	.58	1.92	1.43	.60	11.53
1.48	.48	1.76	1.38	.80	8.10
5.43	1.42	1.47	1.65	.55	49.05
2.17	.79	1.75	1.77	2.07	4.98
1.89	0.80	.98	1.43	.67	4.34
2.18	1.43	1.47	1.05	.61	5.55

Burst duration (min)		Drinking duration first encounter (min)
10 min	50 min	Saline
.54	.24	1.55
.44	.19	1.10
.34	.07	.30
.21	.55	.05
.62	.41	.30
.25	.69	1.25
.27	.46	.05
.49		2.6