URINE OSMOLALITY AND ELECTROLYTE RESPONSES OF ANXIETY TREATED MALE RATS TO EXERCISE

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ABSTRACT

URINE OSMOLALITY AND ELECTROLYTE RESPONSES OF ANXIETY TREATED MALE RATS TO EXERCISE

By

Michael C. Greenisen

This investigation was undertaken to determine the effects of exercise treatments on responses to experimentally induced anxiety by examining the urine osmolality and the Na^+ and K^+ electrolyte concentrations of male rats during a twelve-hour home-cage environment.

Twenty rats (Sprague-Dawley) were observed for 21 days. Four groups of five animals each were used. Group I: A-E was exposed to anxiety conditions by random faradic stimulation through the floor of individual anxiety chambers for three hours daily. A forced exercise treatment, consisting of interval running, immediately followed the anxiety exposure. Group II: SA-E received a sham anxiety exposure followed by the exercise treatment. Group III: A-NE was exposed to the anxiety treatment but did not receive the forced exercise treatment. Group IV: SA-NE was exposed to sham anxiety conditions and did not exercise.

Three-day control data, similar to experimental data, were collected from the animals in each of the experimental treatments but in the absence of any faradic stimulation.

With the inception of the eighteen-day experimental treatment phase, total urine volume was collected during: anxiety or sham anxiety exposure, the exercise treatment or a holding period of the same duration, a recovery period, and the twelve-hour period of sedentary home-cage environment. Body weight and the food and water ingested also were recorded during the recovery and home-cage environment periods.

During the home-cage period, the urine osmolality of the SA-NE group returned to normal values. However, they did demonstrate elevated total urinary Na^+ retention (non-significant) and K^+ excretion patterns ($p \leqq .05$).

Animals exposed to the A-NE and SA-E treatments exhibited increased and similar urine osmolality ($p \leqq .05$). Both groups showed enhanced urine Na^+ retention ($p \leqq .05$), but neither group demonstrated a convincing K^+ imbalance. The A-E group responded with urine osmolalities significantly higher ($p \leqq .05$) than the A-NE and SA-E groups, apparently indicating the additive nature of exercise and anxiety during this study. In addition, the A-E group had increased urinary Na^+ retention and K^+ excretion ($p \leqq .05$).

These findings indicate that exercise does not alleviate experimentally induced anxiety but rather seems to act as a second stressor.

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By

Michael C. Greenisen

A DISSERTATION

Submitted to
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Dedicated to

Virginia Gail Greenisen

my wife

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The challenges of a mountain and a Ph.D. dissertation are more similar to one another than they may appear to the uninitiated. Both require rigorous training in specialized skills. Both offer the personal challenge for man to compete against himself. Both present the uncertainty of accomplishment, for if victory were certain, why bother?

A mountain challenges by its altitude, which makes progress, mentally and physically, more taxing as the summit is in view. It punishes the body with its moraine, scree, jagged rocks, ice and snow. The brain and extremities must work in concert to select the route, pitch by logical pitch, with coordinated reach and step to safely navigate the route, each with its own unexpected moments.

A dissertation challenges by producing a myriad of unexpected data which make progress more laborious as the final two pitches are at hand. It punishes the body over sleepless nights demanding nicotine and caffeine. The brain and extremities must work in concert as they select table, figure, and appendix to navigate a logical route through five hazardous pitches.

In the end, even Everest and McKinley are mere mountain walks during the final steps to the summit. No such pleasures are extended the writer. A dissertation presents its most dangerous terrain at the

summit, where the tired or the unprepared may flounder and be lost forever.

Ultimately the challenge of either is taken up essentially because it is there and blocks our view. Both appear much more overwhelming and hazardous at the base than at the summit.

To challenge a mountain and defeat it provides a few moments of personal victory but, in and of itself, it is an end.

Accepting the challenge of a dissertation and writing it provides fleeting, if any, victory for, in and of itself, it is a beginning.

The mountaineer and the researcher are one and the same--explorers who seek a better view.

I acknowledge the dissertation which, like the mountain, taught me new things about myself and considerably more regarding my profession. In particular, I appreciate the help of:

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ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
A-E	anxiety-exercise
A-NE	anxiety-no exercise
CS	compound symmetry
EV	equal variance
K^+	potassium
Na^+	sodium
PER	percent of expected revolutions
SA-E	sham anxiety-exercise
SA-NE	sham anxiety-no exercise
PER	percent of expected revolutions
TRR	total number of revolutions run

CHAPTER I

INTRODUCTION

An area of investigation relatively unexplored by physical educators is the role of exercise in mammalian psychophysiological adaptation to the environment. Of specific interest is the effectiveness of exercise as a therapeutic measure intended to alleviate concurrent emotional trauma (anxiety).

The stress of exercise or anxiety stimulates increased mammalian pituitary-adrenocortical activity. This increased activity by the pituitary and adrenal glands results in measurable quantitative and qualitative changes in the excreted urine. Considerable data have been assembled regarding posterior pituitary antidiuretic hormone (ADH) responses in urine and/or blood as a result of emotional or exercise stress (7,42,56). ADH acting at the kidney is a direct regulator of urine osmolality (14,55,69). Emotional anxiety and environmental stress have been reported to be reflected in increased urine sodium (Na^+) retention and potassium (K^+) excretion (17,49,89). ADH is further implicated in Na^+ resorption occurring in the distal tubule of the kidney (69). This electrolyte imbalance also implies a hyperfunction of the adrenal cortex, specifically its secretion of aldosterone or corticosterone (55).

These findings lead logically to the conclusion that physiological reactions regarding the effects of exercise on anxiety may be analyzed by urinalysis examining urine osmolality, and the Na^+ and K^+ electrolyte concentrations.

One study which essentially dealt with the prevention of heart disease and/or the reduction of cardiac sympathetic tone seemed to show that physically active and placid individuals respond less to mental and sensory stress than do sedentary and emotionally irritable persons (64). However, the available literature demonstrates a lack of convincing evidence regarding the interrelationships of exercise and anxiety-prone subjects. A need exists to expand human knowledge regarding the direct effects of exercise on those parameters that have been identified as being sensitive to anxiety.

Statement of the Problem

The purpose of this study was to investigate the effects of daily periods of a selected interval running program on male rats following daily exposure to induced anxiety conditions. Home cage urinary Na^+ and K^+ electrolyte concentrations and urine osmolality were the parameters of the investigation.

Rationale

Pioneered by Steinhaus' (82) classical publication, "The Chronic Effects of Exercise", selected positive rewards of exercising on the physiological function of the mammalian organism have become well documented. Specifically, the cardiovascular, respiratory,

and skeleto-muscular systems have been studied in detail. The rationale for this study is to bring another part of the organism into focus by obtaining relevant data regarding the effects of exercise on mammalian emotional adjustment.

The albino laboratory rat was selected as the experimental model for this study, in part to utilize the controlled setting of the laboratory. More dramatically, this author knew of no method by which he could convince any of the animals that exercise would be "beneficial" for them. Therefore, it was reasoned that the physiological responses elicited in the subjects would be uncontaminated by their expectations.

The argument for this investigative approach is based on the following assumption: shifts from control data in the osmolality and the Na^+ and K^+ concentrations of the animals' urine, collected in their home-cage environment, were results of the experimental treatments.

Limitations of the Study

1. Available metabolism cages dictated that a maximum of 20 subjects could be used at one time.

2. The effect of faradic stimulation (foot shock) as the motivator utilized by the electronically controlled running wheel is an unknown, and uncontrolled for, variable.

3. The inability to maintain twenty-four hour home-cage control subjects for the duration of the study is traceable to the availability of metabolism cages. Evaluation of such a control group is thus lacking.

4. Important data may have been lost when urine samples and other measures were not taken during the zero time period each day.

CHAPTER II

REVIEW OF THE LITERATURE

The following literature review cites previous studies linking ADH secretory changes, urine osmolality, and experimental stress. The available research regarding urine osmolality and urinary Na^+ and K^+ mEq/l concentrations with respect to experimentally induced stress will conclude the review.

ADH Relationships to Urine Osmolality

Bernard (93) in 1859 was the first experimenter to observe that emotional stress in dogs and rabbits may suppress the rate of urine secretion even to the degree of total inhibition. Rydin and Verney (67) found that emotional stress, induced by mild faradic stimulation or a frightening noise, results in the inhibition of a water diuresis. To support their theory that the agent responsible for this emotional antidiuresis was a humorally transported antidiuretic substance of posterior pituitary extract, Rydin and Verney performed extensive neural sectioning. They denervated the renal, suprarenal, and splanchnic nerves as well as the entire abdominal sympathetic system of test dogs without finding a reduction in emotional-stress antidiuresis. From these data they concluded that emotional inhibition is mediated by posterior pituitary antidiuretic hormone. In

the same work, they discovered that mild exercise (running 4 to 5 mph for 4 min.) also results in the inhibition of water diuresis. They theorized that the inhibition produced by exercise is due to an emotional factor associated with exercise, for after repeated training sessions this antidiuresis disappeared. After extinction, however, if the dogs were exposed to a loud frightening noise while exercising, an antidiuretic response was elicited once again.

O'Connor and Verney (57) further supported this earlier research by showing that removal of the posterior lobe of the pituitary diminishes the antidiuretic response to only about 5% of its pre-operative inhibition on urine flow induced by emotional stress.

Kozlowski *et al.* (42) reinforced O'Connor and Verney's conclusions using human subjects. He and his co-workers found no increase in blood antidiuretic activity during mild exercise (450 kpm/min. on a cyclometer). This research did show that heavy exercise (1,200 kpm/min.) produces a rise in plasma antidiuretic activity. They hypothesized that the rise was due more to a homeostatic reflex mechanism than to emotional inhibition.

Fendler *et al.* (23) forced female rats to swim in 18°C water until exhausted daily. On days 12, 18 and 29, animals were sacrificed and posterior pituitary extracts from these experimental animals and from control animals were injected into dogs. Extracts from the exercise-treated animals suppressed urine flow in the dogs by an average of 3.5 ml. per five minutes. Extracts from control animals suppressed urine flow an average of only 1.5 ml. per five minutes. Extracts from rats treated for 18 days were found to be the most

dynamic, suppressing urine flow an average of 5.0 ml. per five minutes.

Dempster and Joekes (18) autotransplanted the kidneys of dogs to the dogs' necks in order to more precisely observe the effect of emotional stress on water diuresis inhibition. Using faradic stimulation as the emotional stress, they demonstrated that the kidneys of the animals exhibited the prolonged inhibition of a water diuresis representing ADH involvement.

Corson (7) experimented with chronic conditioned and unconditioned responses. Using Pavlovian type experiments, he demonstrated the participation of ADH in the responses of dogs to psychologic stress. Some dogs produced a marked and persistent release of ADH in response to repeated psychologic stress. Corson's treatment combined auditory tones with faradic stimulation. The secretions of ADH in response to this treatment were indicated by consistently high urine osmolality values (up to 1,500 MOS/1.). Urine concentration remained high, and occasionally increased, even though the dogs received a large water load prior to treatment.

Fendler *et al.* (24) demonstrated that electroshocks (AC, 12 ma) administered daily for 16 days, until a state of lethargy had been developed in male rats, resulted in a significant increase in pituitary ADH. (No mention was made of the number of repetitions of electroshocks per day.)

The evidence seems to be clear that emotional stress activates the supraoptic nucleus, hypothalamus, and posterior pituitary system, thus stimulating the secretion of antidiuretic hormone (ADH) into

the circulating blood. Noble and Taylor (56), in an experiment on venisection using human subjects, demonstrated that all those who fainted excreted ADH in their urine; ADH was not found in their presyncopal urine. ADH was not found in the urine of those subjects who did not faint. Once again, the emotional implication of ADH involvement is clear.

Extracellular Fluid Volume Shifts vs Exercise or Anxiety ADH Secretion

Of major interest to the interpretation of the data presented in this study is the elucidation of the physiological mechanisms which influence the secretion of ADH. Specifically there is concern regarding extracellular fluid volume shifts affecting the normal secretion of ADH during exercise and training induced by a conditioned emotional response.

The acute expansion by endogenous infusing of the extracellular fluid volume in dogs, rats, and man results in an increased urine flow, sodium excretion and osmolar clearance (16,25,28,31,43,50,55,57,60). The immediacy of this response rules out the slow aldosterone mechanisms affecting natriuresis or antinatriuresis (8). Stahl *et al.* (81) demonstrated that the diuretic and natriuretic substances elicited during extracellular fluid volume expansion and stimulation of the intrathoracic volume receptors by immersion in water originated in the liver. Interestingly, ADH destruction occurs in the liver and kidneys (50). This natriuretic substance released by the liver may be the substance X which Homer Smith describes (94).

Expansion of the intrathoracic blood volume by extracellular fluid volume expansion, negative pressure breathing, assuming a supine posture, or immersion in water, activates volume receptors, more specifically those of the left atrium, resulting in a diuretic and natriuretic response (5,38,50,56,61). A diuresis also is elicited by expanding a balloon in the left atrium (30,50). Gauer *et al.* (23) demonstrated that the degree of distention of the left atrium controls water excretion by the antidiuretic mechanism of the posterior pituitary, and that the degree of distention of the right atrium governs the excretion of sodium. This is a complementary mechanism for, unless accompanied by the excretion of sodium, loss of water is limited by the increasing osmolality of the body fluids.

If the stimulation of the intrathoracic volume receptors (specifically in the left atrium) is reduced by a reversal of the maneuvers described (negative to positive pressure breathing, supine position to erect standing, etc.) an antidiuresis ensues accompanied by the return to normal values of sodium and osmolar clearances.

Activation of the intrathoracic volume receptors, by the pooling of central and intrathoracic blood volume, triggers an anti-antidiuresis. This response in turn maintains the homeostatic balance of the living organism in response to these expansion maneuvers. However, this central and intrathoracic blood-pooling is not seen during exercise. This is the first evidence to refute the possibility of an anti-antidiuresis response to exercise.

There is an increase in arterial blood pressure during exercise. Among the first mechanisms to sense this pressure rise are the

aortic and carotid sinuses, which are baroreceptors. Cort (8,9,10,11) found that bilateral occlusion of the common carotid arteries produces a diuresis and natriuresis. This is readily explanable in that lack of the carotid sinus pressor response leads again to central and intrathoracic blood volume pooling, distention of the left and right atria, etc. It is noteworthy that after approximately ten minutes of occlusion, blood pressure begins to return to normal; however, it does not reach pre-occlusion levels. The intact carotid sinus and aortic sinus, when sensing increased blood pressure, demonstrate two responses which interact to reinforce each other: (a) there is a release of vasoconstrictor tone in the periphery of the body which opens vascular beds lowering systemic blood pressure, and (b) there is a decrease in cardiac output due to both bradycardia and a decrease in stroke volume. The latter need not always be present (8). This second point has no relevance to the investigation of exercise. As exercise builds in intensity, blood volume shifts from the thoracic and abdominal regions to the working muscles. As discussed above, muscle vasoconstrictor tone decreases, and muscle capillary beds open to accept this blood volume shift. This situation now permits cardiac output to adjust to the demands of the intensity of the exercise. The baroreceptors and the sympathetic domination of the autonomic nervous system during exercise reinforce this maneuver. As intrathoracic blood pooling declines, so does its effectiveness to impose an anti-antidiuretic situation.

Segar and Moore (73), employing exposure to a hot environment, demonstrated a situation somewhat similar to exercise. As exposure

to heat increases, vascular beds open and increased ADH secretion ensues as a result of decreased distention of intrathoracic stretch receptors. The marked levels of ADH secretion could have resulted from a loss of extracellular fluid as well as a redistribution of blood due to an increased flow to peripheral beds. However, since no measurable changes in plasma sodium or total solute concentration occurred, it is not likely that an osmoreceptor could have mediated the ADH release. The abrupt drop in blood ADH concentration that occurred within 15 minutes after return to normal temperature provides evidence that the changes in ADH concentration were the result of redistribution of blood. This exposure to heat resulted in an antidiuresis and decreased sodium and total solute excretion. If a similar situation did not occur in exercise, there would be a constant urge to void as a result of the previously mentioned anti-antidiuresis response.

The carotid chemoreceptors are sensitive to changes in PCO_2: increased PCO_2 results in enhanced ADH secretion (8,76,77). Share and Levy (77) concluded that under situations in which there is a simultaneous activation of the chemoreceptors which stimulate ADH release and receptors which inhibit ADH release (left atrial receptors and possibly some arterial baroreceptors), the net result will either be no change or a slight reduction in ADH release. This situation is difficult to envision in the intact animal.

It is apparent that a variety of sitmuli excite and inhibit the secretion of ADH. Intact vagi are necessary to transmit afferent volume receptor information to the central nervous system (8,10,77,78).

Presumably, the CNS integrates neural and hormonal responses to maintain body fluid homeostasis in response to this variety of stimuli.

This review has not produced a basis to suspect that exercise or conditioned emotional response treatment will effect an anti-antidiuresis. The immediacy of the response to the various stimuli described would seem to rule out any long-term effect which was not specifically associated with homeostasis. Therefore, any treatment effects should not be normally masked when interpreting the animals' home-cage data which are collected some ten hours after exposure to the treatments.

The effect of anxiety exposure and/or exercise treatments on urine osmolality in the home-cage environment should prove to be meaningful research in the elucidation of the physiological mechanisms regarding the effects of exercise on responses to anxiety.

Exercise or Anxiety Stress Effects on Urine Osmolality

Corson (7), utilizing a combination of auditory tones and electrical stimulation to create anxiety conditions in dogs, found that this experimental treatment resulted in consistently high urine osmolality. These findings remained valid even when the dogs were water loaded prior to the treatment.

Changes in urine osmolality concentrations resulting from exercise seem to be related to the intensity of the exercise. Kachadorian (39) found that urine excreted after moderate exercise reflected a higher osmolality than urine excreted following mild exercise. But, in this same study, urine after heavy exercise was

found to have a lower osmolality than urine after either mild or moderate exercise. Surprisingly, these findings occurred even though the volume of excreted urine was less following heavy exercise than following moderate exercise. Kachadorian's results support earlier investigations by Raisz *et al.* (65) and Schrier *et al.* (71), who found that heavy exercise produced diluted urine osmolality levels. These investigators speculate that an impairment of the renal concentrating mechanism during heavy exercise may account for the low post-exercise urine osmolality concentrations. These findings should stimulate interest among exercise physiologists as they are contrary to the popular view that exercise, in general, not only reduces excreted urine volume but also increases urine osmolality.

Urinary Sodium and Potassium Concentration Reactions to Stress

Dauphinée (17) reports the active retention of Na^+ and K^+ excretion to be a characteristic mammalian response to physical, emotional, or surgical stress.

Moore (49) reports elevated Na^+ retention and K^+ excretion by humans as a result of surgical stress.

Thorn *et al.* (84), although being nonspecific about the stressors, directly relates Na^+ retention and K^+ excretion to environmental stress. Intense Na^+ retention and K^+ excretion also are reported as a result of infusion of ACTH, hydrocortisone, or corticosterone. In an actual environmental stress situation, these researchers postulate that abnormal K^+ excretion would begin three to four hours following the activation of the adrenal cortex to meet the stress.

Hoagland *et al.* (21), employing pursuit meter tests to induce anxiety, examined 20 subjects. These investigators found increases in both urinary Na^+ and K^+ concentrations for those subjects over 20 years of age. Subjects under 20 years of age also had increased Na^+ concentrations. However, the subjects under 20 years of age demonstrated a K^+ retention of 13%.

Gann and Wright (28), collecting urine samples from five dogs after exposure to induced trauma, clearly demonstrated elevated Na^+ retention to be a result of the experimental variable.

Spigel and Ramsay (80) housed thirty-nine 5-year-old male turtles in individual anxiety chambers. The turtles received one second of electrical shock, delivered randomly on the average of one shock every fifty-nine seconds, twelve hours a day for four days. All turtles responded with elevated urinary Na^+ and K^+ excretion. This research further demonstrated a conditioned emotional response in some of the turtles. Those turtles maintained in the anxiety chambers after the conclusion of the experiment continued to reflect elevated urinary Na^+ and K^+ concentrations even though they were no longer receiving shocks. Turtles previously shocked but removed from the anxiety chambers returned to control-level values.

On the other hand, studies have been performed which suggest that Na^+ and K^+ excretion levels do not respond significantly to imposed anxiety.

Frost (19), utilizing nine subjects involved in the 1950 annual Indianapolis 500 Automobile Race, found no consistent Na^+ or K^+ responses in the post-race urine samples of the drivers.

Paré and McCarthy (61), utilizing forty-eight 90-day-old male rats divided into four groups of twelve, conducted a 22-day, 20-hour-a-day stress experiment. The animals were placed in identical individual anxiety chambers and designated as: 1 = tone-shock, 2 = no tone-shock, 3 = no tone - no shock, 4 = home-cage environment controls. Electrical shocks of 3.5 ma intensity were used with one shock occurring on the average of every four minutes, 20 hours a day. This study yielded no significant Na^+ or K^+ concentration changes.

CHAPTER III

EXPERIMENTAL PROCEDURE

The present study was undertaken to determine the effects of daily interval running as an immediate post-treatment for male rats exposed to daily anxiety conditions. Effectiveness of the treatment was determined by measuring the osmolality, the Na^+ and K^+ mEq/l. concentrations of urine excreted in the home cage.

Perspective of the Experimental Design

An overview of the experiment is shown in Figure 1. Twenty subjects were randomly assigned to four groups:

1) Anxiety - Exercise (A-E)
2) Sham Anxiety - Exercise (SA-E)
3) Anxiety - No Exercise (A-NE)
4) Sham Anxiety - No Exercise (SA-NE)

The anxiety treatments (including sham) were administered via individual grid-box anxiety chambers. The exercise groups were placed on a moderate-duration, medium-intensity, interval-running program (Appendix A). This type of program is intended to simulate the training program for middle-distance running training (880-yard or one-mile run) in man. Urine samples were collected daily and frozen for later analysis.

Individual Grid Boxes	Forced Interval Running Exercise Treatment	No Exercise Treatment
Anxiety Exposure —— With tones and electrical foot pad shocking	1. A-E N = 5	3. A-NE N = 5
Sham Anxiety Exposure —— With tones but *no* electrical foot pad shocking	2. SA-E N = 5	4. SA-NE N = 5

FIGURE 1. Perspective of the experiment by groups and treatment protocol.

The purpose of this design was to determine the effects of running exercise as a therapeutic treatment intended to alleviate concurrent emotional trauma.

Receipt and Assignments of Subjects

Twenty-six male Sprague-Dawley rats, 80 days of age, were purchased from Hormone Assay Laboratories in Chicago, Illinois. Upon arrival, each animal was placed in an individual wire-mesh cage, 20.3 x 20.3 x 25.4 cm., with free access to an adjacent activity wheel. The ensuing nine days were reserved for general environmental adaptation and foot-pad conditioning. Mechanical revolution counters attached to each activity wheel allowed individual activity records to be kept daily for each animal. The three most active and the three least active rats were excluded from the study. This procedure was designed to eliminate chronically hyperactive or hypoactive animals.

On the tenth day, the subjects were transferred to individual sedentary Acme Metabolism Cages located in the same quarters. At this time, five subjects were randomly assigned to each of the four treatment groups. Thereafter, all groups received the same general care with the exception of the experimental variables.

Animal Care

During all phases of the experiment, all of the animals were handled at least once daily. The animals were directly exposed to only three researchers during the experiment. All other laboratory

personnel were asked to avoid entering the animal quarters where the experimental home-cage environment was being maintained.

The animals were fed ground Wayne Food blocks *ad libitum*. Food containers were used which permitted the amount of food consumed in any particular phase of the study to be determined. These food containers also prevented food from falling into the stainless steel urine collection funnels of the metabolism cages. Water was provided *ad libitum* from 100 ml. graduated cylinders in order to calculate the amount of water ingested. Cylinder corks were maintained via Dow high vacuum silicone grease. Daily body weights were taken and recorded to the nearest gram.

Room temperature was maintained at 70°F and 50% relative humidity by air conditioning. To minimize diurnal variability, the quarters were maintained on 24 hours per day of continuous light with the initial receipt of the animals.

Pretreatment Protocol

The subjects were maintained under experimental conditions for seven days without receiving the actual treatments. During the first four days, the animals in all groups received their initial exposure to three hours in the individual anxiety chambers. This phase was followed by movement to holding cages for the A-NE and SA-NE groups. The A-E and SA-E groups were placed in individual running wheels but were not allowed to exercise as the wheels were locked. The locked running wheels and holding cages were adjacent to each other and in the same room as the anxiety chambers. During this

pretreatment period the groups remained in their respective positions for the same length of time as that for the first day of the interval running program. These treatments were followed by the recovery period and then the twelve-hour home-cage environment. For the last three days, the same conditions were imposed except that the running wheels were unlocked and could be operated by the animals. This permitted the work-rest cycles for the first day of the exercise program to be presented to the animals. In this manner, they became familiar with the freely moving wheel (work cycle) and its braking action (rest period). At no time during this seven-day period were the animals subjected to any electrical stimulus. The last three days of this pretreatment phase also were used to collect control data in each of the treatment conditions. In addition, measurements were taken on body weight, food and water ingested and urine volume excreted when appropriate.

Urine Collection Periods

Control urine was collected for three days immediately prior to the treatment period. Excreted urine volume was measured and samples were frozen in 20 ml. plastic vials, with snap caps, for later analysis. Samples were taken when the subjects were in the anxicety chambers (6:00 to 9:00 a.m.), during the holding period and/or exercise treatment (9:10 to 10:00 a.m.), during the recovery period (10:15 a.m. to 12:15 p.m.) and during a twelve-hour home-cage environment period (5:45 p.m. - 5:45 a.m.). Urine collection times during the eighteen-day experimental period were identical to those used to obtain the control data.

Urine Collection Technique

Stainless steel funnels were placed under the sedentary home metabolism cages, the anxiety chambers, the holding cages, and the interval training wheels. These funnels caught and collected the excreted urine. The spout of the funnel was protected from feces by a catch tray and roof apparatus. Spun glass was placed in the spout to filter the urine. A glass 50-ml. graduated centrifuge tube was attached to the funnel spout by means of a two-hole rubber stopper. After each sample collection period, the collecting apparatus was washed, rinsed with distilled water and thoroughly dried. The spun glass was replaced each time the apparatus was cleaned. The same glass centrifuge tube was used for each rat in each treatment period.

Experimental Protocols

Anxiety (6:00 to 9:00 a.m.)

Approximately at 5:45 a.m. each morning, all of the animals in each group were transferred from their home-cage quarters to the adjacent but separate animal training room. Body weights to the nearest gram were determined during the transfer process. The A-E and A-NE groups received the following treatment protocol:

Each animal was placed in a 20.3 x 20.3 x 10.16 cm. anxiety chamber for three hours daily. Each chamber had a stainless steel rod floor, plastic walls, and a securable plywood door for a roof. Stainless steel metabolism-tray funnels were placed under each chamber to collect the excreted urine. Anxiety was induced by faradic

stimulation via the stainless steel rod floor. Each electrical shock (60 volts, 15 ma) was preceded by a 1700 Hz tone 0.5 seconds before the shock was administered. Each shock lasted 0.5 seconds and was administered randomly on a 100-second time base. Ninety-five percent of the electrical shocks were from 5 to 20 seconds apart; five percent of the shocks were from 21 to 100 seconds apart. The average number of randomly presented shocks was four per minute over the three-hour period.

Sham Anxiety (6:00 to 9:00 a.m.)

The two sham anxiety groups (SA-E, SA-NE) were placed in identical anxiety chambers as those of the A-E and A-NE groups. These groups were in their chambers during the same time period and in the same room as the A-E and A-NE groups. The two sham anxiety groups, however, received only the auditory tones with no electrical shocks.

Movement from the living quarters, with body weight determinations, occurred at the same time as that for the two anxiety groups.

Exercise (9:10 to 10:00 a.m.)

Two of the experimental groups (A-E and SA-E) received a forced exercise treatment immediately after exposure to anxiety or sham-anxiety conditions. This procedure consisted of a moderate-intensity, medium-duration interval running program (Appendix A), in electronically controlled running wheels. By its description, this program is aerobic in nature and within the physiological work capacity of the rat. Another attractive feature of this training method is that it employs running which is a physical skill common to the rat.

The detailed description of the interval training program found in Appendix A was modified for this study. The purpose of this study was to exercise the animals, not necessarily to train them. A decision was made to follow the training program up to day eight, and then to maintain the exercise level at that intensity through day 18, the final day of the experiment.

Appendix B contains a comprehensive explanation of the electronically controlled interval running wheels. This appendix also describes the motivation stimulus which prompted the animals to run (95).

Body weights before and after the exercise treatment were determined to the nearest gram for each rat.

Holding (9:10 to 10:00 a.m.)

The holding protocol consisted of an intermediate holding area for the A-NE and SA-NE groups. Upon termination of the anxiety or sham anxiety protocols, these groups were immediately transferred to 20.3 x 20.3 x 25.4 cm. wire mesh holding cages within the same room that housed the anxiety chambers and the electronic running wheels. During the holding period food and water were withheld. Urine samples were collected as previously described. Body weights before and after holding were measured.

Recovery (10:15 a.m. to 12:15 p.m.)

At 10:00 a.m. each morning, the two exercise treatment groups and the two non-exercised groups were transferred back to their home cages in the living quarters. The following two hours were

designated as a recovery period. Body weight was determined before and after recovery. Food and water were available *ad libitum*, ingestion levels were measured, as well as the amount of urine excreted.

Zero Phase (12:15 p.m. to 5:30 p.m.)

During this time, the animals remained in their home cages. Food and water were available *ad libitum*. No urine samples were collected.

Home-Cage Environment (5:45 p.m. to 5:45 a.m.)

The twelve-hour home-cage environment period was the focus at which the impact of this current research was aimed. Food and water were available *ad libitum*, the amounts of each that were ingested were measured. Total urine volume excreted was measured and samples were frozen in 20 ml. plastic vials for later analysis.

Sacrifice Protocol

Approximately one hour following the final treatment period on the last experimental day, the animals were sacrificed by decapitation. Their adrenal glands were removed and immediately weighed on a Mettler analytical balance.

Urine Osmolality Analysis

A Precision Systems Osmometer was used to determine urine osmolalities. This is an uncomplicated procedure whereby a 2-ml. sample is placed in the osmometer and its freezing point is determined. At the freezing point, the apparatus produces a digital readout, in

milliosmols per kilogram, of the osmolality of the fluid analyzed. The step by step procedure that was used for analysis was the standard method provided by the manufacturer of the osmometer.

Urine Na^+ and K^+ Electrolyte Concentrations

Na^+ and K^+ electrolyte concentration gradients (mEq/l.) were measured using a Coleman Flamephotometer. The standard procedure described by the manufacturer was employed in the operation of the apparatus for these analyses. The procedure is straightforward; however, care must be taken to recalibrate the flamephotometer every ten to fifteen samples in order to insure accurate measures.

Statistical Analysis

In order to standardize the data so that meaningful comparisons could be made, the raw data for each animal were divided by the animal's body weight and then multiplied by 100 (osmolality values were not multiplied by 100). This procedure converts all data to values which are relative. As a result of this procedure, the data for each animal were expressed in terms of units per gram body weight; i.e., a reported mean osmolality of 6.212 was the amount of osmolality per gram of body weight. After compiling the results for each rat, mean values for each group in each observation period were obtained.

It was decided that the collected data lent itself to a repeated measures analysis of variance. This analysis requires that there must be more subjects per group than there are observations on each subject (97). There were five animals in each group; therefore the 21 days of control and experimental data collection were divided

into four observation intervals. Observation interval 1 was the three-day control data interval. The remaining three observation intervals consisted of six days each. That is, observation interval 2 consisted of treatment days 1-6. Based on research by Danford *et al.* (15), concerning the analysis of repeated measurements experiments, the data were tested for equal variances (EV) and compound symmetry (CS). If the data met the assumption of equality of variance and compound symmetry, a one-way repeated measurements univariate analysis of variance was performed. If the data had equal variances, but failed the test for compound symmetry, the same analysis was performed using the "conservative F-test." Table 1 shows the variables investigated and the results of these tests of assumptions.

The probability of making a type I statistical error was set at the .05 level. A Scheffé Post-Hoc procedure was performed on the data in order to identify the observation periods where significant changes had occurred.

TABLE 1

Results of Statistical Tests on the Investigated Variables to Determine Their Meeting the Assumptions of Equal Variance and Compound Symmetry

	Variable	EV	DS
1.	Home-cage urine osmolality	yes	no
2.	Home-cage urine Na^+ in mEq/l.	yes	no
3.	Home-cage urine K^+ in mEq/l.	yes	no
4.	Home-cage food digested	yes	yes
5.	Home-cage HOH digested	yes	yes
6.	Home-cage urine excreted	yes	no
7.	Home-cage body weight	yes	no
8.	Recowery HOH digested	yes	yes
9.	Recovery urine excreted	yes	yes
10.	Home-cage total urine Na^+	yes	no
11.	Home-cage total urine K^+	yes	yes
12.	Home-cage urine Na^+/K^+ ratios	yes	no

CHAPTER IV

RESULTS

The results presented here were based upon the repeated measurements univariate analysis of variance, further clarified by a Scheffé Post-Hoc test, as described in the experimental methods section. The probability of chance occurrence was set at the .05 level of significance. Unless otherwise indicated, all of these results reflect the animals' home-cage environment.

When the overall treatment and duration-of-treatment effects were statistically significant ($p \leq .05$) for the variables investigated, in the rats' home-cage environment, specific treatment and duration effects tests were run. Therefore, the specific treatment results between groups within observation periods and the duration of treatment effects between observation periods within groups are tabulated for the appropriate variables as they appear in the results section.

Response to the Running Exercise

Two of the four groups (A-E, SA-E) were exercised daily by a program of interval running conducted in electrically controlled interval running wheels. These data demonstrate that the animals reacted favorably to the imposed interval running exercise. The

first six days of running was, essentially, when the rats learned to operate the wheels and also represented their initial, programmed, physical conditioning. During this initial six-day period (second observation) the A-E group accumulated an average of 479 total revolutions run per day (TRR = 479), which was 107 percent of the revolutions run (PER = 107). These results are shown in Figure 2. During this same period the SA-E group had TRR = 462 for PER = 103. During the third observation interval, the A-E group averaged 850 TRR, which was 112 PER, while the SA-E group averaged 784 TRR for 105 PER. For the fourth observation, the A-E group compiled 830 TRR and 110 PER, while the SA-E group had 783 TRR, which was 105 PER. The differences between the groups were not statistically significant.

These findings lend further validity to the effectiveness of the electronically controlled interval running wheel as a reliable method of exercising laboratory rats.

Body Weight

Body weight comparisons between the groups (Figure 3) showed that all groups except the SA-NE group lost weight during the course of the study. However, this weight loss was statistically significant for only the SA-E group. A general weight loss by young postpubertal animals was surprising. Possibly this was the initial evidence of the imposed stress the animals were experiencing.

Urine Osmolality Responses

All of the groups showed statistically significant and similar increases in urine osmolality (Figure 4, Table 2) by the second

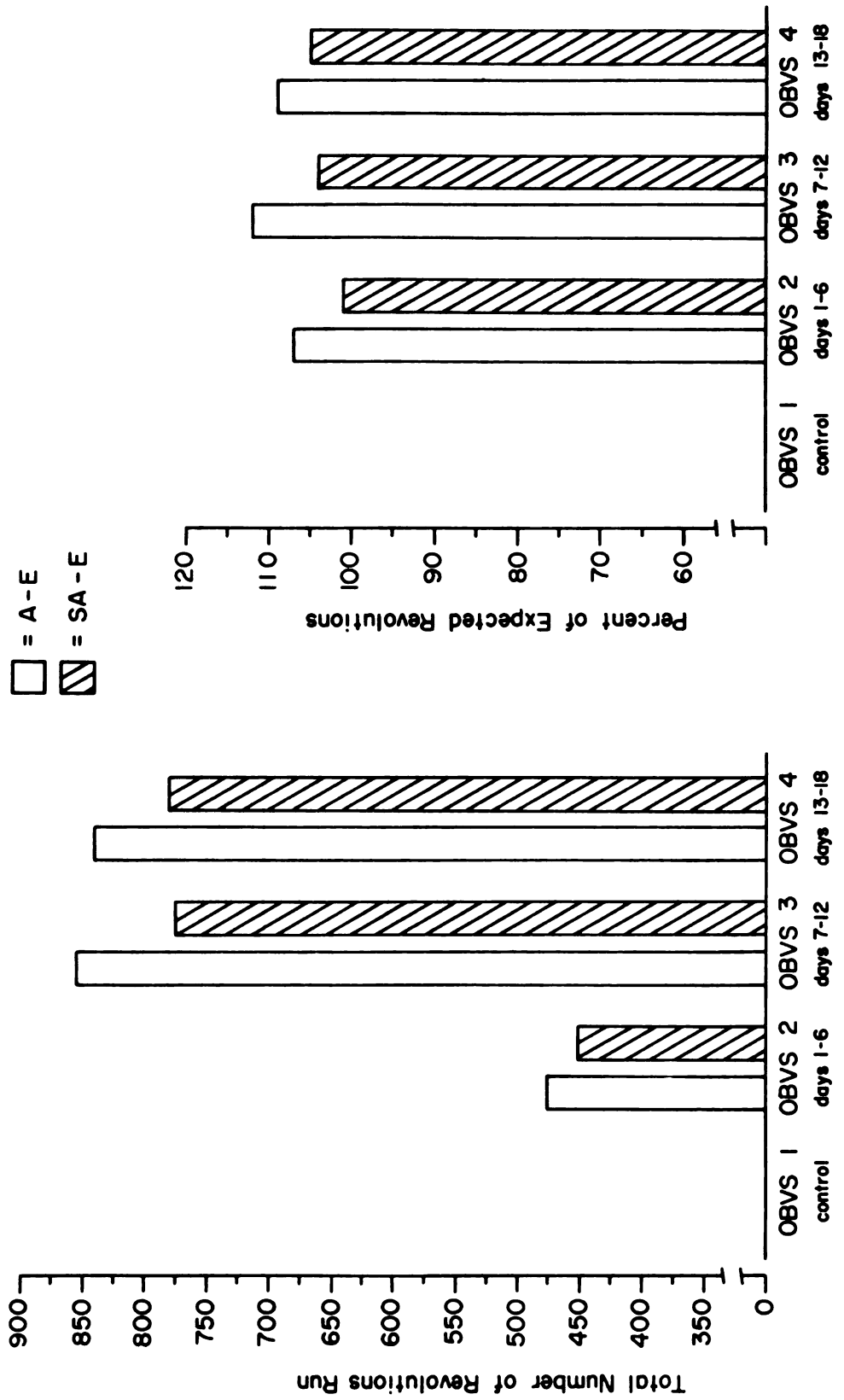

Figure 2. Average percent expected revolutions (PER) and total number of revolutions run (TRR) of the exercise treated rats over the three experimental observation intervals.

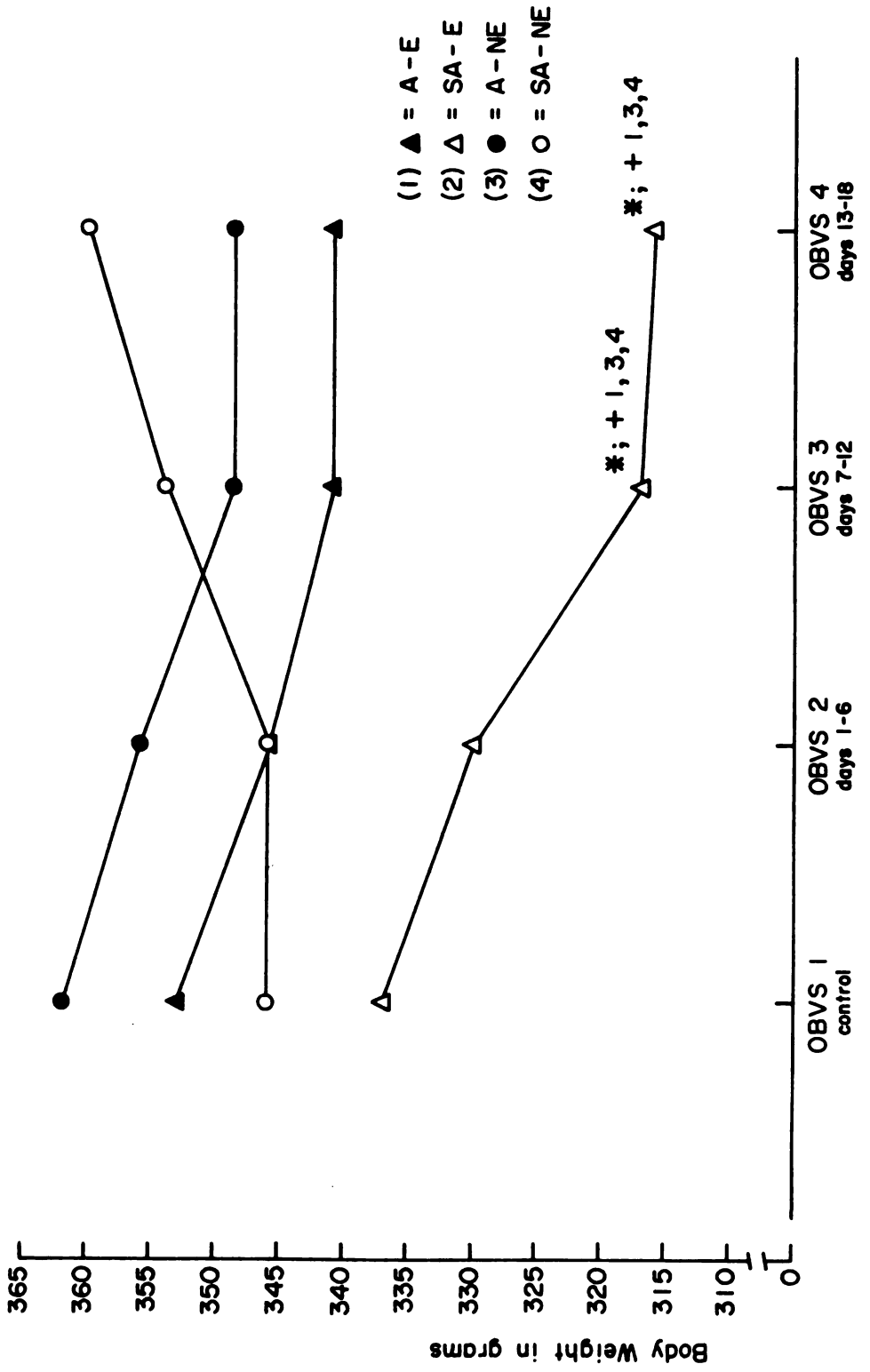

Figure 3. Mean body weight comparisons by groups across the study.

* = Significantly different from control data (p=.05)
+ = Significantly different from indicated groups (p=.05)

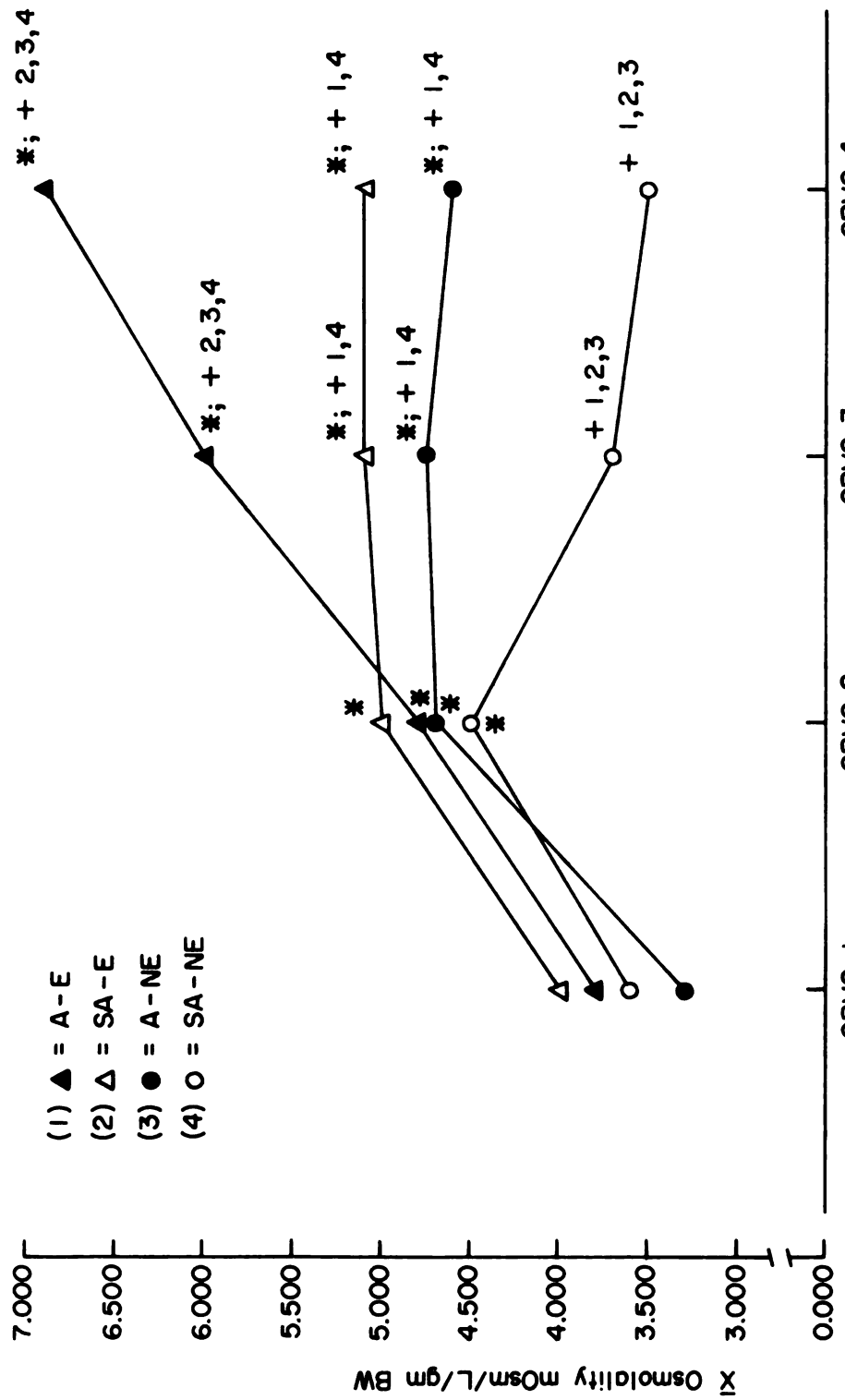

Figure 4. Mean home cage urine osmolality in mOsm/L/gm BW during control and experimental observations.

* = Significantly different from control data (p=.05)
+ = Significantly different from indicated groups (p=.05)

TABLE 2

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Osmolality

Treatment group		Observation period 1	2	3	4
A-E	Mean	3.634	4.960	6.596	6.987
	S.D.	.506	.868	1.038	.461
SA-E	Mean	3.608	4.904	5.244	5.234
	S.D.	.234	.798	.788	.615
A-NE	Mean	3.270	4.883	4.933	4.841
	S.D.	.400	1.173	.931	.307
SA-NE	Mean	3.296	4.649	3.625	3.549
	S.D.	.234	.465	.378	.331

Duration of treatment effects, between observations, within groups

	F	P
A-E	75.886	$\leq$ .01
SA-E	12.938	$\leq$ .05
A-NE	23.058	$\leq$ .01
SA-NE	6.047	$\leq$ .05

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	.902	.388	9.322	21.427
P	NS	NS	$\leq$ .05	$\leq$ .01

TABLE 2 (continued)

Scheffé Post-Hoc Test

Observations			Treatment		
Treatment groups	Periods contrasted	Mean differences	Observation period	Groups contrasted	Mean difference
A-E	2-1	1.326*	3	A-E - SA-E	1.352*
	3-1	2.962*		A-E - A-NE	1.563*
	4-1	3.353*		A-E - SA-NE	2.171*
				SA-E - A-NE	.311
SA-E	2-1	1.296*		SA-E - SA-NE	1.619*
	3-1	1.686*		A-NE - SA-NE	1.308*
	4-1	1.626*			
			4	A-E - SA-E	1.753*
A-NE	2-1	1.613*		A-E - A-NE	2.146*
	3-1	1.663*		A-E - SA-NE	3.338*
	4-1	1.571*		SA-E - A-NE	.393
				SA-E - SA-NE	1.685*
SA-NE	2-1	1.353*		A-NE - SA-NE	1.292*
	3-1	0.329			
	4-1	0.253			

*Mean difference = 1.277 required for significance at $p \leq .05$.

*Mean difference = 1.277 required for significance at $p \leq .05$.

observation interval. The A-E treatment group continued to reflect increasing urine osmolality over the next two observation intervals, nearly doubling their control urine osmolality. They also reflected significantly higher home-cage urine osmolalities than either of the other groups at observation intervals three and four. The SA-E and A-NE groups remained at about the same elevated urine osmolalities over the third and fourth observation intervals; these osmolalities were nearly fifty percent greater than their control urine osmolalities. These two groups were not significantly different from each other, but their home-cage treatment urine osmolalities at observations three and four were significantly different from those of A-E and SA-NE groups. The SA-NE group returned to control level urine osmolality by the third observation interval. Of particular interest is that the increased osmolality at the second observation interval was accompanied by a failure to gain weight (see Figure 3). These associated findings warrant further investigation.

Urine Na^+ Concentration in mEq/l.

All groups demonstrated a statistically significant Na^+ retention (Figure 5, Table 3) which was evident by the second observation interval. There were no significant differences between the groups with respect to Na^+ retention based on concentration gradients alone. The Na^+ electrolyte retention factor will be brought more into focus when the total Na^+ retention patterns are examined.

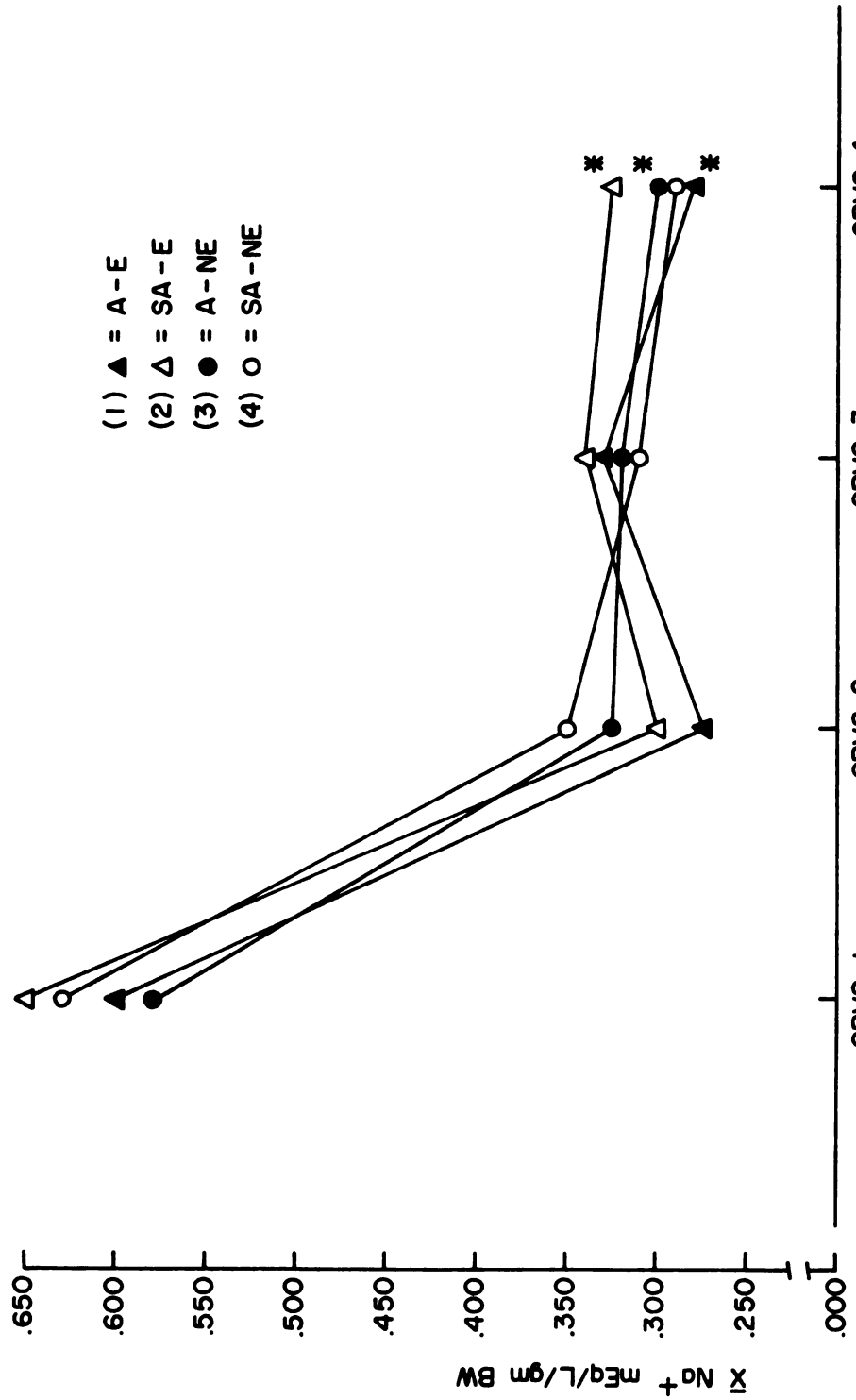

Figure 5. Mean home cage urine Na^+ concentration in mEq/L/gm body weight x 100 for the control and experimental observations.

* = Significantly different from control data (p = .05)

TABLE 3

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Na^+ Patterns

Treatment group		Observation period 1	2	3	4
A-E	Mean	.626	.283	.309	.274
	S.D.	.023	.064	.072	.070
SA-E	Mean	.659	.313	.338	.320
	S.D.	.057	.046	.030	.048
A-NE	Mean	.596	.339	.334	.290
	S.D.	.036	.059	.035	.095
SA-NE	Mean	.640	.340	.303	.289
	S.D.	.055	.027	.031	.091

Duration of treatment effects, between observations, within groups

	F	P
A-E	79.238	$\leq .01$
SA-E	77.765	$\leq .01$
A-NE	53.507	$\leq .01$
SA-NE	52.601	$\leq .01$

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	1.105	1.131	.489	.897
P	NS	NS	NS	NS

TABLE 3 (continued)

Treatment groups	Observations: Periods contrasted	Mean differences
A-E	2-1	-.343
	3-1	-.317*
	4-1	-.352*
SA-E	2-1	-.346*
	3-1	-.321*
	4-1	-.339*
A-NE	2-1	-.257*
	3-1	-.262*
	4-1	-.306*
SA-NE	2-1	-.300*
	3-1	-.337*
	4-1	-.351*

*Mean difference = 0.146 required for significance at $p \leq .05$.

Urine K^+ Concentration in mEq/l.

All groups showed an enhanced K^+ excretion (Figure 6, Table 4) by the third observation interval. The SA-NE group returned to its control-level urine K^+ concentration gradient during the fourth observation interval. A more detailed examination of the responses of this group will be found in the section on urine volume excretion patterns. At the fourth observation interval, the K^+ excretions of the other three groups returned toward normal levels but remained significantly above their control levels.

Total Urinary Na^+

Total urinary Na^+ refers to the concentration in mEq/l. multiplied by the volume of urine excreted for that analyzed sample. For example, a concentration of Na^+ equal to 200 mEq/l. for a sample taken from a urine volume of 15 ml. would yield a total urinary Na^+ of 3,000 mEq.

All of the groups showed a Na^+ retention (Figure 7, Table 5) over the course of the experiment, as demonstrated by their total Na^+ data. There were no significant differences between groups, but all groups except the SA-NE group had statistically significant total Na^+ retentions across all three of the experimental observation intervals. The SA-NE group failed to exhibit significance based on total urine volume calculations. They were the one group which displayed stable and even increasing urine excretion rates (Figure 12) over the duration of the investigation.

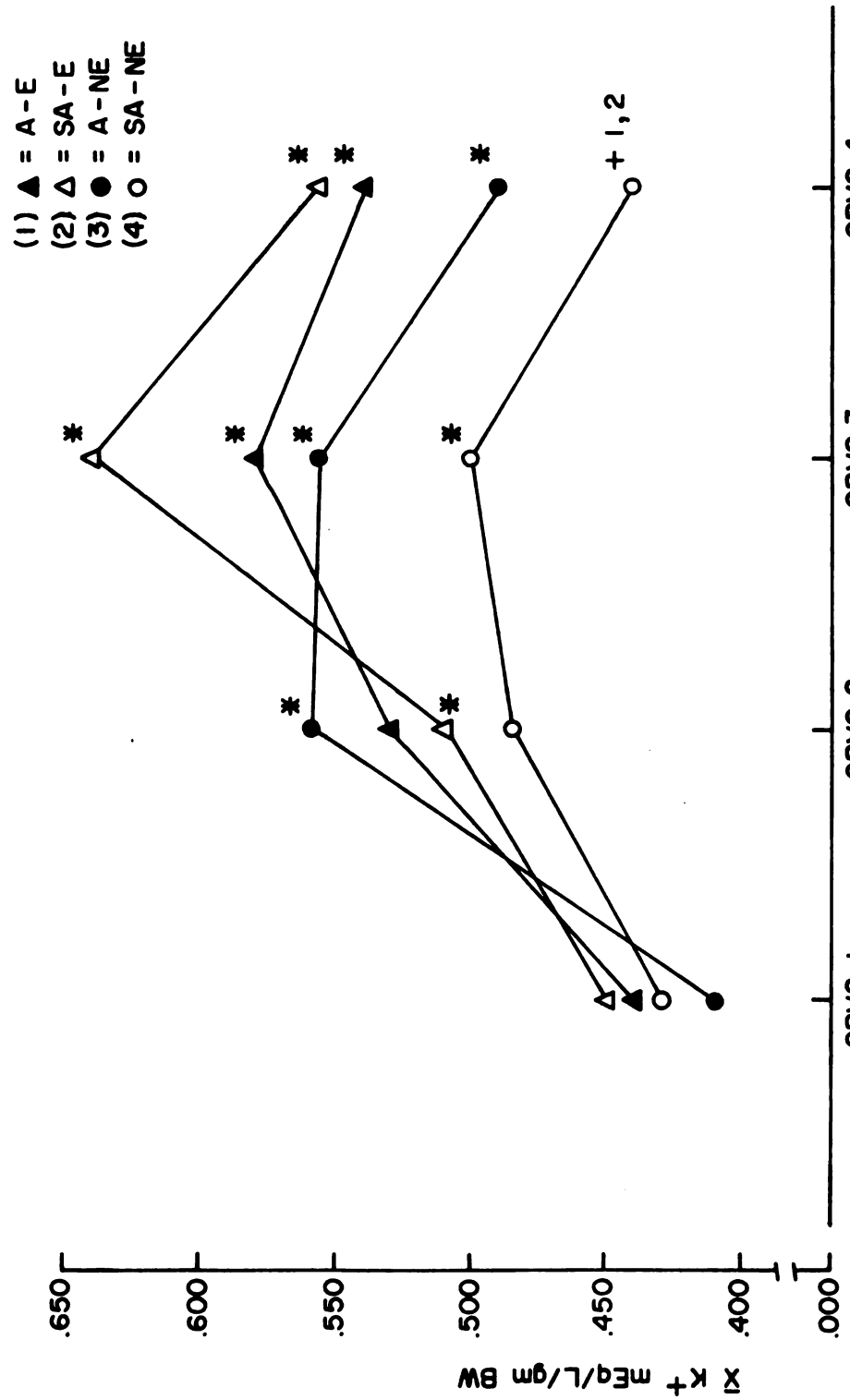

Figure 6. Mean home cage urine K^+ concentration in mEq/L/gm body weight x 100 for the control and experimental observations.

* = Significantly different from control data (p = .05)
+ = Significantly different from indicated groups (p = .05)

TABLE 4

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine K^+ Patterns

Treatment group		Observation period 1	2	3	4
A-E	Mean	.425	.549	.610	.560
	S.D.	.032	.091	.063	.104
SA-E	Mean	.450	.538	.642	.580
	S.D.	.036	.080	.028	.109
A-NE	Mean	.405	.565	.598	.535
	S.D.	.033	.080	.041	.139
SA-NE	Mean	.420	.526	.550	.427
	S.D.	.036	.087	.062	.072

Duration of treatment effects, between observations, within groups

	F	P
A-E	9.129	$\leq .05$
SA-E	9.801	$\leq .05$
A-NE	9.364	$\leq .05$
SA-NE	4.283	NS

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	.226	.247	1.435	1.859
P	NS	NS	NS	NS

TABLE 4 (continued)

Scheffé Post-Hoc Test

Observations				Treatment	
Treatment groups	Periods contrasted	Mean differences	Observation period	Groups contrasted	Mean difference
A-E	2-1	.124	4	A-E - SA-E	.020
	3-1	.185*		A-E - A-NE	.025
	4-1	.135*		A-E - SA-NE	.133*
				SA-E - A-NE	.045
SA-E	2-1	.188*		SA-E - SA-NE	.153*
	3-1	.192*		A-NE - SA-NE	.108
	4-1	.130*			
A-NE	2-1	.100*			
	3-1	.198*			
	4-1	.130*			
SA-NE	2-1	.106			
	3-1	.130*			
	4-1	.007			

*Mean difference = .128 required for significance at $p \leq .05$.

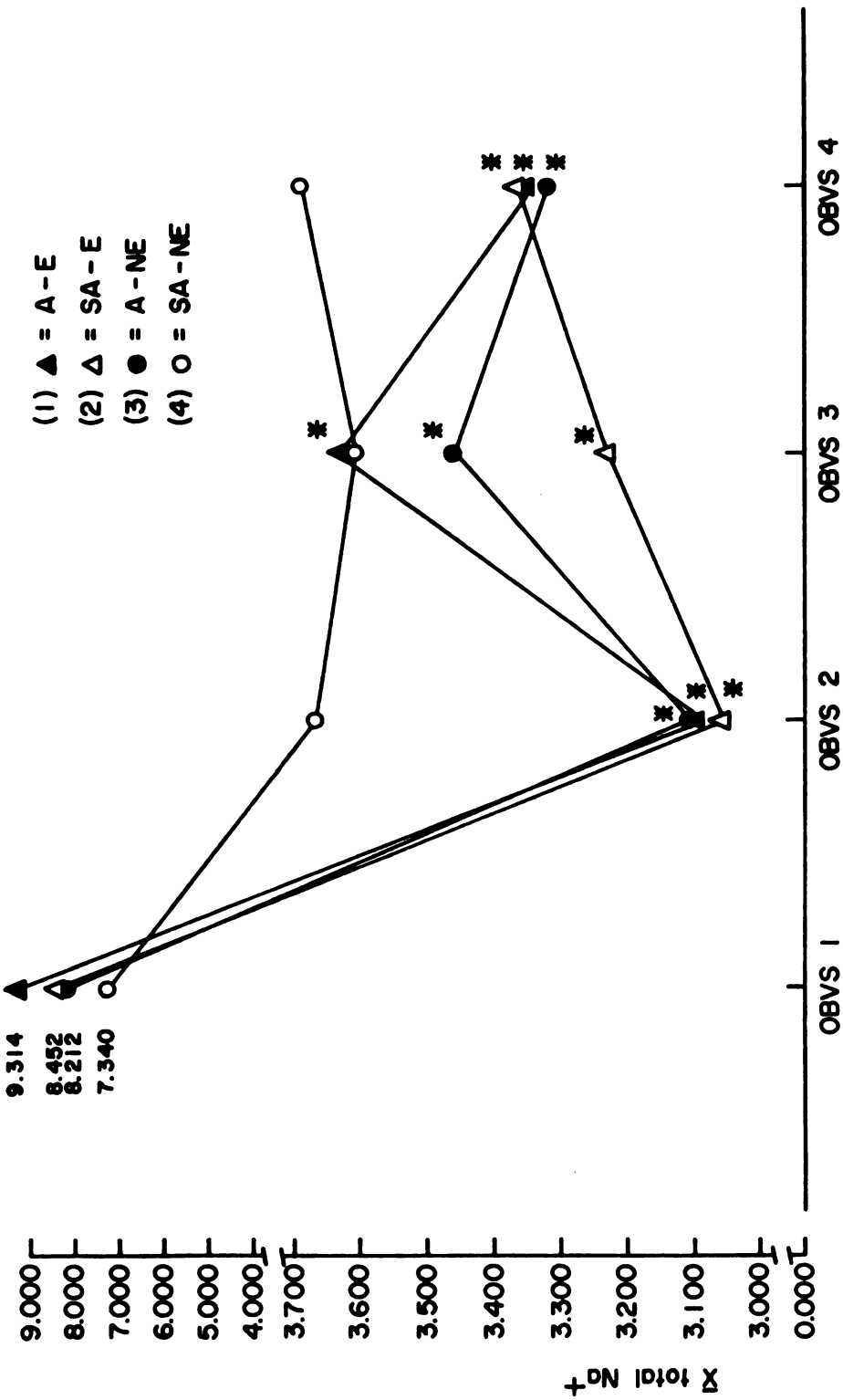

Figure 7. Mean home cage total urine Na^+ (Na^+ concentration in mEq/L x urine volume) in mEq/gm body weight x 100 during the control and experimental observations.

* = Significantly different from control data (p=.05)

TABLE 5

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Total Na^+ Patterns

Treatment group		Observation period 1	2	3	4
A-E	Mean	9.314	3.100	3.688	3.632
	S.D.	1.817	.373	.255	.284
SA-E	Mean	8.452	3.052	3.258	3.376
	S.D.	.872	.791	.345	.159
A-NE	Mean	8.212	3.160	3.460	3.352
	S.D.	3.628	.259	.752	.426
SA-NE	Mean	7.340	3.686	3.622	3.698
	S.D.	1.782	.261	.235	.260

Duration of treatment effects, between observations, within groups

	F	P
A-E	33.084	$\leq .01$
SA-E	26.313	$\leq .01$
A-NE	23.042	$\leq .01$
SA-NE	12.969	$\leq .05$

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	2.347	.309	.131	.110
P	NS	NS	NS	NS

TABLE 5 (continued)

Treatment groups	Scheffé Post-Hoc Test Observations Periods contrasted	Mean difference
A-E	2-1	-6.214*
	3-1	-5.626*
	4-1	-5.682*
SA-E	2-1	-5.400*
	3-1	-5.194*
	4-1	-5.076*
A-NE	2-1	-5.052*
	3-1	-4.752*
	4-1	-4.860*
SA-NE	2-1	-3.654
	3-1	-3.718
	4-1	-3.642

*Mean difference = 3.859 required for significance at $p \leq .05$.

Total Urinary K^+

Regarding total urine K^+ (Figure 8, Table 6), the A-E and SA-NE groups showed a statistically significant urinary excretion of total K^+ at observation intervals three and four. The A-NE group demonstrated a similar significant rise in total K^+ excretion during observation period three. The SA-E group was the only group not to reflect a statistically significant rise in K^+ excretion.

The F ratios (7.429) were not as high as those for urine osmolality (71.409) or Na^+ mEq/1. concentration (29.304); however, the total K^+ data produced thought-provoking information. Interest is aroused as to why the SA-E group responded at observation interval two with a K^+ retention (not significant) and otherwise had an essentially stable K^+ excretion pattern. The possibility of K^+ excretion being sensitive essentially only to emotional stress should be examined. This is emphasized further by the fact that the SA-NE group did not demonstrate a total Na^+ retention but clearly showed a K^+ wasting effect when their total excreted quantity of the K^+ electrolyte was examined.

Na^+/K^+ Ratio

Na^+/K^+ ratios were depressed for all of the groups across all three experimental observation intervals (Figure 9, Table 7). The depressed concentration ratios were significantly different from the control Na^+/K^+ ratios. All groups responded similarly.

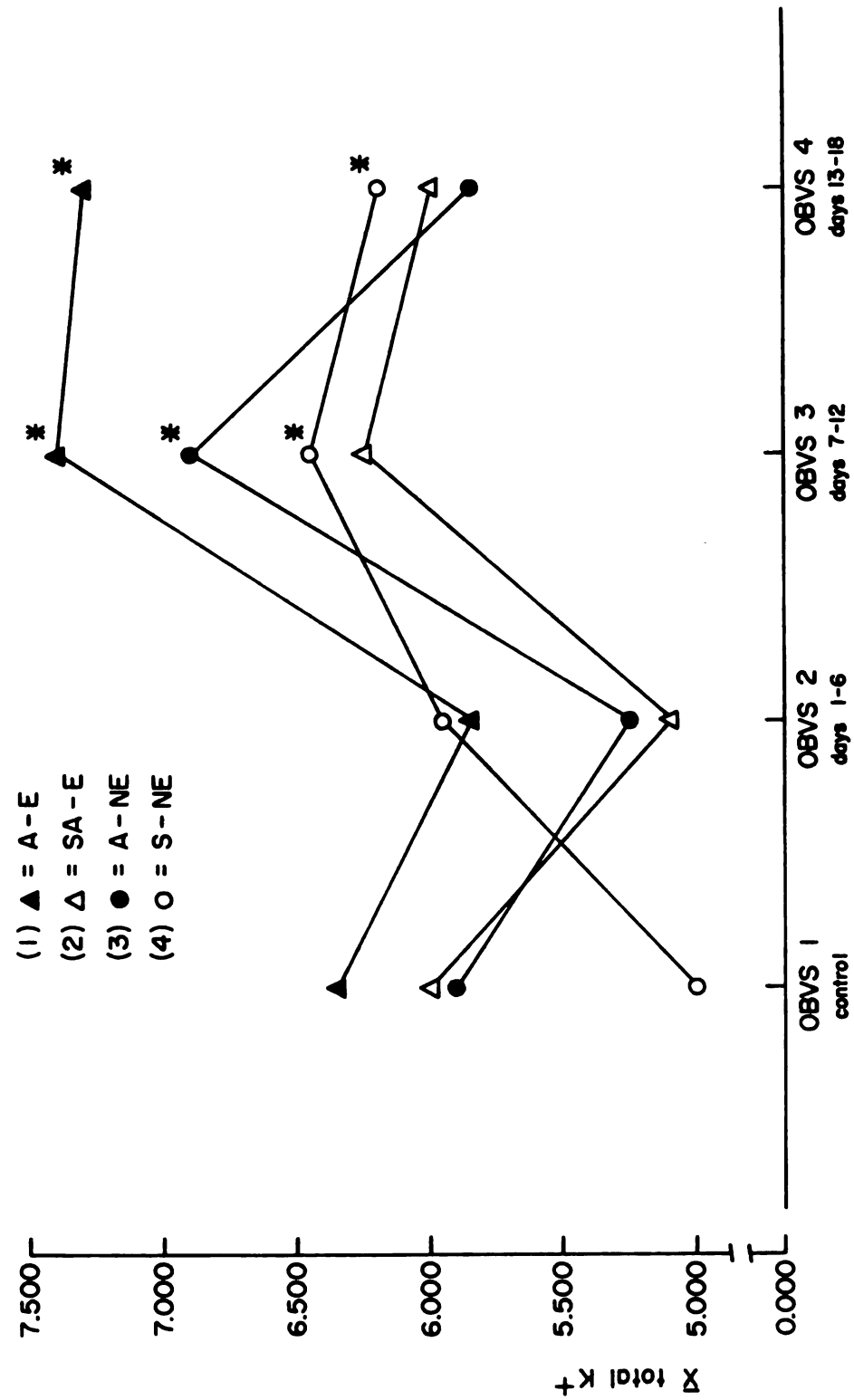

Figure 8. Mean home cage total urine K^+ (K^+ concentration in mEq/L x urine volume) in mEq/gm body weight x 100 for the control and experimental observations.

* = Significantly different from control data (p=.05)

TABLE 6

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Total K^+ Patterns

Observation group		Observation period 1	2	3	4
A-E	Mean	6.301	5.876	7.608	7.650
	S.D.	1.127	.841	1.327	.472
SA-E	Mean	5.960	5.176	6.232	5.963
	S.D.	.806	.978	.706	.382
A-NE	Mean	5.906	5.358	7.351	5.872
	S.D.	2.196	1.135	2.281	.534
SA-NE	Mean	5.098	5.938	6.492	6.398
	S.D.	.998	.455	.555	.290

Duration of treatment effects, between observations, within groups

	F	P
A-E	5.767	$\leq .05$
SA-E	1.328	NS
A-NE	2.810	NS
SA-NE	4.989	$\leq .05$

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	1.193	.588	1.208	1.887
P	NS	NS	NS	NS

TABLE 6 (continued)

Treatment groups	Scheffé Post-Hoc Test Observations Periods contrasted	Mean differences
A-E	2-1	-1.425
	3-1	1.307*
	4-1	1.349*
SA-E	2-1	-0.784
	3-1	.278
	4-1	.002
A-NE	2-1	.548
	3-1	1.445*
	4-1	.034
SA-NE	2-1	.840
	3-1	1.394*
	4-1	1.300*

*Mean Difference = 1.281 required for significance at $p \leq .05$.

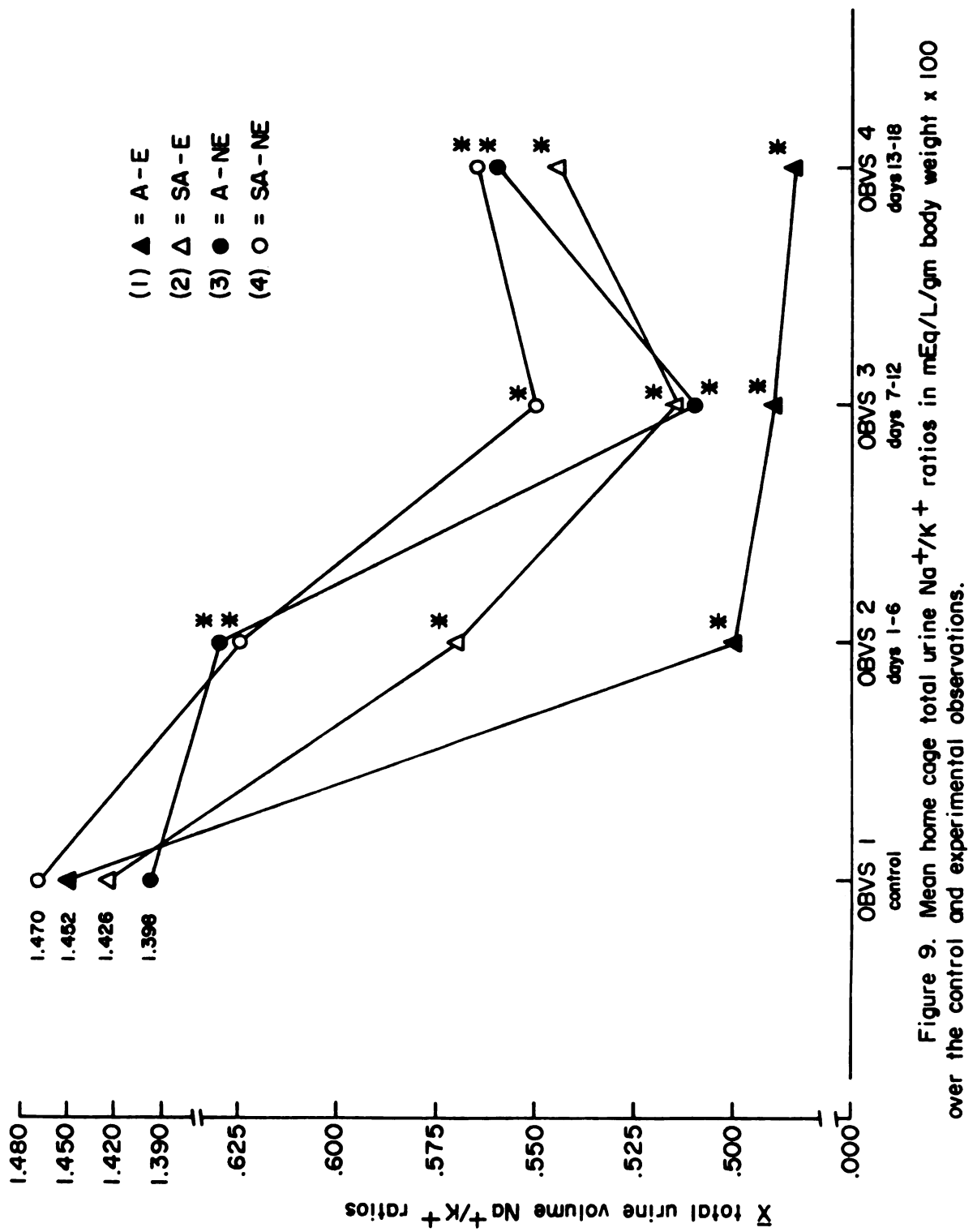

Figure 9. Mean home cage total urine Na^+/K^+ ratios in mEq/L/gm body weight x 100 over the control and experimental observations.

* = Significantly different from control data (p=.05)

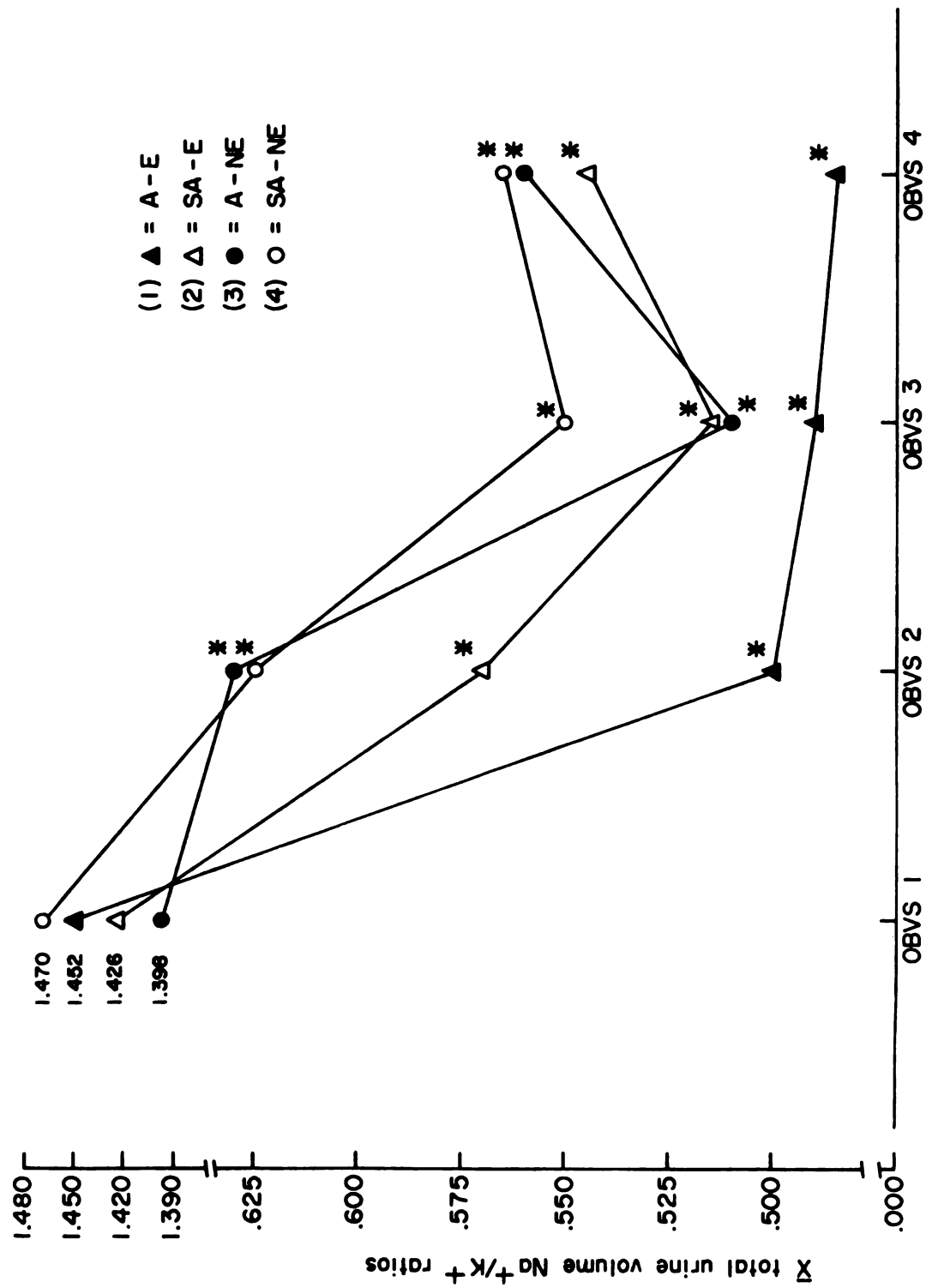

Figure 9. Mean home cage total urine Na^+/K^+ ratios in mEq/L/gm body weight x 100 over the control and experimental observations.

* = Significantly different from control data (p=.05)

TABLE 7

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Na^+/K^+ Ratios

Treatment group		Observation period 1	2	3	4
A-E	Mean	1.452	.502	.496	.490
	S.D.	.062	.047	.059	.043
SA-E	Mean	1.426	.574	.516	.556
	S.D.	.047	.053	.036	.036
A-NE	Mean	1.398	.628	.514	.566
	S.D.	.062	.065	.059	.038
SA-NE	Mean	1.470	.624	.550	.568
	S.D.	.052	.034	.019	.020

Duration of treatment effects, between observations, within groups

	F	P
A-E	504.146	$\leq .01$
SA-E	504.397	$\leq .01$
A-NE	454.295	$\leq .01$
SA-NE	519.307	$\leq .01$

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	2.157	3.549	1.112	3.004
P	NS	NS	NS	NS

TABLE 7 (continued)

Treatment groups	Scheffé Post-Hoc Test Observations Periods contrasted	Mean differences
A-E	2-1	-.950*
	3-1	-.956*
	4-1	-.962*
SA-E	2-1	-.852*
	3-1	-.910*
	4-1	-.870*
A-NE	2-1	-.770*
	3-1	-.884*
	4-1	-.832*
SA-NE	2-1	-1.146*
	3-1	-.920*
	4-1	-.920*

*Mean difference = .146 required for significance at $p \leq .05$.

Relative Adrenal Weight

This comparison (Table 8) revealed that the relative adrenal weight for the A-E group was significantly greater than were those of the SA-NE and A-NE groups, but similar to that of the SA-E group. In turn, the SA-E and A-NE groups had similar relative adrenal weights. Both were significantly greater than that of the SA-NE group ($p = .05$).

TABLE 8

Means for Relative Adrenal Weight

Groups	A-NE	SA-NE	SA-E	A-E
Mean	1.784	1.402	1.987	2.150

Food Ingestion Comparisons

All groups had similar and statistically significant drops in food ingestion which were evident at the second observation interval (Figure 10, Table 9). By the third and fourth observation intervals, food ingestion had returned to the control-period intake (adjusted for body weight changes, i.e., gram food per gram body weight). The A-E group demonstrated a significantly elevated food ingestion at the fourth observation interval.

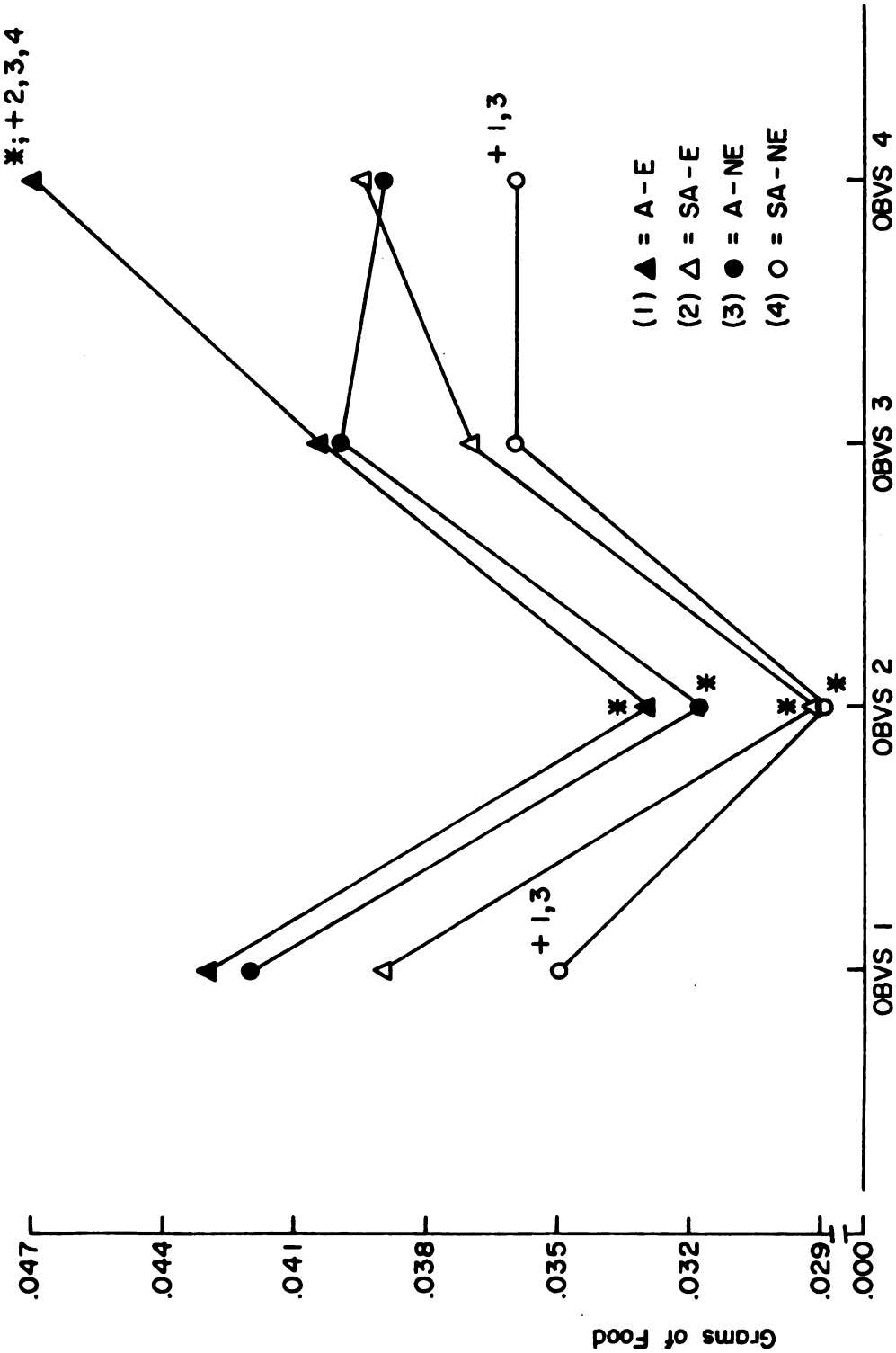

Figure 10. Mean home cage food ingestion levels in grams/gm body weight x 100 over the control and experimental observations.

* = Significantly different from control data (p=.05)
+ = Significantly different from indicated groups (p=.05)

TABLE 9

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Food Ingestion

Treatment group		Observation period 1	2	3	4
A-E	Mean	.043	.033	.043	.048
	S.D.	.006	.005	.002	.002
SA-E	Mean	.039	.029	.037	.039
	S.D.	.004	.005	.005	.004
A-NE	Mean	.042	.032	.040	.041
	S.D.	.006	.002	.005	.005
SA-NE	Mean	.035	.029	.036	.036
	S.D.	.006	.001	.005	.007

Duration of treatment effects, between observations, within groups

	F	P
A-E	13.464	≤ .01
SA-E	8.980	≤ .01
A-NE	6.808	≤ .01
SA-NE	4.000	≤ .05

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	2.877	1.020	1.083	5.259
P	NS	NS	NS	≤ .05

TABLE 9 (continued)

Scheffé Post-Hoc Test

Observations			Treatment		
Treatment groups	Periods contrasted	Mean differences	Observation period	Groups contrasted	Mean difference
A-E	2-1	-.010*			
	3-1	.000	4	A-E - SA-E	-.009*
	4-1	-.005*		A-E - A-NE	-.007*
				A-E - SA-NE	-.012*
SA-E	2-1	-.010*		SA-E - A-NE	-.002
	3-1	-.002		SA-E - SA-NE	-.003
	4-1	.000		A-NE - SA-NE	-.005*
A-NE	2-1	-.010*			
	3-1	-.002			
	4-1	-.001			
SA-NE	2-1	-.006*			
	3-1	.001			
	4-1	.001			

*Mean difference = .005 required for significance at $p \leq .05$.

*Mean difference = .005 required for significance at $p \leq .05$.

Water Consumption Comparisons

All groups had a significant and similar decrease in water ingestion at the second observation interval (Figure 11, Table 10). Water ingestion was only partially restored by the third observation interval. During the fourth observation interval, all groups returned to control levels of water ingestion, with the exception of the A-E group, which continued to exhibit depressed water intake. The water consumption of the A-E group was significantly different from that of the other groups in the fourth observation interval. This is consistent with the fact that they were also statistically different from the other three groups during the control period.

Urine Volume Excretion Patterns

The urine excretion pattern for all of the groups followed a trend similar to that of their water consumption (Figure 12, Table 11). All groups had significantly depressed urine volume excretions which were evident by the second observation interval and which continued through the third observation interval (except for the SA-NE group). During the fourth observation interval, the A-E and A-NE groups returned to control urine excretion volumes. However, the SA-E group urine excretion rates remained depressed while the SA-NE group demonstrated a significantly increased urine volume during the fourth interval.

The urine excretion patterns verify the validity of utilizing total electrolyte patterns. If only a mEq/l. concentration gradient analysis had been employed, it would have mistakenly appeared that

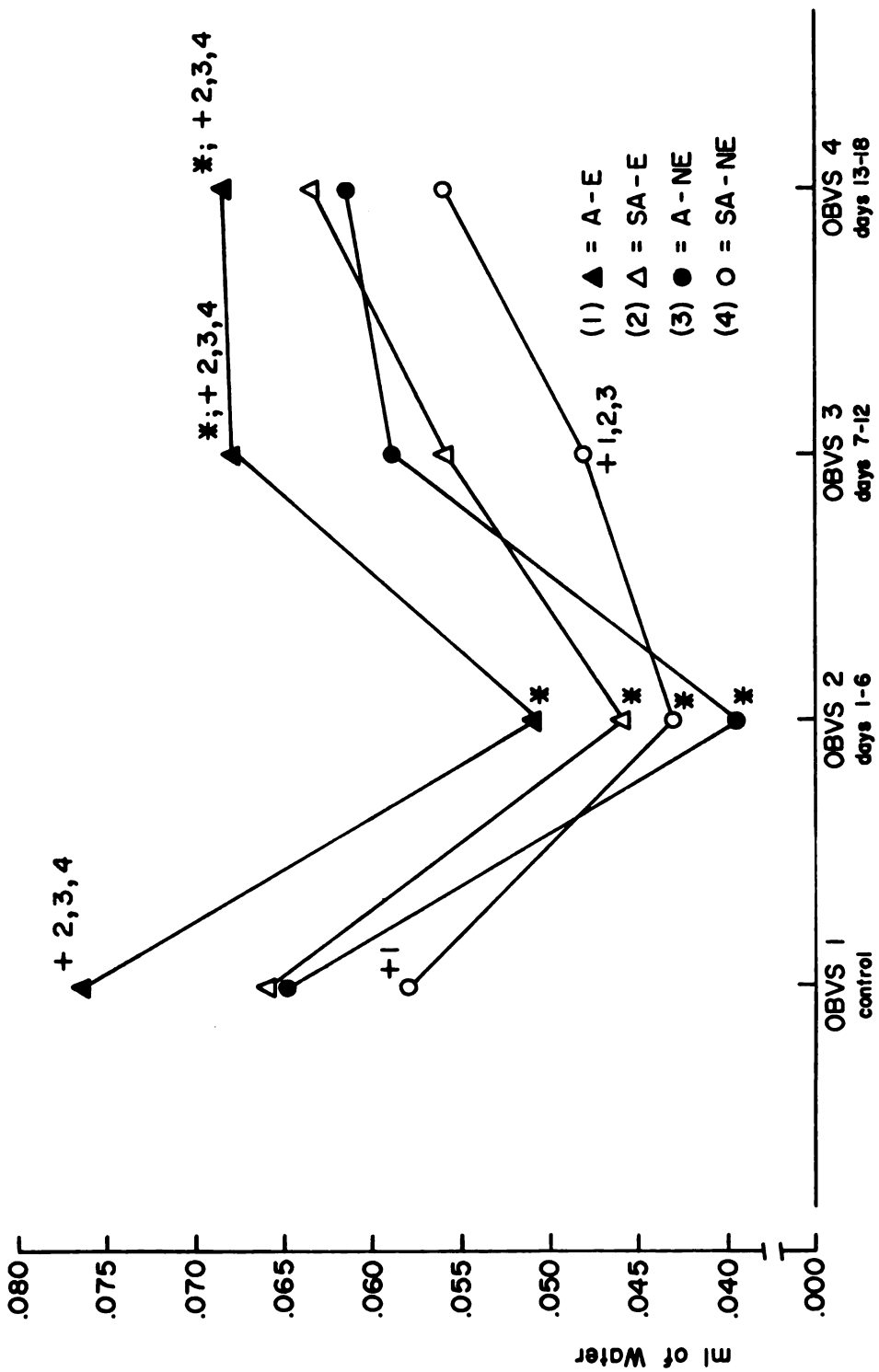

Figure 11. Mean home cage water consumption in ml/gm body weight x 100 during the control and experimental observations.

* = Significantly different from control data (p=.05)
+ = Significantly different from indicated groups (p=.05)

TABLE 10

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Water Consumption

Treatment group		Observation period 1	2	3	4
A-E	Mean	.076	.051	.068	.069
	S.D.	.009	.010	.007	.017
SA-E	Mean	.065	.046	.056	.064
	S.D.	.011	.013	.010	.006
A-NE	Mean	.065	.040	.059	.062
	S.D.	.016	.008	.020	.014
SA-NE	Mean	.058	.043	.049	.056
	S.D.	.008	.004	.008	.006

Duration of treatment effects, between observations, within groups

	F	P
A-E	11.344	$\leq$ .01
SA-E	7.831	$\leq$ .01
A-NE	13.485	$\leq$ .01
SA-NE	4.938	$\leq$ .05

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	4.103	.927	1.199	3.247
P	$\leq$ .05	NS	NS	$\leq$ .05

TABLE 10 (continued)

Scheffé Post-Hoc Test

Observations			Treatment		
Treatment groups	Periods contrasted	Mean differences	Observation period	Groups contrasted	Mean difference
A-E	2-1	-.025*	1	A-E - SA-E	-.011*
	3-1	-.006*		A-E - A-NE	-.011*
	4-1	-.007*		A-E - SA-NE	-.018*
				SA-E - A-NE	.000
SA-E	2-1	-.019*		SA-E - SA-NE	-.001
	3-1	-.009*		A-NE - SA-NE	-.003
	4-1	-.001			
			3	A-E - SA-E	-.012*
A-NE	2-1	-.025*		A-E - A-NE	-.009*
	3-1	-.006*		A-E - SA-NE	-.019*
	4-1	-.003		SA-E - A-NE	-.003
				SA-E - SA-NE	-.007*
SA-NE	2-1	-.015*		A-NE - SA-NE	-.010*
	3-1	-.009*			
	4-1	-.002	4	A-E - SA-E	-.005*
				A-E - A-NE	-.007*
				A-E - SA-NE	-.013*
				SA-E - A-NE	-.002
				SA-E - SA-NE	-.003
				A-NE - SA-NE	-.002

*Mean difference = .004 required for significance at $p \leq .05$.

*Mean difference = .004 required for significance at $p \leq .05$.

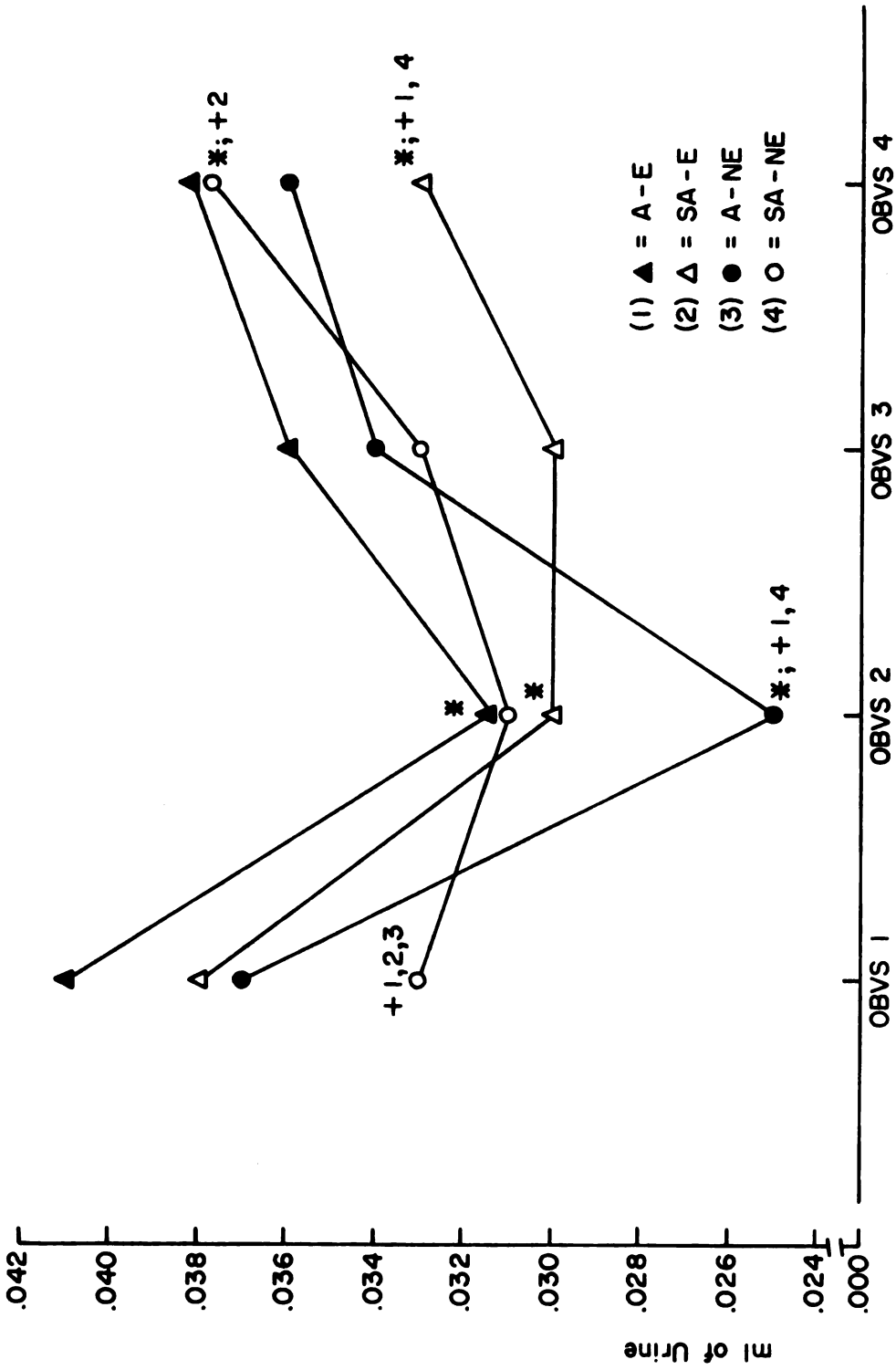

Figure 12. Mean home cage urine excretion patterns in ml/gm body weight x 100 for the control and experimental observations.

* = Significantly different from control data (p = .05)
+ = Significantly different from indicated groups (p = .05)

TABLE 11

Analysis of Variance Summary and Scheffé Post-Hoc Test on Home-Cage Urine Excretion Volume

Treatment group		Observation period 1	2	3	4
A-E	Mean	.041	.031	.036	.040
	S.D.	.008	.008	.008	.006
SA-E	Mean	.038	.029	.029	.033
	S.D.	.004	.008	.004	.004
A-NE	Mean	.037	.025	.034	.036
	S.D.	.016	.007	.014	.011
SA-NE	Mean	.033	.031	.033	.038
	S.D.	.007	.004	.005	.006

Duration of treatment effects, between observations, within groups

	F	P
A-E	5.478	$\leq .05$
SA-E	4.722	$\leq .05$
A-NE	7.690	$\leq .05$
SA-NE	2.487	$\leq .10$

Treatment effects, between groups, within observations

	Observation period 1	2	3	4
F	.852	.469	.655	.454
P	NS	NS	NS	NS

TABLE 11 (continued)

Scheffé Post-Hoc Test

Observations			Treatment		
Treatment groups	Periods contrasted	Mean differences	Observation period	Groups contrasted	Mean difference
A-E	2-1	-.010*	1	A-E - SA-E	-.003
	3-1	-.005*		A-E - A-NE	-.004
	4-1	-.001		A-E - SA-NE	-.008*
				SA-E - A-NE	-.001
SA-E	2-1	-.009*		SA-E - SA-NE	-.005*
	3-1	-.009*		A-NE - SA-NE	-.005*
	4-1	-.005*			
			2	A-E - SA-E	-.002
A-NE	2-1	-.012*		A-E - A-NE	-.006*
	3-1	-.005*		A-E - SA-NE	-.003
	4-1	-.001		SA-E - A-NE	-.001
				SA-E - SA-NE	-.006*
SA-NE	2-1	-.002		A-NE - SA-NE	-.003
	3-1	.000			
	4-1	.005*	4	A-E - SA-E	-.007*
				A-E - A-NE	-.004
				A-E - SA-NE	-.002
				SA-E - A-NE	-.004
				SA-E - SA-NE	-.005*
				A-NE - SA-NE	-.003

*Mean difference = .005 required for significance at $p \leq .05$.

*Mean difference = .005 required for significance at $p \leq .05$.

the SA-NE group had returned to a normal urine K^+ electrolyte balance. It was not until examining this group's K^+ mEq/l. with respect to their total excreted urine volume that an accurate picture of their K^+ excretion levels was apparent. The same situation occurred for the SA-NE group regarding their retention of Na^+. It is the total amount of electrolytes which is important, not just the concentration gradient. Examining electrolyte concentration gradients alone may be misleading. For example, if two rat urine samples were being compared, one sample at 400 mEq/l. of K^+ and the other at 200 mEq/l. of K^+, a quick estimate would be that the rat with the 400 mEq/l. concentration was excreting the most K^+. However, if the 400 mEq/l. concentration was from a 10 ml. sample and the 200 mEq/l. concentration was from a 25 ml. sample, the rat with the lower concentration gradient actually would be excreting the most K^+.

Recovery Period Water Consumption

All of the groups except the SA-NE group showed a significant increase in water consumption by the second observation interval (Figure 13). Only the A-E group retained this increase across all three experimental observation intervals.

Recovery Period Urine Excretion

The decrease in urine excretion for all groups was pronounced during the recovery period (Figure 14). The groups were not statistically different from each other across the three experimental observation intervals, but all had values which were significantly different from their control excretion levels.

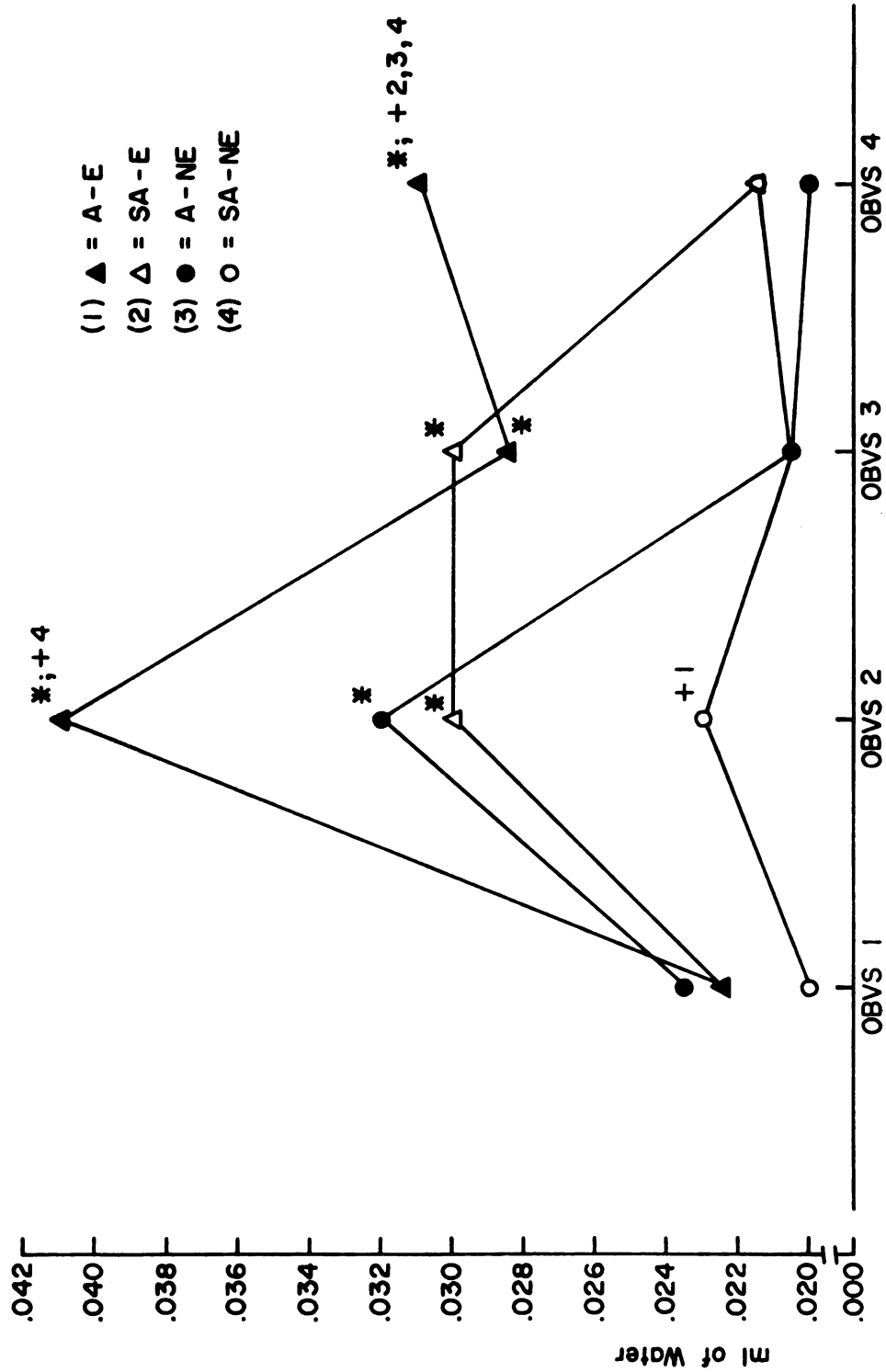

Figure 13. Mean recovery period water consumption in ml/gm body weight x 100 over the control and experimental observations.

* = Significantly different from control data (p = .05)
+ = Significantly different from indicated groups (p = .05)

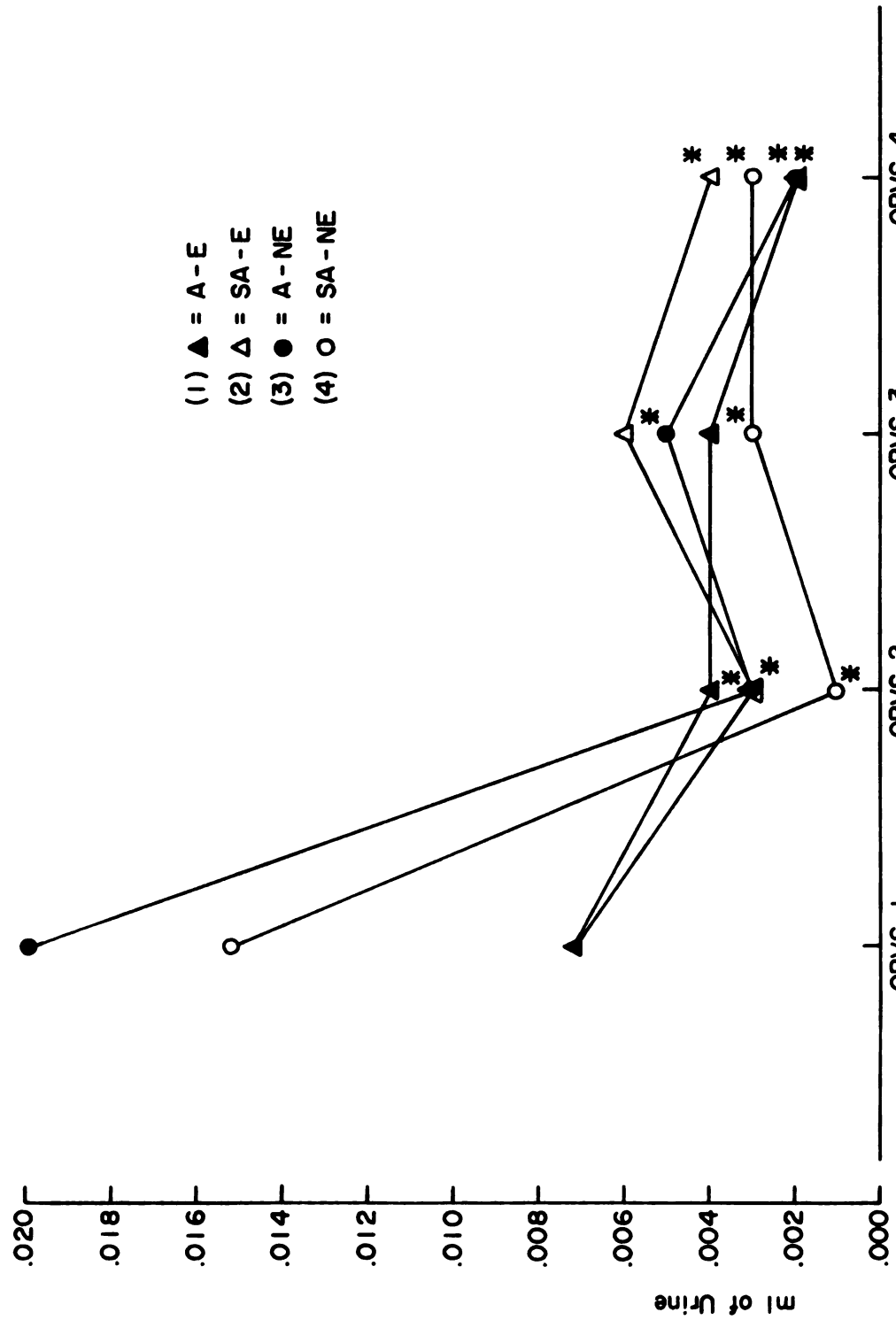

Figure 14. Mean recovery period urine excretion volume in ml/gm body weight x 100 over the control and experimental observations.

* = Significantly different from control data (p = .05)

Recovery urine excretion and water ingestion were included to support and clarify the corresponding home-cage parameters. Once more the SA-NE group presented thought-provoking data. These animals appeared to be demonstrating an antidiuretic effect similar to those exhibited by the groups that received the experimental treatments.

Discussion

An evaluation of the exercise data shows that the animals were able to successfully complete the imposed interval-running program. These data provide supporting evidence for the effectiveness of the Controlled Running Wheel (37,95) as a reliable method of exercising and/or training research rats.

The results of the body weight comparisons of these animals were puzzling. Generally, exercised animals weigh less than sedentary animals as was the case in this study. However, this investigator was unable to uncover any other studies where young postpubertal rats lost weight as a result of similar experimental treatments. The loss of weight by the animals in this study was the first indication of the possible pronounced reaction they may have had to the induced stress.

Urine osmolality data in this study directly support Corson's (7) findings in dogs. It is interesting that the A-NE and SA-E groups, which theoretically were exposed to one stress each (shocking and exercising, respectively), had approximately 50% increases in urine osmolality. The A-E group which was exposed to two stresses

(shocks and exercise) had approximately a 100% increase in urine osmolality by the fourth observation interval. The implication that can be drawn from these results seems to be that increases in urine osmolality reflect increases in imposed stress--at least in that part of the osmolality continuum observed in this investigation.

The Na^+ and K^+ electrolyte data of this study are in agreement with earlier data published by Dauphinée (17), Moore (50), Thorn *et al.* (89) and Gann and Wright (28). However, the findings of this study contradict those of Spigel and Ramsay (80), who found significant Na^+ as well as K^+ excretion. Hoagland *et al.* (21) also found elevated Na^+ and K^+ excretion in human subjects over 20 years of age whereas, for those under 20 years, they reported elevated K^+ retention accompanied by increased Na^+ excretion.

Paré and McCarthy (61), in a somewhat similar study, reported no disturbance of the urinary Na^+-K^+ electrolyte balance. This may be attributable to the fact that they used only 3.5 ma of shock in their study.

It is important to note the Na^+ and K^+ electrolyte changes which occurred in the current investigation. All of the groups except the SA-NE group had increased total Na^+ retention. Only the A-E and SA-NE groups had consistently elevated total K^+ excretions, but the A-NE group also had an elevated K^+ excretion during the third observation period. The SA-E group was unique in that they did not respond with elevated K^+ excretion and even showed a non-significant retention in the second observation period.

One could speculate that urine K^+ responds only to severe stress were it not for the data of the SA-NE group. Taking this group's responses into consideration, it becomes entirely plausible that K^+ electrolyte excretion changes may be more associated with emotional stress than physical stress (exercise). This assumes that SA involves a significant amount of emotional stress. This theory becomes more interesting as it is noted that the SA-NE group was the only group which did not consistently retain Na^+ during the study.

The mechanism directly responsible for these Na^+ and K^+ electrolyte shifts seems to be associated with mineralocorticoid aldosterone rather than corticosterone. While it is known that aldosterone may be secreted independently of pituitary function(55,69), there is a rapid increase in aldosterone secretion following anxiety stress or physical stress (45). ACTH, which is secreted by the anterior pituitary, stimulates the adrenal cortex to secrete elevated levels of aldosterone in a stress situation (55). This evidence leads to the conclusion that, in the current investigation, aldosterone was stimulated by ACTH to respond to the induced stress situations and was the agent responsible for the electrolyte imbalances. Enhanced aldosterone secretion also is reported to result in a depressed Na^+ to K^+ ratio (55). Figure 11 shows a significant Na^+ to K^+ ratio depression for all of the groups, with that of A-E group being the furthest depressed. This writer chooses to disagree with those investigators (16,61,78) who have reported that aldosterone is not implicated in the stress reaction.

Relative adrenal weight data reveal adrenal hypertrophy for the A-E, SA-E, and A-NE groups. These data, along with the urinary osmolality and Na^+ and K^+ imbalances, suggest significant hyperfunction of the pituitary and the adrenal cortex as responses to the induced experimental stress situations. The relative adrenal weights support the findings of Paré and McCarthy (61) for male Sprague-Dawley rats of the same age.

It is clear that the A-E group responded dramatically to the experimental protocol as evidenced by statistically significant increases in urinary osmolality, Na^+ retention and K^+ excretion. The SA-NE group, however, yielded equally dramatic data. During the first six treatment days, their home-cage urine osmolality increased in a manner similar to those of the three treatment groups, but it did return to control level by the end of the study. However, their recovery urine data showed a marked antidiuretic effect equivalent to that found in the other groups across all experimental periods. While they did not demonstrate significant home-cage total Na^+ retention, their total K^+ excretion pattern paralleled that of the A-E group. Significant alterations occurred in both groups over time.

This SA-NE group provides further supporting evidence concerning the role of aldosterone in stress. ADH secretions probably are not responsible for their Na^+ retention pattern since their home-cage urine osmolality returned to normal while their Na^+ retention remained consistently elevated. Aldosterone is approximately 30 times more potent than corticosterone in influencing Na^+ retention and 5 times

greater in its influence on K^+ excretion (55). It seems reasonable, therefore, since ADH responses may be elicited only by some physical source which involves a direct treatment such as physical activity, loud noises, pain or fear of pain, hemorrhage, etc., that the marked antidiuretic effect in the SA-NE group recovery period was mediated by ADH. This enhanced ADH secretion probably was a result of direct stress in response to the loud tones in combination with the squeals of the shocked animals during the experimental treatment immediately prior to the recovery period.

The Na^+ and K^+ imbalances detected in their home-cage urine were not the results of directly applied stressors but rather may have reflected stress by association. The fact that the SA-NE animals were in the same home-cage environment as the stressed animals may have produced an association stress reaction in them. This stress could have been via olfactory stimuli given off by the treated animals. If this hypothesis is tenable one would have to assume that over the days of the study the other treatment groups learned that removal from the treatment environment merely signaled a delay before the onset of the next treatment cycle. Thus, for the A-E and A-NE animals the fear of pain and/or a general environmental threat may have remained with them in the home-cage environment. This could explain their significantly increased home-cage urine osmolalities. An extension of this thinking is that general, undefined anxiety which is detected by olfaction may stimulate changes in the Na^+ and K^+ electrolyte levels. In addition, this observation may show that it is possible

to produce anxiety in the absence of a direct anxiety stressor. Laboratory rats may be susceptible to emotional anxiety merely by being directly associated with other animals who are living under high anxiety conditions. These olfactory stimuli, possibly detected by the SA-NE group, could have triggered elevated ACTH secretions which, in turn, may have maintained enhanced aldosterone secretions. This hypothesis is not contradicted by Ganong *et al.* (48), who showed that lesion of the median emminence of the hypothalamus results in markedly decreased ACTH and aldosterone secretion plus adrenal cortical atrophy. Aldosterone is further implicated here as a result of the SA-NE group's significantly depressed Na^+/K^+ ratio.

The A-NE and SA-E groups responded with thought-provoking data. Their urine osmolalities were significantly elevated and similar; their Na^+ retention patterns were similar; their K^+ excretions were similar except that the third observation period the A-NE group had a significantly elevated total K^+ excretion. The A-NE group's elevated K^+ excretion at the third observation period supports the possibility of K^+ excretion reacting as a response to emotional stress since only the SA-E group did not alter its K^+ excretion. The A-NE and SA-E groups seemed to clearly demonstrate similar responses to a single stress even though the stressors were different (anxiety and exercise, respectively). This supports the opinion of those investigators who claim that response to stress is nonspecific. In this study, however, differences in K^+ electrolyte response must be ignored in order to categorically accept that hypothesis.

In conclusion, the urine data gathered in the home-cage environment and the body weight comparisons indicate that both experimental treatments produced pronounced stress reactions in the experimental animals. Urine osmolality seems to represent a reliable and sensitive measure of physical and emotional stress. Posterior pituitary secreted ADH appears to be the logical mechanism controlling this response. The fact remains, however, that A-E, SA-E, and A-NE treatment groups all may have been responding to physical stress. The electrical shocking protocol may not have elicited an emotional stress situation but rather another form of physical stress. This would mean that the SA-NE group might have been the only actual anxiety treatment group. The existing literature and the data available from this investigation make this a difficult position to support.

The Na^+ retention by all of the groups and the K^+ excretion by the A-E, A-NE and SA-NE groups make it reasonable to infer that the mineralocorticoids do play a role in the physiological response to stress. ACTH stimulated aldosterone is probably the controlling factor mediating this electrolyte response. These electrolyte imbalances, supported by adrenal hypertrophy and increased urine osmolality, also imply a hyperfunction of the hypothalamus, anterior pituitary and adrenal cortex as responses to anxiety and/or exercise stress.

The data resulting from this study provided physiological evidence that the stresses of exercise and anxiety are additive in nature and elicit an apparent double stress syndrome. This evidence

is *contrary* to the popular view that exercise alleviates emotional anxiety. The implications these findings may have regarding cardiac rehabilitation alone warrant continuing research with respect to the relationships between exercise and anxiety. For example, the hearts of the animals used in this investigation were examined in a companion study of the possibility that myocardial damage might have resulted from the experimental treatments the animals received. After completion of the current investigation, the animals were immediately sacrificed by decapitation. Following quick freezing of the hearts, five sets of four transverse sections (eight microns thick) were taken from the heart muscles at equal intervals from the lower half of the ventricles. These sections then were stained and rated for possible myocardial damage. The hearts of the SA-NE group were used as the control heart tissue. The difference in heart damage ratings between the SA-NE and the A-E groups was found to be significant ($p \leq .10$). Slight damage also was recorded in the SA-E and A-NE groups, but this damage was not statistically significant (86). These findings provide supporting evidence regarding the severe stress the A-E animals were confronting.

CHAPTER V

SUMMARY, CONCLUSIONS, RECOMMENDATIONS

The purpose of this study was to determine the effectiveness of exercise as a therapeutic measure for alleviating emotional trauma (anxiety). Home-cage excreted urine osmolality, sodium (Na^+) and potassium (K^+) electrolyte concentrations were examined as the primary variables.

Twenty male albino rats (Sprague-Dawley) were randomly assigned to four experimental groups and studied for 21 days. The four treatment groups consisting of 5 rats each were as follows:

"A-E" Group: Anxiety (A) treatments were mediated via random faradic stimulation through the stainless steel grid floor of individual anxiety chambers for three hours daily. Exercise (E) was by forced interval running (Appendix A) immediately following the anxiety treatments.

"SA-E" Group: Sham Anxiety (SA) was presented at the same time as the anxiety treatments. These animals were placed in similar individual anxiety chambers located in the same room as, and adjacent to, the treated animals' chambers. However, faradic stimulation was not administered to the sham anxiety group. Exercise (E) was by forced interval running immediately following the sham anxiety treatments.

"A-NE" Group: The Anxiety (A) treatment was the same as for the A-E group. No exercise (NE) followed this group's exposure to anxiety conditions.

"SA-NE" Group: Sham Anxiety (SA) was imposed on this group in the same manner as for the SA-E group. No exercise (NE) followed this group's exposure to the sham anxiety treatment.

The daily experimental treatment protocol began with anxiety or sham anxiety treatments from 6:00 to 9:00 a.m. All four treatment groups were placed in individual anxiety chambers during this time period and all were in adjacent chambers in the same room. Anxiety was induced by random electrical shocks (60 volts, 15 ma) preceded by a 1700 Hz tone 0.5 secs. before the shock was administered. The two sham anxiety groups, however, received only the auditory tones with no electrical shocks.

Immediately after anxiety or sham anxiety, 9:10 to 10:00 a.m., the two groups scheduled for exercise (A-E, SA-E) followed an interval running program of moderate intensity, medium duration in electronically controlled running wheels. Since the purpose of this study was to exercise the animals, not necessarily to train them, the description of the interval training program found in Appendix A was modified. It was decided to follow the training program up to day 8 and then to maintain the exercise level at that intensity through the final day of the experiment. All of the exercise rats ran simultaneously in individual wheels. These electronically controlled interval running wheels employ operant conditioning utilizing a light (60 volts, 120 watts) as the conditioned stimulus and faradic stimulation (.2 ma) through the running surface as the unconditioned stimulus. During this same time those animals who were to receive no exercise (A-NE, SA-NE) were transferred to wire mesh holding cages. This holding cage protocol was conducted in the

same room and at the same time as the exercise animals were running.

From 10:15 a.m. to 12:15 p.m., all four groups were transferred back to their home cages in the living quarters. These two hours were designated as a recovery period.

The zero phase was from 12:15 p.m. to 5:30 p.m. During this time period the animals remained in their home cages and no data were collected.

The period of home-cage environment in the living quarters was from 5:45 p.m. to 5:45 a.m.; this was the time interval on which the current research was focused.

Body weights were taken before and after: 1) anxiety or sham-anxiety treatments, 2) exercise or holding protocol, 3) recovery and 4) home-cage environment. Urine samples (total volume) were collected during all of the treatment periods using stainless steel metabolism trays and frozen for later analysis. Food and water were available *ad libitum* during the recovery period and in the home-cage environment. Daily ingestion levels of both food and water were recorded for each animal. Daily records of running performance also were maintained for the two exercise groups.

The animals were exposed initially to the experimental protocol for four days prior to the onset of control data collection. Control data were then collected for three days immediately prior to the 18-day treatment period. The technique used for control data collection was exactly the same as that used to collect data during the treatment periods with one exception: at no time during the

control data collection period were the animals subjected to any electrical stimuli.

All animals were decapitated approximately one hour following their final treatments. The adrenal glands of each rat were removed and weighed.

Urine osmolality was determined utilizing an osmometer. Milliequivalents of Na^+ and K^+ were measured by flame photometer.

The data were statistically analyzed by a one-way repeated measurements univariate analysis of variance. The results were further analyzed, whenever appropriate, by the Scheffé Post-Hoc procedure.

Conclusions

The conclusions that can be drawn from this study are as follows:

1. Exercise, as administered in this study, does not reduce coinciding emotional trauma (anxiety).

2. Exercise is an additive stress on an animal which is also exposed to anxiety.

3. Urine osmolality is a reliable and sensitive indicator of emotional or physical stress.

4. Urine sodium electrolyte imbalance (retention) is a sensitive indicator of emotional or physical stress.

5. Urine potassium electrolyte imbalance (excretion) may be a reliable measure of emotional stress.

Recommendations

1. Further studies should utilize an additional control group which is continuously located in the home-cage environment.

2. A similar study on previously trained rats, employing a larger sample size, should be undertaken.

3. Rats previously exposed to an anxiety treatment should be exercised with the anxiety regimen continuing during the conduct of the daily exercise program.

4. Rats exposed to an anxiety treatment should be exercised with the anxiety regimen terminating at the start of the daily exercise program.

5. The ordering of the treatments in items 3 and 4 should be reversed, i.e., exercise followed by anxiety.

6. Resting heart rate and systolic blood pressure should be additional variables examined.

7. Human studies which closely parallel these animal studies should be considered.

It is this writer's opinion that further research should be undertaken to clarify the role of exercise in mammalian psychophysiological adjustments to the environment.

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APPENDICES

APPENDIX A

STANDARD EIGHT-WEEK, MEDIUM-DURATION, MODERATE-INTENSITY ENDURANCE TRAINING PROGRAM FOR POSTPUBERTAL AND ADULT MALE RATS IN CONTROLLED-RUNNING WHEELS

Wk.	Day of wk.	Day of Tr.	Acceleration time (sec)	Work time (sec) or (min)	Rest time (sec)	Repetitions per bout	No. of bouts	Time bet. bouts (min)	Shock (ma)	Run speed (ft/sec)	Total time of prog. (min: sec)	Total work time *TWT* (sec)	Total exp. revolutions *TER*
1	M	1	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
	T	2	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
	W	3	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
	T	4	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
	F	5	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
2	M	6	2.5	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
	T	7	2.5	10s	10	40	3	5.0	1.2	2.0	49:30	1200	600
	W	8	2.5	10s	10	40	3	5.0	1.2	2.5	49:30	1200	750
	T	9	2.5	10s	10	40	3	5.0	1.2	3.0	49:30	1200	900
	F	10	2.5	10s	10	40	3	5.0	1.2	3.0	49:30	1200	900
3	M	11	2.0	10s	10	40	3	5.0	1.2	3.0	49:30	1200	900
	T	12	2.0	10s	10	30	4	5.0	1.2	3.5	54:20	1200	1050
	W	13	2.0	10s	10	30	4	5.0	1.2	3.5	54:20	1200	1050
	T	14	2.0	10s	10	30	4	5.0	1.2	3.5	54:20	1200	1050
	F	15	2.0	10s	10	30	4	5.0	1.2	3.5	54:20	1200	1050
4	M	16	1.5	10s	10	30	4	5.0	1.2	3.5	54:20	1200	1050
	T	17	1.5	15s	15	20	4	5.0	1.2	4.0	54:00	1200	1200
	W	18	1.5	15s	15	20	4	5.0	1.2	4.0	54:00	1200	1200
	T	19	1.5	15s	15	20	4	5.0	1.2	4.0	54:00	1200	1200
	F	20	1.5	15s	15	20	4	5.0	1.2	4.0	54:00	1200	1200

APPENDIX A (continued)

Wk.	Day of wk.	Day of Tr.	Acceleration time (sec)	Work time (sec) or (min)	Rest time (sec)	Repetitions per bout	No. of bouts	Time bet. bouts (min)	Shock (ma)	Run speed (ft/sec)	Total time of prog. (min:sec)	Total work time *TWT* (sec)	Total exp. revolutions *TER*
5	M	21	1.5	15s	15	20	4	5.0	1.2	4.0	54:00	1200	1200
	T	22	1.5	20s	20	15	4	5.0	1.2	4.0	53:40	1200	1200
	W	23	1.5	20s	20	15	4	5.0	1.2	4.0	53:40	1200	1200
	T	24	1.5	20s	20	15	4	5.0	1.2	4.0	53:40	1200	1200
	F	25	1.5	20s	20	15	4	5.0	1.2	4.0	53:40	1200	1200
6	M	26	1.5	20s	20	15	4	5.0	1.0	4.0	53:40	1200	1200
	T	27	1.5	25s	25	13	4	5.0	1.0	4.0	56:40	1300	1300
	W	28	1.5	25s	25	13	4	5.0	1.0	4.0	56:40	1300	1300
	T	29	1.5	25s	25	13	4	5.0	1.0	4.0	56:40	1300	1300
	F	30	1.5	25s	25	13	4	5.0	1.0	4.0	56:40	1300	1300
7	M	31	1.5	25s	25	13	4	5.0	0.8	4.0	56:40	1300	1300
	T	32	1.5	30s	30	11	4	5.0	0.8	4.0	57:00	1320	1320
	W	33	1.5	30s	30	11	4	5.0	0.8	4.0	57:00	1320	1320
	T	34	1.5	30s	30	11	4	5.0	0.8	4.0	57:00	1320	1320
	F	35	1.5	30s	30	11	4	5.0	0.8	4.0	57:00	1320	1320
8	M	36	1.5	30s	30	11	4	5.0	0.8	4.0	57:00	1320	1320
	T	37	1.5	35s	35	10	4	5.0	0.8	4.0	59:20	1400	1400
	W	38	1.5	35s	35	10	4	5.0	0.8	4.0	59:20	1400	1400
	T	39	1.5	35s	35	10	4	5.0	0.8	4.0	59:20	1400	1400
	F	40	1.5	35s	35	10	4	5.0	0.8	4.0	59:20	1400	1400

APPENDIX B

DESCRIPTION OF ELECTRONICALLY CONTROLLED INTERVAL RUNNING WHEEL FOR SMALL ANIMALS

Motivation to run is provided by a low-intensity, controlled shock current which is terminated when the animal reaches a specified speed. A light always precedes the shock so the animal may avoid shock entirely by responding promptly. At the beginning of each running period, a light is turned on above the wheel and remains on for a predetermined acceleration period. If a specified speed has not been reached by the end of the acceleration period, the light is turned off and a controlled shock current is applied to the animal through the running surface of the wheel. As soon as the animal reaches the specified speed, the shock is discontinued. If this speed is attained within the acceleration period, the light is turned off and no shock is applied. As long as the animal continues to run at or faster than the specified speed, he avoids being shocked; if he slows down below the specified speed during the running period, the light and shock sequence is repeated. Initially, the animals run in response to the shock. By the end of the third 40-min learning period, most animals learn that the light always precedes the shock and will run in response to the light.

A typical running program consists of alternate periods of work and rest. During work periods, the wheel is free to turn; during rest periods, it is braked automatically to prevent spontaneous activity. A specified number of work and rest periods (repetitions) constitutes 1 bout of exercise. A single training period may include several such bouts separated by relatively long periods of inactivity (time between bouts), during which the wheel remains braked. A buzzer signals when the running program has been completed.

A bank of controlled-running wheels consists of 1 master control unit and up to 12 wheels. The master control unit . . . can be programmed for a given training period as follows: 1) Acceleration time can be set for 0.5-5.0 sec at intervals of 0.5 sec. These times are accurate to $\pm$ 0.02 sec. 2) Work time can be set for 5 sec-60 min in intervals of 5 sec. These times are accurate to $\pm$ 0.02 sec. 3) Rest time can be set for 5-60 sec in intervals of 5 sec. These times are accurate to $\pm$ 0.02 sec. 4) The number of repetitions (of work and rest periods) per bout can be set for 1-399. 5) The number of bouts can be set for 1-10. 6) Time between bouts can be set for 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0 and 30.0 min. These times are accurate to $\pm$ 0.02 sec. 7) Shock level can be set for 0.0-1.2 ma at intervals of 0.2 ma. Shock levels are accurate from + 0 to -10% regardless of whether the animal is wet or dry. 8) Running speed can be set for 1.0-6.0 ft/sec at intervals of 0.5 ft/sec. Speed settings are accurate to $\pm$ 2%.

The controlled-running wheel. . . is made of light-weight plastic and has aluminum rods as the running surface to minimize

angular inertia. The circumference of the running surface is 122 cm (4 ft); the width is 11 cm. The wheel is supported by bearings in a steel frame. Fecal droppings are caught in a paper-covered metal tray below. Light baffles are provided to avoid confusion between adjacent animals due to nonsynchronous light signals. The top light baffle can be raised to permit easy removal of the wheel for washing. A simulus-control cage is provided in which a matched simulus-control animal receives light signals and shock simultaneously with the animal in the wheel.

A result unit. . . is attached to the frame of each controlled-running wheel. The following data are collected and displayed in digital form by the result unit: a) the total number of revolutions run (TRR) by the experimental animal, and b) the cumulative duration of shock (CDS) received by both the experimental and control animals. Two calculated variables have been derived to allow a comparison to be made between animals on different training programs: a) the percent of expected revolutions (PER), which is a measure of work performed relative to work expected, and b) the percent shock-free time (PSF), which is the percent of the total work time during which the animal avoided shock by running at or faster than the speed specified on the master control unit.

The master control unit provides electrical power to itself and to a maximum of 12 wheels. It also provides work and shock information, shock current, and coded signals representing speed and acceleration time to each wheel. . . .

If an animal's speed drops below the set speed during a work period, the light must turn on for a period equal to the acceleration time set on the master control unit. Since no 2 wheels will decelerate simultaneously, timing of the light must be accomplished independently within each result unit. Thus, a signal representing acceleration time must be continuously available to each result unit. Providing this signal is the function of the acceleration time control circuit.

A synchronizing pulse generator develops pulses at a rate of 2 per second. These pulses are derived from the 60-Hz power line and serve as a timing reference for the entire system. Pulses from the synchronizing pulse generator are counted by the timing unit composed of 4 silicon controlled-rectifier (SCR) ring counters. The timing unit generates intervals of .5 sec-60 min for acceleration time, work time, rest time, and time between bouts. Five distinct modes of operation are recognized in the master control unit: work, shock, rest, time between bouts, and program complete. The mode logic unit selects the appropriate mode in the proper sequence at intervals determined by the timing unit.

The repetition counter consists of 3 silicon controlled-rectifier ring counters which count work periods to a number preset on the repetitions switches. The bout counter consists of 1 SCR ring counter which counts bouts to a number preset on the bouts switch.

An output is provided on the rear of the master control unit for connection to an external alarm speaker. A second output

permits interconnection of a master control unit for synchronized oepration of as many as 24 wheels.

Light from a small bulb passes through one side of the plastic wheel and illuminates 2 photocells. Light to one cell is interrupted by a black stripe on the wheel during half of each revolution. The output from this cell is used in counting revolutions. Light to the other cell is interrupted 8 times per linear foot of running surface (32 times per revolution). The output of this cell represents wheel speed. . . .

The tachometer section of the result unit monitors the output of the second photocell (wheel speed) and compares it with the speed set on the master control unit. The tachometer output is a DC signal: 0 volts if set speed exceeds wheel speed and -12 volts if wheel speed exceeds set speed.

The logic section determines whether light, shock, or neither shall be applied. Assuming the master control unit is in either the work or shock modes, the logic section will turn on the light whenever wheel speed falls below set speed. Whenever the light comes on, the acceleration time generator begins timing the acceleration period according to instructions from the acceleration time control circuit in the master control unit. If wheel speed reaches set speed during the acceleration period, the logic section turns the light off. Otherwise, at the end of the acceleration period, the logic section switches the light off and the shock on. As soon as wheel speed reaches set speed, the logic section switches the shock off.

An alarm circuit in the result unit is activated whenever shock current is applied continuously for more than 3 sec. A buzzer in the master control unit signals that 1 or more animals is in difficulty. A red light on each result unit directs the operator to the appropriate animal. The usual cause of difficulty is fecal matter short-circuiting the bars of the wheel so that little or no current flows through the animal. In rare cases, an animal may be sick or injured and require attention. The alarm also is activated by failure of the photocell driver bulb. In this case, light and shock are inhibited. If this were not done, the animal would be unable to avoid shock by running.

The alarm reset switch serves a dual purpose. In the center position, the result unit functions normally. Pressing the switch down resets the alarm circuit. In its upper position, the switch prevents application of either light or shock stimulus. In this mode, voluntary exercise may be observed since the wheel is free-running during all work periods and the TRR is recorded.

Shock stimulus arrives at the result unit as a voltage (0-600 volts AC). It was found in preliminary work with the CRW that shock must be applied as a controlled current rather than a controlled voltage. An animal's skin resistance varies greatly according to whether it is wet or dry, causing corresponding variations in shock current when controlled current assures uniform shock stimulus to all animals at all times.

In the result unit, shock current is switched by the shock relay. When the relay is activated by the logic section, current

passes through the relay contacts, a neon light, and the animal. The neon light indicates that current actually is passing through the animal (95).