PLASMA CONDITIONS AT THE INITIATION OF DRINKING IN SALT INJECTED RATS

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY

Charles Robert Almli

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Plasma Conditions at the Initiation of Drinking in Salt Injected Rats

by

Charles Robert Almli

Abstract of Master's Thesis

Completed Spring Term, 1968

The problem under investigation in this thesis was the determination of blood plasma conditions which lead to the initiation of drinking in water satiated rats. Also of interest was the stability of these conditions as rats were adapted to a drinking schedule.

Previous research has shown that satiated rats injected subcutaneously with 16% sodium chloride solution, reinitiate drinking between 3 and 11 min. postinjection. However, no data are available to show the reliability of this postinjection latency to drink for a given rat. The reliability of postinjection latency to drink was determined in Experiment 1. The determination of blood conditions at the initiation of drinking behavior (Experiment 2), took advantage of this phenomenon as measured in Experiment 1. Accordingly, blood samples were taken when the rat would have normally reinitiated drinking behavior following injection.

In Experiment 1, adaptations to recurrent deprivation schedules, separated by ad lib recovery periods, were studied. It was found that latency to drink decreased over subsequent deprivation periods, indicating that this was a

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learned component of adaptation behavior. Water intake and body weight, in contrast, required the same adjustment during each deprivation period, indicating that these components of adaptation were not influenced by prior learning. The results are discussed in the context of a prior controversy in the literature.

In Experiment 2, latency to drink following subcutaneous injection of 16% sodium chloride solution was measured on Days 0, 1, 2, 3, 5, and 10, of two equivalent deprivation periods separated by five days of ad lib conditions. It was found that across and within treatment days, postinjection latencies to drink were highly consistent ($r=0.711$). Therefore, a mean was computed for each rat, based on two postinjection latencies. Each mean represented a 'predicted' postinjection latency to drink for that rat.

A blood sample was drawn at the 'predicted' postinjection latency to drink, using this as representative of when the rat would normally reinitiate drinking following salt injection. Across days of adaptation (0, 1, 2, 3, 5, and 10), with the exception of transition days 1 and 2, a postinjection increase of 2-3% over non-injected controls, in plasma osmotic pressure was sufficient to instigate drinking behavior. These threshold conditions are discussed in terms of regulatory mechanisms, and in relation to other pertinent research on the subject.

Approved: Glenn I. Hatton Date: May 1968

Glenn I. Hatton, Chairman
John I. Johnson Jr.
Ralph Levine

PLASMA CONDITIONS AT THE INITIATION OF DRINKING IN SALT INJECTED RATS

By

Charles Robert Almli

A THESIS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures Page

LIST OF APPENDICES

GENERAL INTRODUCTION

When water supply is restricted, profound changes occur in most mammals (extensively reviewed by Chew, 1965). These changes can be observed in the behavior, physiology, and basic chemistry of the animal. Depending upon the regimen of water restriction, there may result distinct short-term and long-term alterations which have pervasive influence on the animal's interaction with its environment. In studying animals as they adapt to some regimen, information is obtained regarding both these short-term and long-term conditions, as well as transitional states through which the animal may pass in the process of adaptation. Of specific relevance to this thesis are certain aspects of water-oriented behavior, and the blood chemistry closely associated with that behavior, of rats as they adapt to a schedule of water deprivation.

The ultimate goal of this thesis was to determine blood plasma conditions at the initiation of drinking behavior of rats as they adapt to 23.5 hr. water deprivation. However, unlike most behavioral phenomena, this variable is not readily accessible to direct observation. Therefore, an indirect method of determining blood conditions at the initiation of drinking behavior was necessary. The indirect method used in this study consisted of obtaining a 'predicted'

postinjection (16% sodium chloride) latency to drink, and withdrawing a blood sample at the time when the rat would normally be initiating drinking behavior.

In order to obtain a predicted postinjection latency to drink for rats as they were adapted to 23.5 hr. water deprivation, it was necessary to establish that, in terms of water intake, successive adaptations to water deprivation schedules were equivalent and comparable. This problem is presented in Experiment 1. It was also necessary to establish that postinjection latencies to drink at each of several different levels of adaptation to deprivation, for a given rat, were consistent. This problem is presented in Experiment 2.

Once the previous two problems were resolved, indicating that successive adaptations to deprivation schedules were equivalent, and that latencies to drink following injection at each of several different levels of adaptation were consistent, a 'predicted' postinjection latency to drink could be computed for each rat. The predicted postinjection latency to drink, representing the time when the rat would normally initiate drinking, could then be used to determine when the blood sample would be drawn. In this way, blood conditions at the initiation of drinking behavior could be determined for each of the different levels of adaptation to deprivation. This was accomplished in Experiment 2.

EXPERIMENT I

The physiological and behavioral changes accompanying adaptation to water deprivation schedules have received limited attention by researchers. There is, however, widespread use of animals on such schedules in the fields of learning, motivation, and regulation.

This experiment represents an attempt to resolve the first problem toward the determination of blood conditions at the initiation of drinking behavior. It was necessary to establish whether or not rats had to readapt to successive deprivation periods, when the deprivation periods were separated by ad lib recovery periods. This problem was significant because in Experiment 2, identical treatments would be administered on specified days of two separate adaptation to deprivation periods. Thus, on selected days of separate adaptation periods, it would be necessary to have rats at the same state (as indexed by amount drunk) of adaptation each time.

According to Ghent (1957), however, this would not be possible because she has interpreted the increasing amount drunk with successive days of adaptation to deprivation as a learning phenomenon. If this were the case, the rats would not have to readapt to each successive deprivation period because of the learning that had taken place in the

previous deprivation periods. In contrast, Beck (1962) has concluded that the increasing water intake over successive days of adaptation to deprivation was due to an increased need for water. His conclusion was based on the finding that when rats were deprived of water one day and allowed two recovery days on ad lib, they drank the same amount of water each time, indicating that the water intakes were not influenced by prior learning.

The present experiment attempted to resolve this controversy by using; a) 10-day adaptation to water deprivation periods, within which drinking is known to stabilize, and b) constant external stimulus conditions during the ad lib recovery periods, which eliminates confounding factors associated with a novel drinking situation. By continuing to place the rats in the drinking boxes during the ad lib periods, the stimulus complex of the drinking situation would retain its familiarity and the rats would not have to readapt to the stimulus complex during the following deprivation periods. Thus, the latency to drink and the amount drunk during successive adaptation to deprivation periods should reflect the degree of dehydration experienced by the rats, and will not be confounded by a novel drinking situation each time.

Method

Subjects

Eleven male albino rats of the Holtzman strain, were housed in individual wire cages in a temperature controlled room (73°-78° F.), under constant light conditions. Wayne Lab Blox were constantly available in the home cage throughout the experiment. The rats were approximately 100 days old and weighed 360 ± 25 grams at the start of the experiment.

Apparatus

Two six-unit drinking boxes were used for the observations of the drinking behavior. The boxes were constructed of wood, with individual Plexiglas covers and hardware cloth floors. Each unit was fixed with a 100 ml. gas collecting tube, graduated in 0.2 ml. (For a more complete description of the drinking boxes, see Appendix).

A 0.01-sec. timer was used to measure latency to drink. A foot pedal switch started and stopped the timer.

A six compartment carrying-cage was used for transporting the subjects from the weighing scales to the drinking boxes. (For a description of the carrying-cage, see Appendix).

Procedure

The procedure consisted of three 10-day periods of adaptation to 23.5 hr. water deprivation, with 5-day ad lib periods interpolated between the deprivation periods. The experimental design is presented in Figure 1.

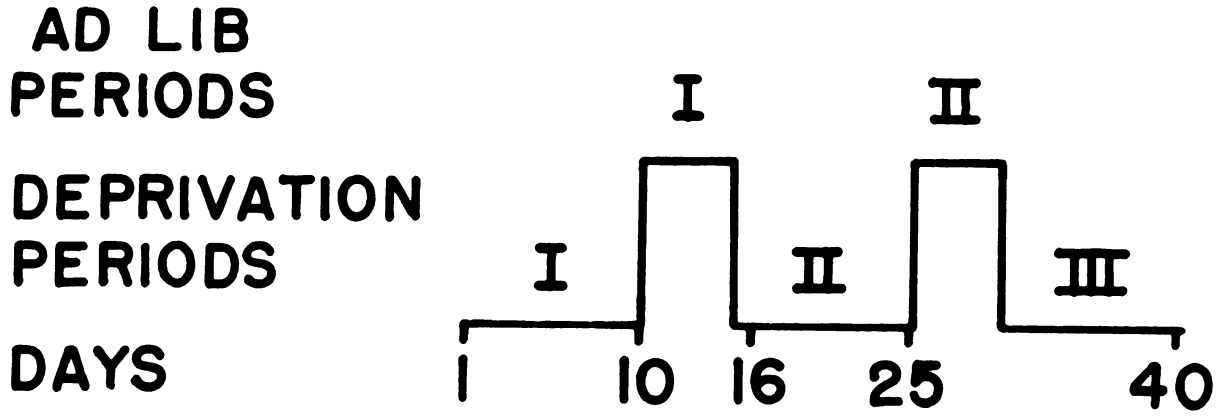


Figure 1

Experimental design for Experiment 1. Ad lib and Adaptation to deprivation periods are superimposed on days of the experiment.
I, II, III: refer to 10-day adaptation to 23.5 hr water deprivation periods.
I, II: refer to 5-day ad lib recovery periods.

Upon arrival at the laboratory the subjects were randomly divided, six were assigned to one group and five were assigned to a second group. They were then maintained on ad lib food and water.

After three days on ad lib, the subjects were weighed and the water bottles were removed from the home cages. For the next 10 days, the subjects were weighed and given 0.5 hr. free access to water in the drinking boxes. Latency to drink after being placed in the drinking box and water intake were measured daily.

Following the tenth day on this schedule, ad lib conditions were begun. One group (6 Ss) was placed in the drinking boxes for 0.5 hr. at their usual daily drinking time, while the second group (5 Ss) was placed in individual compartments in a carrying-cage for that 0.5 hr. This continued for 5 days, during which all subjects were weighed as before and water intakes were recorded for those subjects which were in the drinking boxes. A second 10-day adaptation period, identical to the first, followed the five days of ad lib conditions, after which the subjects were again returned to ad lib conditions. The second ad lib period was like the first, except that all subjects were placed in the drinking boxes for 0.5 hr. at their usual daily drinking time. A third 10-day adaptation period was then given, and was identical to the first two described above.

On Day 10 of Adaptation periods 2 and 3, a 16% saline injection was administered subcutaneously to all rats immediately following the drinking period. The subjects

were then returned to the drinking boxes where postinjection latency to drink was measured. The injections were given in an attempt to determine the reliability of postinjection latency to drink, on two separate occasions, for a given rat. This method was used in Experiment 2 to obtain a 'predicted' postinjection latency to drink. This predicted postinjection latency was used to estimate when the awake rat would drink, and blood samples were drawn at that time under anesthesia.

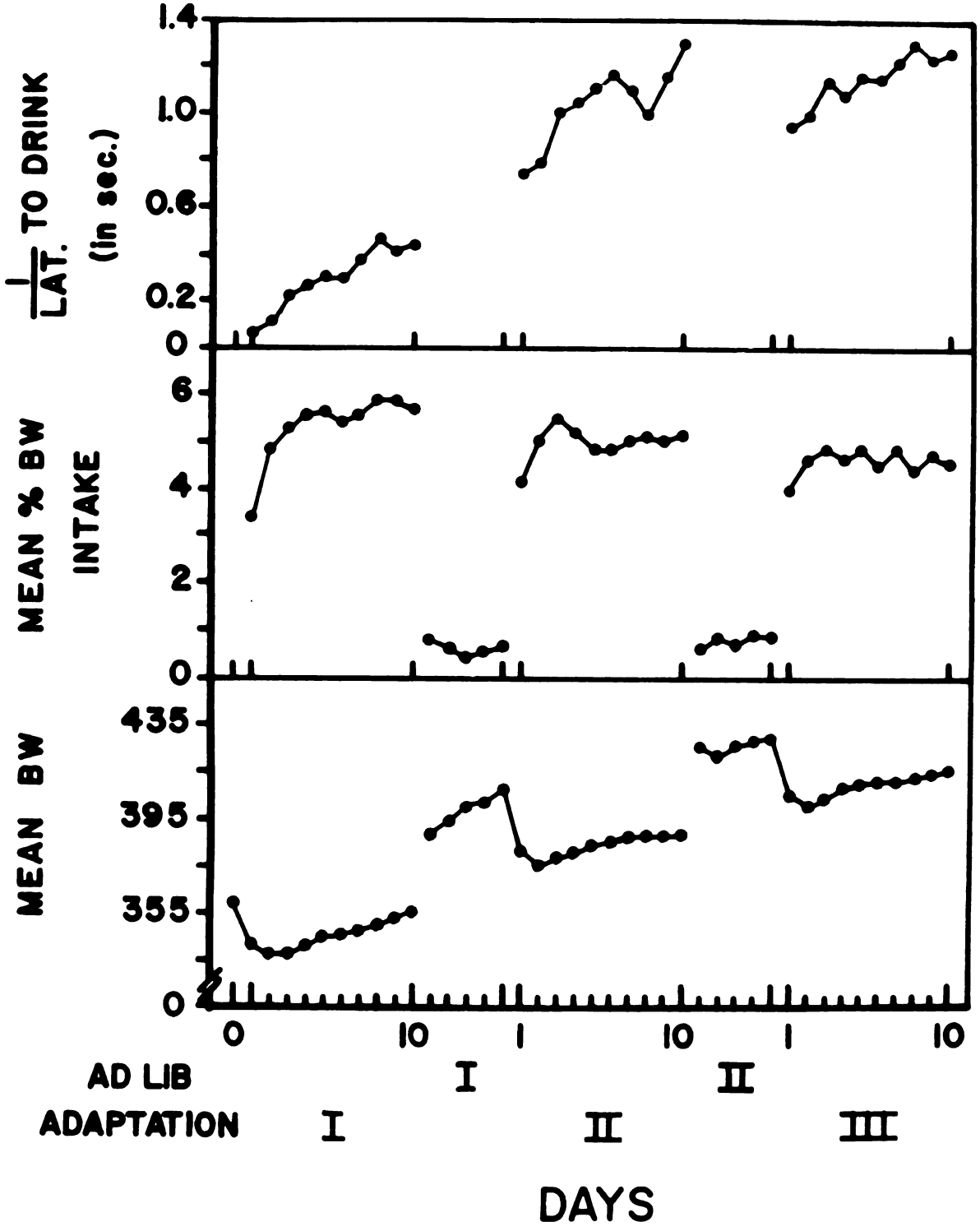
Results

The results of the experiment, for both groups combined, are summarized in Figure 2. The top panel of the figure represents the means for reciprocal latency to drink as a function of days of the experiment. There was no over-lap of the distributions of reciprocal latencies for Day 1 and Day 10 of Adaptation 1. No reliable difference was found between the 'carrying-cage' subjects and the 'drinking box' subjects in latency to drink on the first day of Adaptation 2 ($t=1.21$, $df=9$), although there was an overall significant difference between Day 10 of Adaptation 1 and Day 1 of Adaptation 2 ($t=2.08$, $df=10$, $p<.05$). The latencies continue to shorten during Adaptation 2, but on Day 1 of Adaptation 3, mean reciprocal latency was reliably lower than it was on the last day of the previous Adaptation period ($t=2.46$, $df=10$, $p<.025$).

The center panel of the figure summarizes the water intake, expressed as a percentage of body weight. The differences between Day 1 and Day 10 means for each Adaptation

FIGURE 2

Mean reciprocal latency to drink, mean percent body weight intake, and mean body weight, of the 11 albino rats during adaptation to 23.5 hr. water deprivation and during ad lib recovery periods, plotted as a function of days of the experiment. Ad lib periods (I and II), and Adaptation periods (I, II, and III), are as indicated below the baseline.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period were all significant at $p<.005$ or beyond ($t=12.36$, $df=10$, $p<.001$; $t=5.98$, $df=10$, $p<.001$; $t=3.30$, $df=10$, $p<.005$). The mean intake on the first day of Adaptation 1 was reliably below the means for the corresponding day of the second and third Adaptation periods ($F=4.37$, $df=2/30$, $p<.025$).

Plotted in the bottom panel of the figure are the mean body weights for the duration of the experiment. The body weight loss from the last day of ad lib conditions to the second day of each corresponding Adaptation period was significant at $p<.001$ ($t=13.57$, $df=10$; $t=24.11$, $df=10$; and $t=19.05$, $df=10$). These weight losses were roughly equivalent in all three cases, the largest being associated with Adaptation 2.

Discussion

The data concerning latency to drink definitely show that some familiarization with the drinking situation was important in obtaining rapid orientation to the water spout. Further, the increased speed of starting to drink on Day 1 of Adaptation 2 indicates that the initial familiarization produced behavioral changes which not only persisted, but became even more pronounced during the ad lib recovery period. By the last day of the second Adaptation period, reciprocal latency to drink appeared to reach a maximum value. At the beginning of Adaptation 3, unlike Adaptation 2, there was a significant decrease in reciprocal latency to drink, with a subsequent gradual return to the established maximum. Thus, rapid approach to the water spout could be interpreted as analogous to, or as actually being

a motor skill, which takes time to develop to a high level. Decrements, such as the one at the beginning of Adaptation 3, are to be expected if practice is interrupted as it was in this case. This interpretation is fairly consistent with that of Ghent (1957). Explanations of the decrement at the beginning of Adaptation 3, which are based on some altered reinforcement value of the drinking boxes during the ad lib recovery periods, run into the difficulty presented by the increment shown in Adaptation 2.

The intake data are consistent with the finding of Beck (1962). The subjects do not seem to learn to increase their Day 1 intakes with successive adaptations, as evidenced by the fact that the means for Day 1 of Adaptation 2 and 3 are not different. The lower intakes associated with the first day of Adaptation 1 was to be anticipated on the basis of the novelty of the drinking situation. This result is consistent with the findings of Fink and Patton (1953). Drinking on the first day of Adaptation 2 was slightly, but significantly higher for the 'carrying-cage' subjects than it was for the 'drinking box' subjects. It seems that this result can be accounted for by the following considerations. Neither group found water in the home cage upon returning there on the last day of ad lib conditions. Thus, subjects retained in the carrying-cage experienced 0.5 hr. more of water deprivation, during which time all subjects of the other group drank from 1-5 ml. of water.

It seems that Beck's reasoning from increased water need is correct on the basis that, as adaptation to

deprivation proceeds, rats gain weight. This they do by slowly adjusting their eating times, so that the major part of their food intake immediately follows the daily drinking time. The osmotic stress caused by the increased food intake results in higher water intake on the following day, which in turn results in higher food intake, etc. Apparently, this process continues with diminishing overshoots until asymptotic intake and weights are reached. Many regulatory control systems show gradual damping effect of this sort (Bayliss, 1966).

In sum, the decreasing latency to drink with repeated testing indicates that this is a learned component of adaptation behavior. Water intake and body weight, conversely, require the same adjustment process during each adaptation, indicating that these components are not influenced by prior learning.

As stated earlier, the injection treatments were administered on Day 10 of Adaptation periods 2 and 3 in order to determine the reliability of postinjection latency to drink on two separate occasions. It was found that the postinjection latencies were consistent, and that the mean of the two latencies would be a reasonable predictor of postinjection latency to drink. This method was used in Experiment 2.

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EXPERIMENT II

Regulatory processes are dependent on detection of a state of physiological imbalance and correction of these imbalances. Detection of a state of dehydration and the initiation of drinking behavior are two necessary processes in the regulation of water balance.

A state of water imbalance followed by drinking behavior can be produced, experimentally, by injection of hypertonic solutions, e.g., Hatton and Thornton (1968); Wayner, Wetrus, and Blonk (1962); and Young, Heyer, and Richey (1952). This behavioral response reflects the detection of the imbalance and leads to the initiation of corrective behavior.

Uniform osmotic pressure[1] exists between the intracellular and extracellular fluid compartments; this uniformity is governed by the passage of water across membranes under the forces of hydrostatic and osmotic pressure. If the concentration of the extracellular compartment rises, as in

[1]Osmotic pressure is defined as that particular hydrostatic pressure which must be applied to a solution to prevent entry of solvent. An osmotic pressure develops only when a solution is separated from a more dilute solution by a semipermeable membrane (Pitts, 1965; page 32). In a biological system, however, and its relation to thirst, it is more meaningful to speak of 'effective' osmotic pressure. Effective osmotic pressure is the ratio of the number of dissolved particles unable to freely penetrate a membrane to the total number of solute particles in a solution (Wolf, 1958; page 20).

the case of water deprivation and hypertonic injections, the resulting extracellular hypertonicity produces several effects which tend to counteract the imbalance. There is an osmotically induced flow of water from the intracellular to the extracellular compartments. Antidiuretic hormone is released from the posterior pituitary which increases water reabsorption in kidney tubules, thereby conserving body water (Verney, 1947). There is also a behavioral effect; most mammals will drink (Greenleaf, 1966).

Gilman (1937) attempted to determine whether or not the osmotic pressure of the blood, per se, was critically involved in drinking behavior. He administered isosmolar concentrations of sodium chloride (20%) or urea (40%) solutions to dogs and measured the amount drunk following the intravenous injection. It was found that the saline and urea solutions did not have the same effect on the amount of water drunk. Injection of saline solution potentiated drinking, whereas the urea solution had almost no effect. Gilman thus concluded that an increment in 'effective' osmotic pressure of the extracellular fluid was an important condition for drinking behavior. Effective osmotic pressure is produced by particles unable to freely penetrate the cell membrane; sodium chloride has this characteristic, whereas urea moves freely across the cell membrane.

When hypertonic saline solutions are added to the extracellular compartment, the extracellular osmolality increases, and this produces movement of water out of the cells into the extracellular compartment. Over time, when equilibrium

is finally reached, the osmolality and volume of the extracellular compartment has increased. At the same time, the osmolality of the intracellular compartment has increased, but with a decreased volume (Ruch and Patton, 1965; page 891). Increased osmolality and volume of the plasma, following hypertonic injection, are processes which occur simultaneously. The pulling of water out of the cells into the plasma is a homeostatic mechanism which seems to buffer plasma osmotic pressure changes.

At any point in time, it should be possible to withdraw blood samples and determine the osmolality and volume changes of the plasma. An indication of plasma osmolality can be obtained by freezing-point depression, whereas plasma volume can be indexed by protein concentration. Because protein is not free to move through the membrane, an increase in protein concentration can be used to indicate a decrease in plasma volume (Pitts, 1965, page 27; Stricker, 1966).

Hatton and Thornton (1968) found that latency to drink following a 16% saline injection was approximately 5 min., with a range of 1 min. 59 sec. to 10 min. 47 sec. This latency to drink was obtained from albino rats that were fully adapted to 23.5 hr. water deprivation, and known to be satiated before the injection. Based on the range of latencies to drink, they withdrew blood samples from another group of rats at 2, 5, 8, and 11 min. postinjection. Again, the rats were fully adapted to 23.5 hr. water deprivation and known to be satiated before the injection. For 16%

saline injections they found that plasma osmolality increased, while plasma protein concentration decreased (volume increased), as a function of minutes postinjection.

The behavioral observation of Hatton and Thornton (1968), that postinjection latency to drink was approximately 5 min., is of interest when compared with a neurophysiological finding of Hatton (1965). Changes in the electrical activity of the lateral hypothalamus of satiated rats were observed to occur approximately 5 min. after an injection of 16% saline solution. Perhaps this time lapse represents the time required for the injection to dehydrate the body sufficiently to activate the mechanisms which control drinking behavior.

Verney (1947) in his studies of antidiuresis in the dog, estimated that a 1-2% increase in plasma concentration would be sufficient to trigger central detector cells and produce an antidiuretic response. Would it be reasonable to expect that a similar increase in plasma concentration would lead to another form of corrective behavior, i.e., drinking behavior?

According to Fitzsimons (1963), an increase in effective osmotic pressure of body fluids is a physiological stimulus of drinking behavior. By slowly infusing 1-M sodium chloride intravenously in rats, he found a mean increase in osmotic pressure of $1.6 \pm 0.11\%$ at the initiation of drinking behavior. This percentage increase was calculated from an assumed initial body fluid osmolality and volume.

Now that is has been established that an increment in effective osmotic pressure of the extracellular fluid is an

important condition for drinking behavior (Fitzsimons, 1963 and Gilman, 1937), and that plasma conditions (osmolality and protein concentration) change as a function of time post-injection (Hatton and Thornton, 1968), it is important to know more precisely, the blood plasma conditions at the time of initiation of drinking behavior.

In order to determine blood conditions at the time of detection, as indicated by initiation of intake, it was necessary to establish that rats must readapt, physiologically, to recurrent deprivation periods. It was found in Experiment 1 (Hatton and Almli, 1967a), that in terms of weight losses and gains and water intake, rats must readapt to each ensuing deprivation period when they are separated by ad lib recovery periods.

With the knowledge that successive deprivation periods require the same adaptation process, it is now possible to determine two relationships. The first is the temporal relationship between a hypertonic saline injection and the initiation of drinking behavior, while on ad lib conditions and while adapting to 23.5 hr. water deprivation. And the second is blood plasma conditions at the initiation of drinking behavior as a function of days of adaptation to 23.5 hr. water deprivation.

This study was designed, a) to verify the temporal relationship found by Thornton (1966), between a subcutaneous injection of 16% saline solution and the initiation of drinking in satiated rats, b) to determine the reliability of this temporal relationship over two separate but similar

occasions, and c) to determine the blood plasma conditions at the initiation of drinking behavior. These variables were studied as a function of days of adaptation to 23.5 hr. water deprivation.

Method

Subjects

Thirty-six* male albino rats of the Holtzman strain, were housed in individual wire cages in a temperature controlled room (73°-78° F.), under constant light conditions. Wayne Lab Blox were constantly available in the home cage throughout the experiment. The rats were approximately 100 days old and weighed 387 ± 40 grams at the start of the experiment.

Apparatus

Observations of drinking were made using the apparatus described in Experiment 1.

The subcutaneous injections were delivered through 24 ga. needles in 2 cc. glass syringes. All injections were given in the area of the hind quarters, and at a constant volume of 1 cc. The solution injected was made up of 16 grams of sodium chloride per 100 ml. of solution, the solvent being distilled water. A sponge lined animal restrainer was used to administer the subcutaneous injections. (For a description of the animal restrainer, see Appendix).

* 1 rat died on the fiftieth day of the experiment due to an overdose of ether, data from this Day 5 animal was dropped from all analyses. Therefore, the results of the experiment are based on N=35.

The subjects were transported to an adjoining room, where the blood samples were taken, in a carrying-cage.

The blood samples were drawn in 1 or 2 cc., heparinized, glass syringes. Blood plasma was analyzed in a refractometer (protein concentration) and in a freezing-point osmometer (osmolality).

Procedure

The basic design used here was identical to that used in Experiment 1. This consisted of 10-day periods of adaptation to 23.5 hr. water deprivation, with 5-day ad lib periods interpolated between the deprivation periods. The design is presented in Figure 3. The following manipulations were added to the procedure of Experiment 1; 1) one more 5-day ad lib period, and 10-day 23.5 hr. water deprivation period was added. 2) all subjects were given 0.5 hr. free access to water in the drinking boxes during all ad lib periods. 3) on the last day of the first and second ad lib periods (referred to as Day 0) and on Days 1, 2, 3, 5, and 10, of the second and third deprivation periods, the rats were injected following the drinking period, returned to the drinking boxes, and latency to drink postinjection was recorded. Thus, two postinjection latencies to drink were obtained for each animal, and a mean latency to drink was computed for each animal. And 4) on the last day of the third ad lib period (Day 0) and on Days 1, 2, 3, 5, and 10, of the fourth adaptation period, blood samples were drawn, following injection, at the subject's mean postinjection latency to drink.

On the day of arrival in the laboratory, thirty-six rats were randomly assigned to six equal sized treatment groups. After three days of ad lib food and water, the subjects were weighed and the water bottles were removed from the home cages. For the next 10 days, the subjects were weighed and given 0.5 hr. free access to water in the drinking boxes. Following the tenth day on this schedule, ad lib conditions were begun. During the ad lib period the rats were placed in the drinking boxes for 0.5 hr. at their usual daily drinking time. On the fifth, and last day of the ad lib period, six rats were removed from the drinking boxes after their 0.5 hr. drinking period. They were immediately injected and returned to the drinking boxes where latency to drink following the injection was measured. This group constituted the Day 0 treatment condition.

Following the ad lib period, the subjects were again given a 10-day adaptation to deprivation period. The second deprivation period was identical to the first deprivation period with the exception of the following; one group of subjects per day (representing Days 1, 2, 3, 5, and 10) received an injection following their usual 0.5 hr. free access to water. They were immediately returned to the drinking boxes and latency to drink, postinjection, was recorded.

All subjects, following their injection treatment day, continued on the deprivation schedule for the remaining days of the 10-day deprivation period.

Following the tenth day of the deprivation period, ad lib conditions were again begun. The ad lib conditions were identical to the previous ad lib period. On the fifth day of the 5-day ad lib period, and on the specified days (1, 2, 3, 5, and 10) of the following 10-day deprivation period, the rats were again given injection treatments identical to those given in the previous periods. Each subject was injected, following drinking, on the same day of the schedule at it had in the previous treatment period, i.e., on Days 0, 1, 2, 3, 5, or 10. By repeated injection treatments over two separate but similar occasions, the reliability of postinjection latency to drink was determined.

A mean latency to drink, following injection, was computed for each subject from the two postinjection latencies to drink. The mean postinjection latency to drink for a given subject was used in the following periods to determine when the blood samples would be drawn.

During the following ad lib and deprivation periods, each subject, on his treatment day was removed from the drinking boxes following the drinking period and taken to an adjoining room. The rat was immediately etherized and then injected subcutaneously with 16% sodium chloride solution. At the subject's mean postinjection latency to drink, a heart puncture on the rat's left side was performed, and a blood sample was drawn under ether anesthesia. (For a complete description of the heart puncture procedure, see Appendix).

The blood samples were drawn in heparinized syringes, immediately centrifuged and the plasma drawn off. A small part of the plasma was analyzed for protein concentration in a refractometer. The remaining plasma was quickly frozen in sealed vials and later analyzed for osmolality in a freezing-point osmometer.

Results

Water intake, expressed as a percentage of body weight, is plotted in Figure 4 as a function of days of the experiment. During the initial deprivation period and during the deprivation periods following the ad lib conditions, the phenomenon of adaptation and readaptation took place. The readaptation to each ensuing deprivation period was highly similar to the results obtained in Experiment 1. Even with injection treatments on Days 0, 1, 2, 3, 5, and 10, of the second and third deprivation periods, water intake increased for approximately four days and then leveled off for the remaining days of the deprivation period.

The data for latency to drink after injection treatment were transformed into reciprocals for purposes of statistical analysis. When reciprocal latencies are used, latency to drink is referred to as 'readiness to drink'.

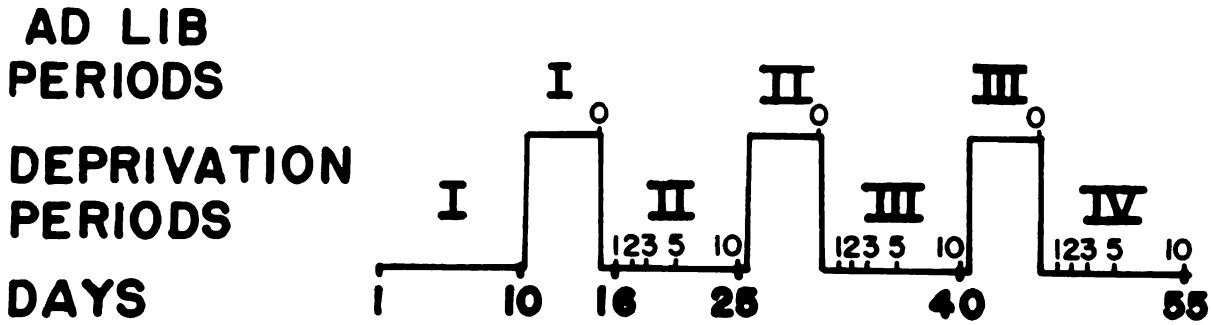
Two-way analysis of variance of readiness to drink following injection treatment, revealed that the difference between the means of the first and second injections were not significantly different ($F=1.484$, $df=1/60$).

Figure 3

Experimental design for Experiment 2. Ad lib and adaptation to deprivation periods are superimposed on days of the experiment. Treatments administered on days indicated.
I, II, III: refer to 5-day ad lib periods.
I, II, III, IV: refer to 10-day 23.5 hr. water deprivation periods.
0, 1, 2, 3, 5, 10: refer to treatment days. Injections administered during ad lib periods I and II, and during deprivation periods II and III. Injections followed by blood samples administered during ad lib period III and deprivation period IV.

Figure 4

Mean percent body weight water intake, of 35 albino rats, during adaptation to 23.5 hr. water deprivation and during ad lib recovery periods, plotted as a function of days of the experiment. Ad lib and adaptation to deprivation periods are indicated below the baseline.


AD LIB
PERIODS
DEPRIVATION
PERIODS
DAYS
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II
III
IV
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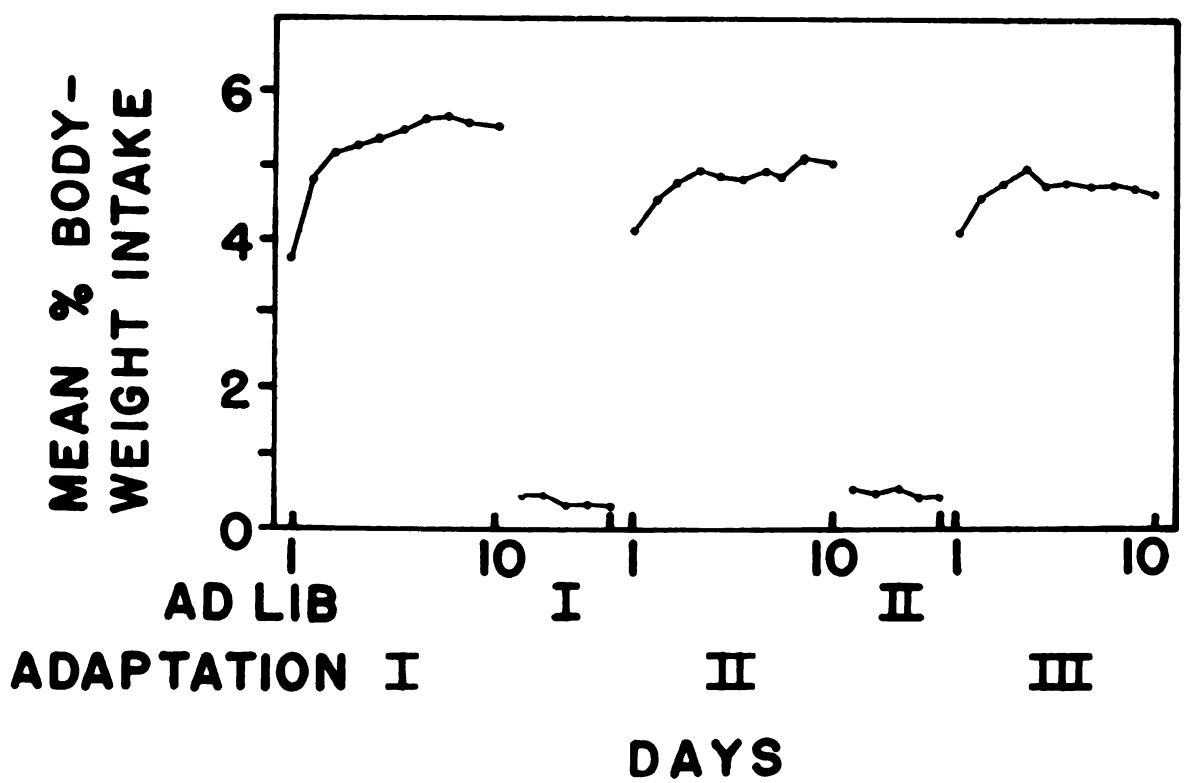
40
55


MEAN % BODY-
WEIGHT INTAKE
6
4
2
0
1
10
AD LIB
I
II
ADAPTATION I
II
III
DAYS

Means for readiness to drink on different days of the adaptation to deprivation schedule were likewise not significantly different ($F=2.109$, $df=5/60$). In Table 1, the mean post-injection latencies to drink for Days 0, 1, 2, 3, 5, and 10, are presented. Finally, the interaction of the two injections with days of adaptation were not significant ($F=0.290$, $df=5/60$).

Since the means for the two injection treatments were not significantly different, and since the means for days of adaptation to the deprivation schedule were also not significantly different, the latency to drink data were combined and are presented as a bar graph in Figure 5.

A high degree of consistency in readiness to drink was obtained between injection 1 and 2 ($r=0.711$). The scattergram in Figure 6 represents readiness to drink for injection 2 plotted as a function of readiness to drink for injection 1. A mean shift of 16.53 sec. in latency to drink, from 5 min. 45 sec. to 6 min. 1 sec. was not statistically significant.

The means, medians, and ranges of latency to drink following the first and second injection, and for the two injections combined are presented in Table 2.

During the fourth adaptation to deprivation period (preceding the withdrawal of the blood samples), the means for amount drunk on Days 1, 2, 3, 5, and 10, were statistically different ($F=3.769$, $df=4/24$, $p<.05$). The means for readiness to drink, on the other hand, were not statistically reliable ($F=1.529$, $df=4/24$).

Table 1

Means and standard errors of postinjection latencies to drink (in sec., and min. and sec.).*

Day	Mean in sec.	Mean $\frac{1}{\text{Lat. (in sec.)}}$	Std. error (in sec.)	Mean Min.:Sec.
0	385	.0028	35.41	6:25
1	349	.0029	16.49	5:49
2	375	.0031	46.00	6:15
3	388	.0026	17.04	6:28
5	313	.0033	20.25	5:13
10	294	.0034	14.94	4:54

*Note: Means were computed from latencies to drink following the first and second injections for 35 albino rats.

Figure 5

Latency to drink of 35 albino rats immediately after injection of 16% saline solution for the first and second injection treatments, and the means obtained from both treatments combined. Postinjection latency to drink grouped as to time, irrespective of Day group.

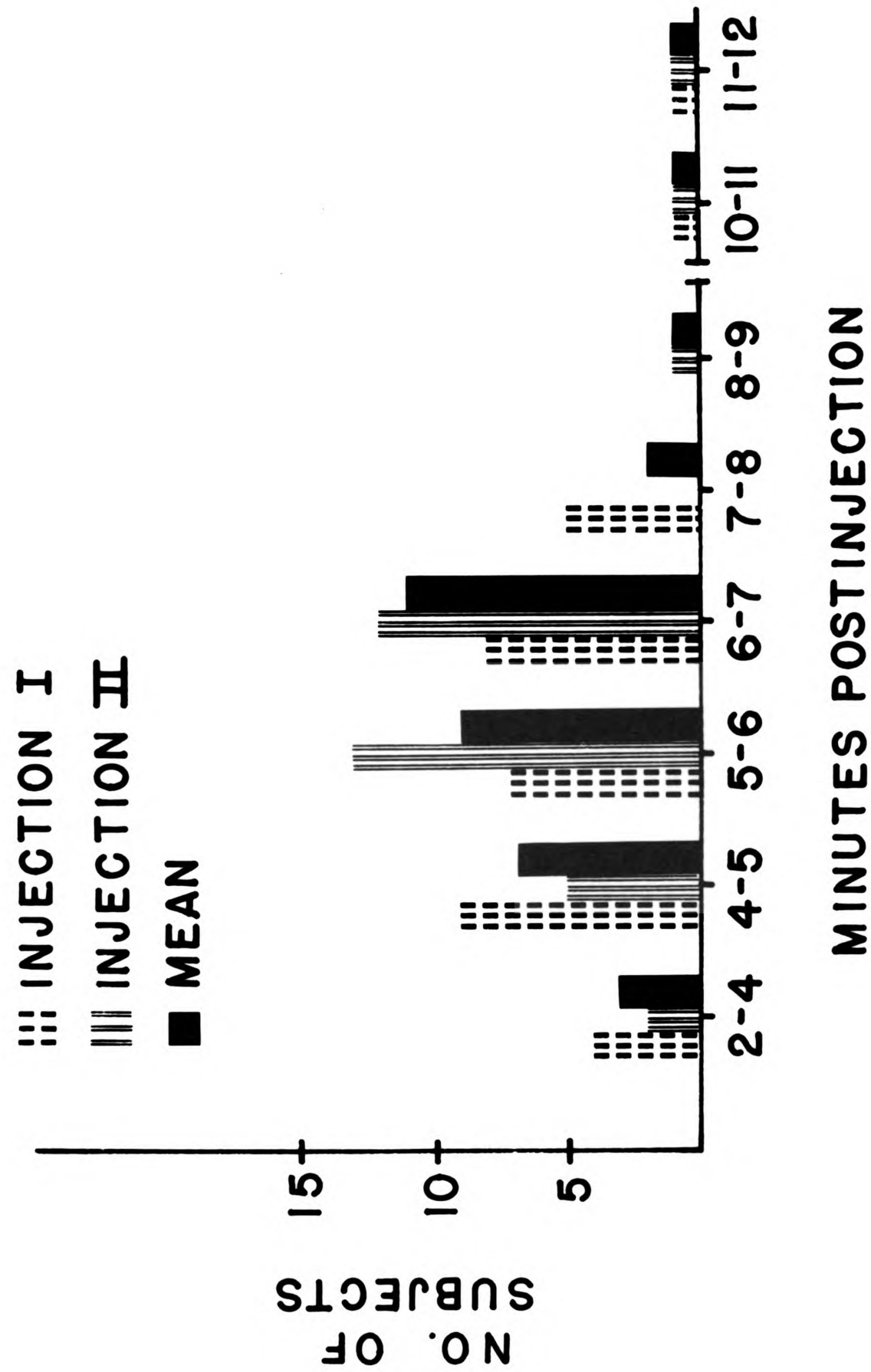

INJECTION I
INJECTION II
MEAN
NO. OF SUBJECTS
15
10
5
2-4
4-5
5-6
6-7
7-8
8-9
10-11
11-12
MINUTES POSTINJECTION

Figure 6

A scattergram showing the relationship between the post-injection latency to drink following injection one and postinjection latency to drink following injection two, for 35 albino rats. Day of adaptation to 23.5 hr. water deprivation is indicated in the key. In the upper right hand corner, the correlation coefficients between latency from injection one and latency following injection two are plotted as a function of days of adaptation to 23.5 hr. water deprivation.

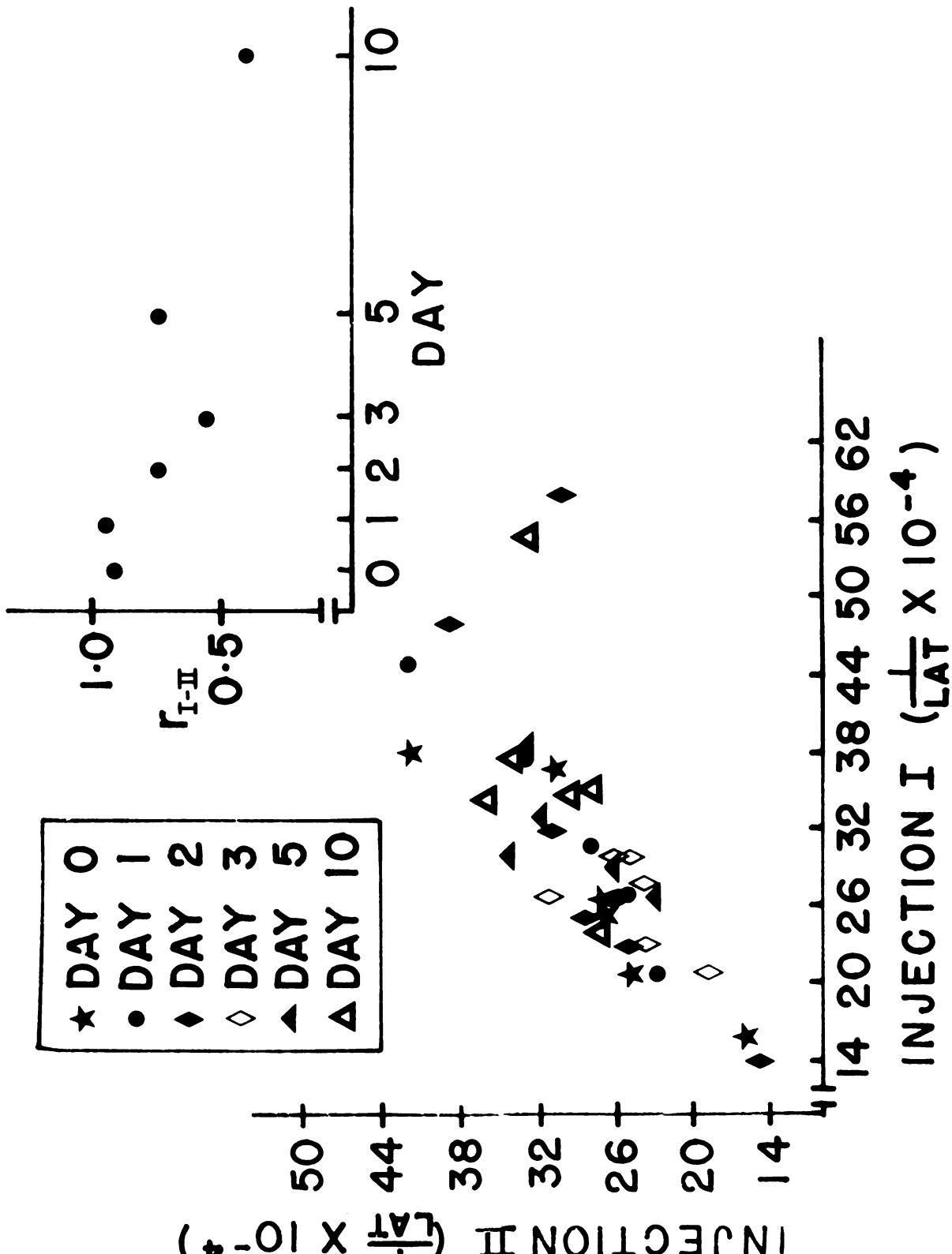

DAY 0
DAY 1
DAY 2
DAY 3
DAY 5
DAY 10
INJECTION I ($\frac{1}{LAT}$ X 10^{-4})
14 20 26 32 38 44 50 56 62
INJECTION II ($\frac{1}{LAT}$ X 10^{-4})
50 44 38 32 26 20 14
r_{I-II}
1·0
0·5
0 1 2 3 5 10
DAY

Table 2

Means, medians, and ranges of postinjection latencies to drink following the first and second injection treatments, and for the two injection treatments combined. N=35.*

Injection treatment	Mean (min:sec)	Median (min:sec)	Range (min:sec to min:sec)
Injection I	5:45	5:28	2:51 to 11:26
Injection II	6:01	5:44	3:52 to 11:06
Injections I and II combined	5:53	5:42	2:51 to 11:26

*Note: Latencies to drink are grouped irrespective of Day group.

The means for blood plasma osmolality (mOsm./l.) at the initiation of drinking behavior, are plotted as a function of days of adaptation to the deprivation period in Figure 7. An analysis of variance computed on the means indicated that they were significantly different ($F=5.787$, $df=5/29$, $p<.01$). Duncan's test (Edwards, 1960), showed that plasma osmolality on Day 0 was reliably higher than on Days 2, 3, 5, and 10 of adaptation ($p<.01$).

Plasma protein concentration (grams/100 ml.) was used as an index of plasma volume, increases in plasma protein concentration indicating decreased plasma volume (Stricker, 1966).

In Figure 8 are plotted the means for protein concentration at the initiation of drinking behavior as a function of days of adaptation to the deprivation period. An analysis of variance computed on the means yielded a significant difference in mean protein concentration ($F=5.00$, $df=5/29$, $p<.01$). Duncan's test, indicated that protein concentration at the initiation of drinking behavior on Day 0 was significantly higher than Days 2, 3, and 5 ($p<.01$). Also, mean protein concentration for Day 1 was significantly higher than the mean concentration for Day 3 ($p<.01$).

The lack of relationship between protein concentration and osmolality is plotted in scattergram form in Figure 9, protein concentration being plotted as a function of osmolality.

Figure 7

Mean plasma osmolality at the initiation of drinking behavior, following injection of 16% saline solution, of 35 albino rats, plotted as a function of days of adaptation to 23.5 hr. water deprivation. Standard error indicated by flags.

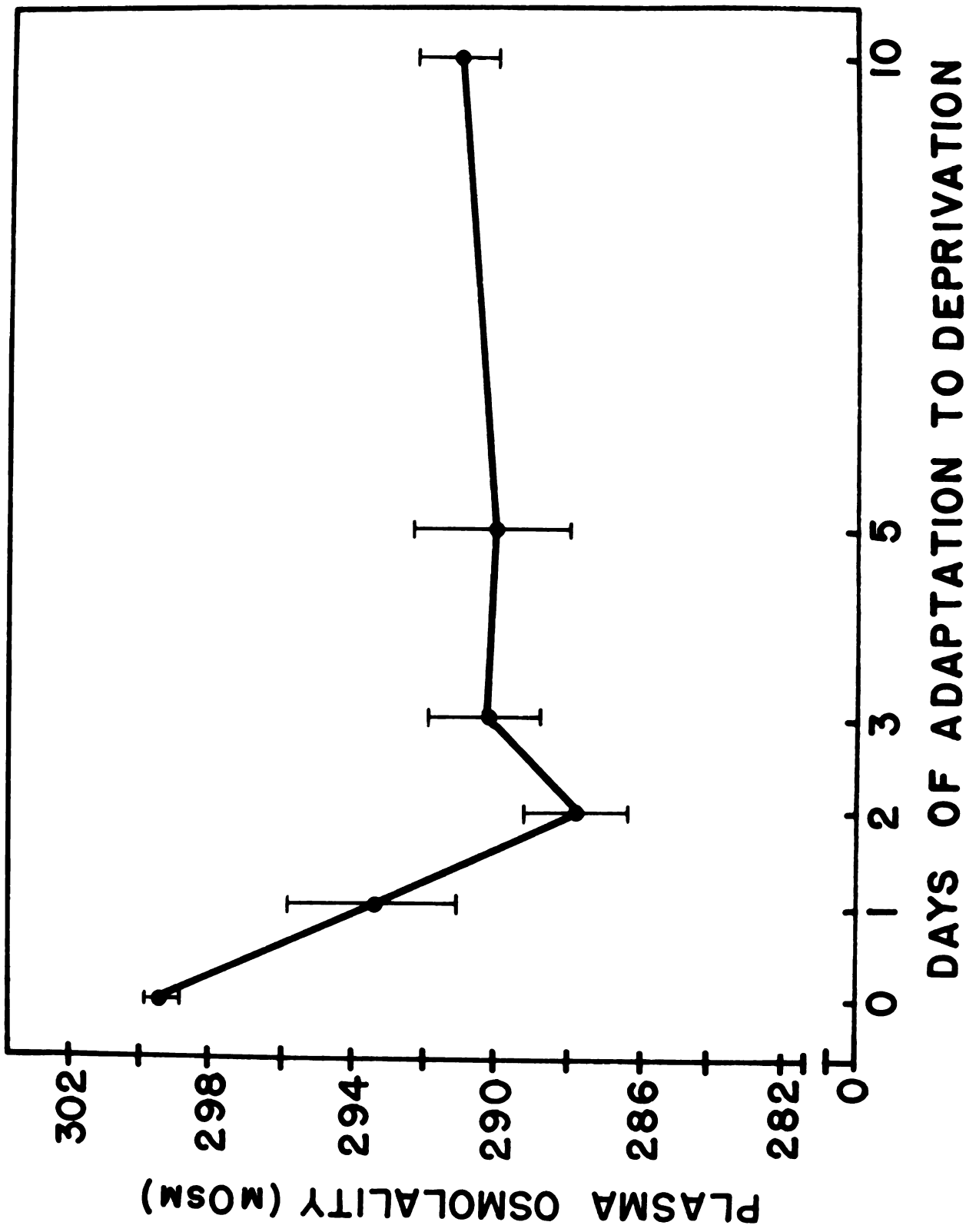

PLASMA OSMOLALITY (MOSM)
302
298
294
290
286
282
0
1
2
3
5
10
DAYS OF ADAPTATION TO DEPRIVATION

Figure 8

Mean plasma protein concentration at the postinjection (16% saline) initiation of drinking behavior, of 35 albino rats, plotted as a function of days of adaptation to 23.5 hr. water deprivation. Standard error indicated by flags.

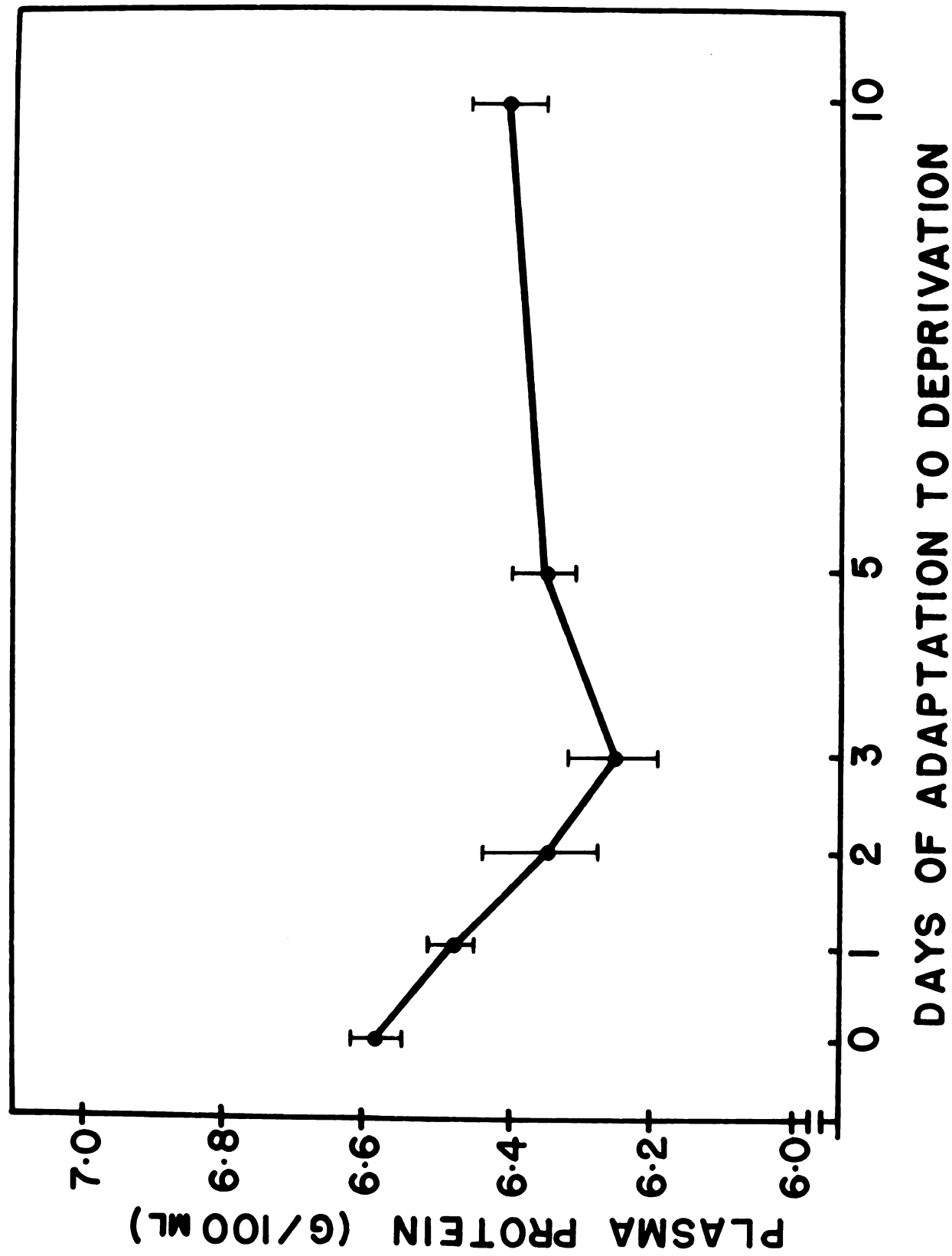

PLASMA PROTEIN (G/100 ML)
7.0
6.8
6.6
6.4
6.2
6.0
0
1
2
3
5
10
DAYS OF ADAPTATION TO DEPRIVATION

Figure 8

Mean plasma protein concentration at the postinjection (16% saline) initiation of drinking behavior, of 35 albino rats, plotted as a function of days of adaptation to 23.5 hr. water deprivation. Standard error indicated by flags.

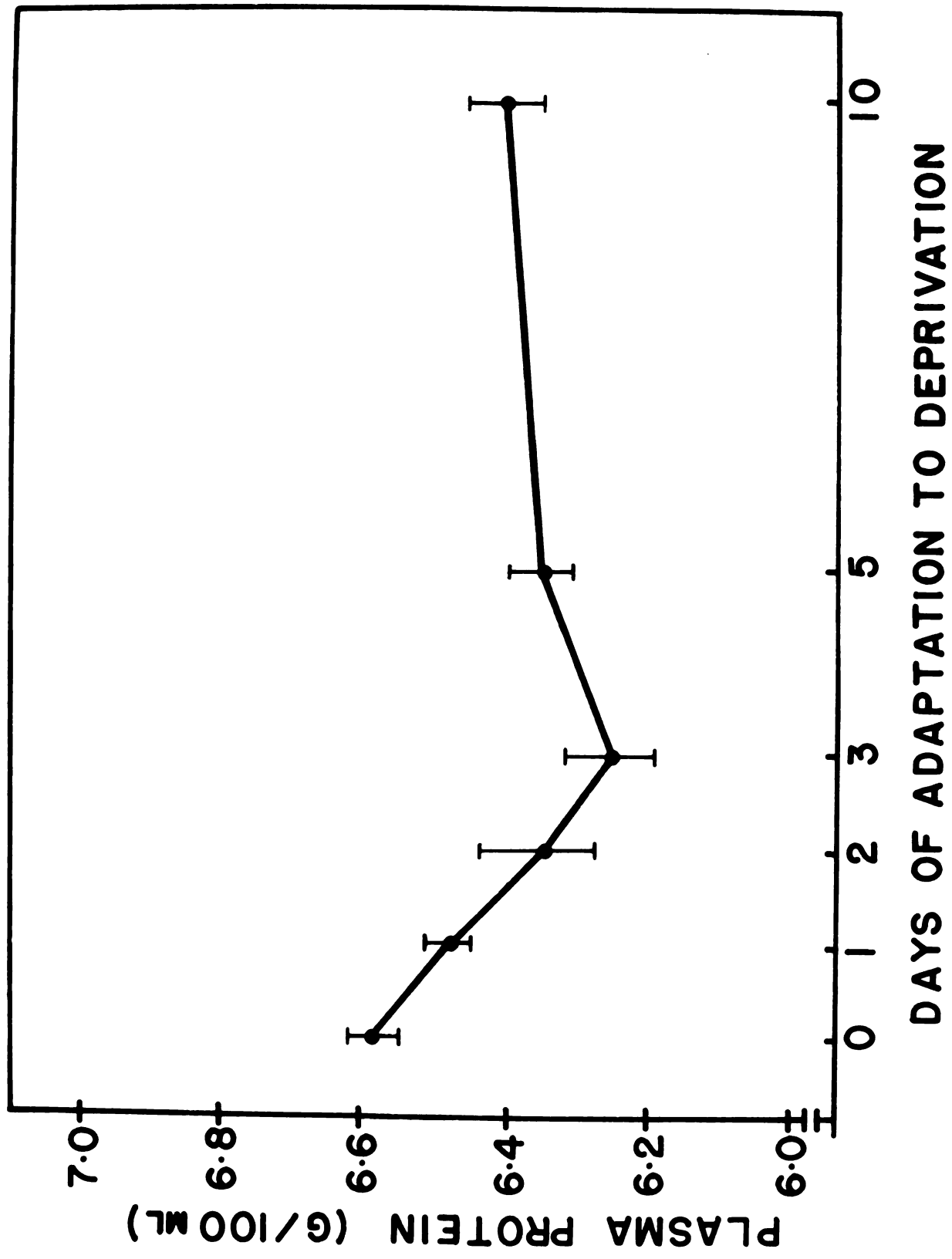
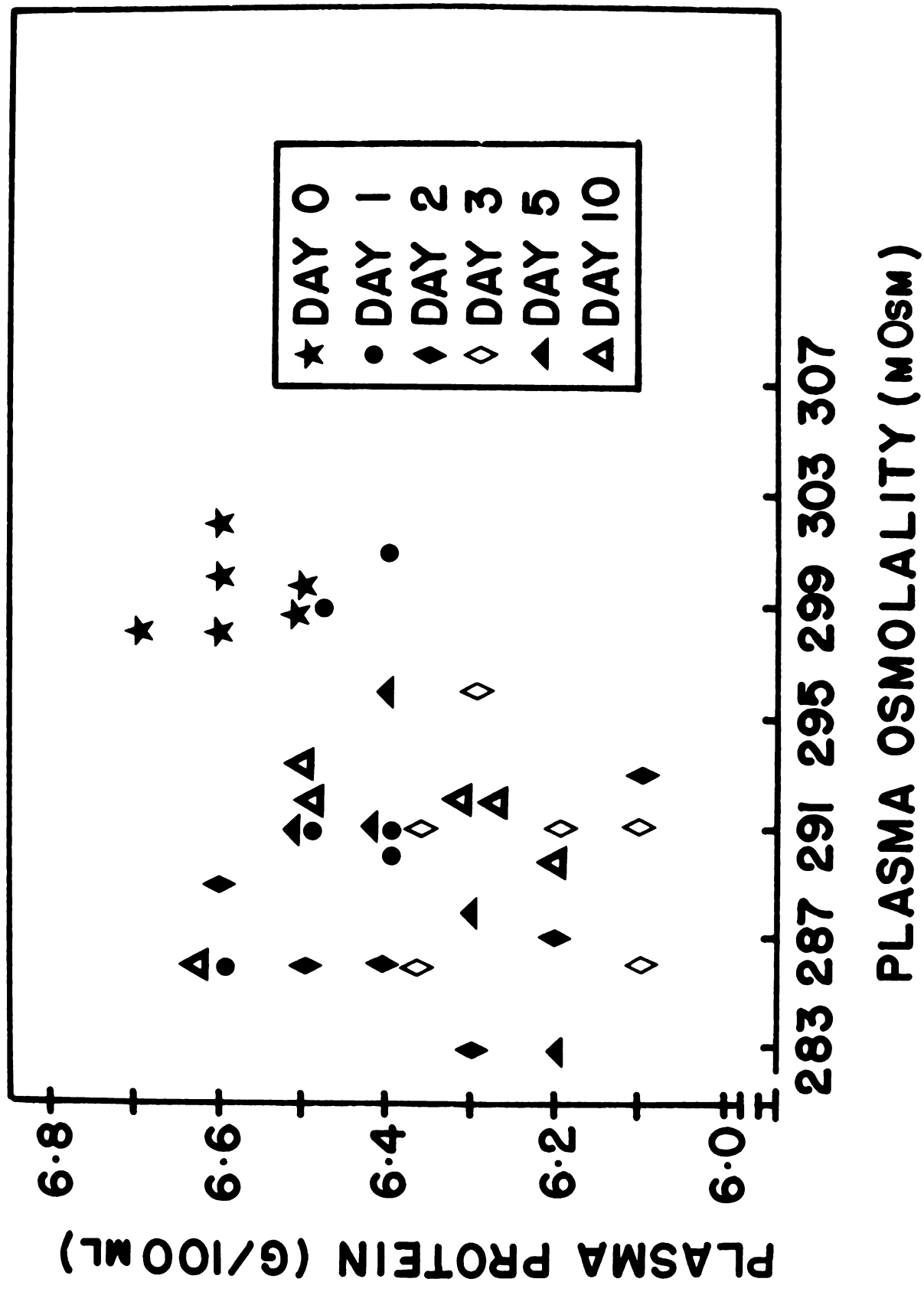

PLASMA PROTEIN (G/100 ML)
7.0
6.8
6.6
6.4
6.2
6.0
0
1
2
3
5
10
DAYS OF ADAPTATION TO DEPRIVATION

Figure 9

A scattergram showing the lack of relationship between plasma osmolality and plasma protein concentration at the postinjection initiation of drinking behavior for 35 albino rats. Day of adaptation to 23.5 hr. water deprivation is indicated in the key.


★DAY 0
● DAY 1
◆ DAY 2
◇ DAY 3
◀ DAY 5
△ DAY 10
PLASMA PROTEIN (G/100 ML)
6.8
6.6
6.4
6.2
6.0
283 287 291 295 299 303 307
PLASMA OSMOLALITY (MOSM)

In Figure 10, plasma osmolality and plasma protein concentration are plotted as a function of minutes postinjection in which the blood samples were obtained.

Discussion

Detection of a state of water imbalance can be indexed by the initiation of corrective behavior, e.g., drinking behavior. Unfortunately, the degree of dehydration necessary for the detection process to occur is not as easily indicated. Here, we cannot simply look at the behavioral act of ingestion; the physiological conditions which influence corrective behavior must be tapped.

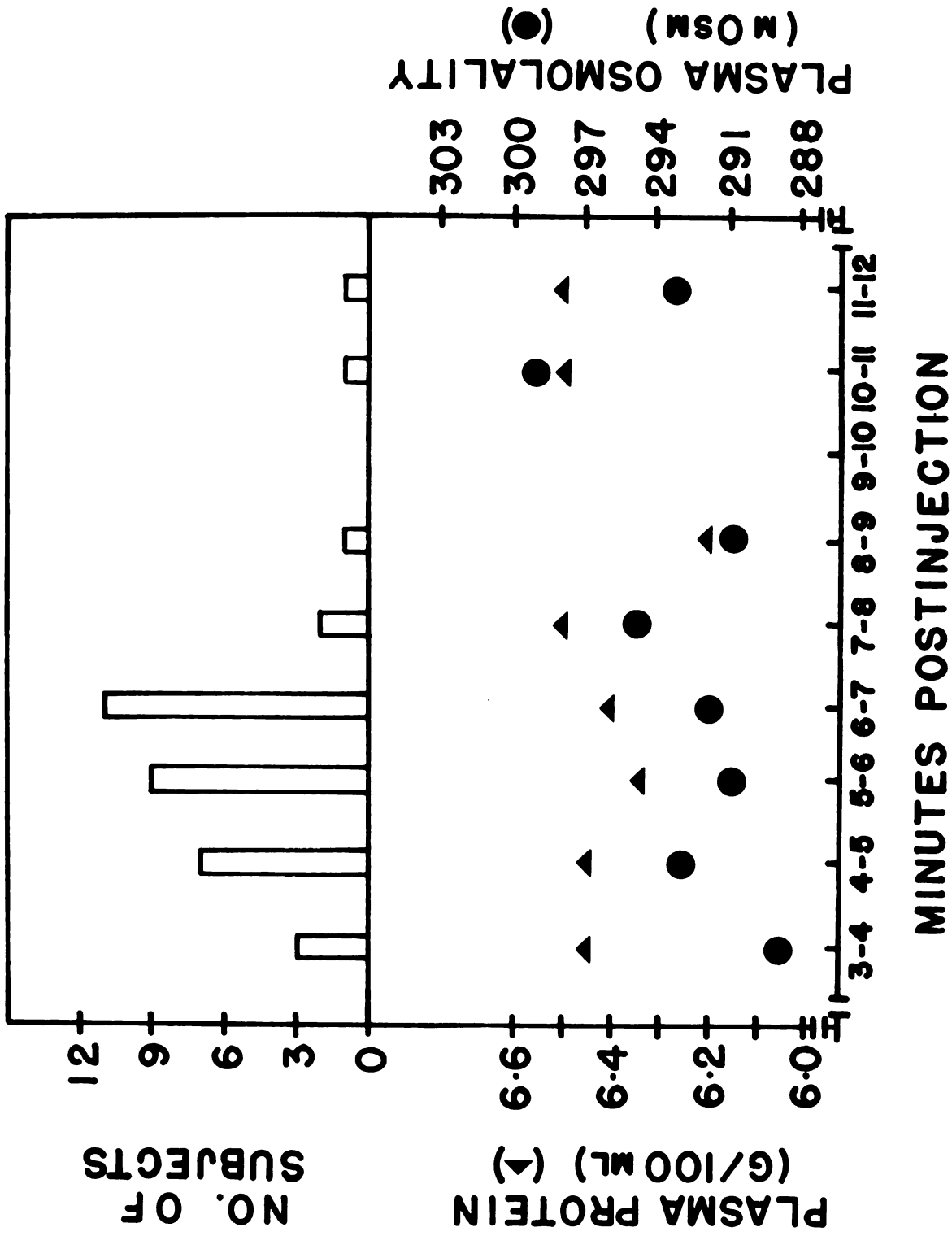
Increased blood plasma concentration, and the cellular changes thereby produced, have been considered to be of critical importance for the instigation and maintenance of water ingestion (Fitzsimons, 1963; Kutscher, 1966; Wolf, 1948). If this is true, latency to drink following a salt injection may reflect the time required for blood conditions to change sufficiently to stimulate the detector mechanism.

In this experiment, four main questions were asked and attempts were made at their resolution. The questions asked, and the answers obtained from this experiment are presented in the following text.

The first question to be resolved was: "What is the latency to drink of rats, satiated immediately before injection of 16% saline solution, while on ad lib and while adapting to 23.5 hr. water deprivation?"

Figure 10

Plasma protein concentration and plasma osmolality at the postinjection initiation of drinking behavior of 35 albino rats, plotted as a function of time postinjection when the blood samples were withdrawn. The bar graph represents the number of observations per time period.


NO. OF SUBJECTS
12
9
6
3
0
PLASMA PROTEIN (G/100 ML) (▲)
6.6
6.4
6.2
6.0
PLASMA OSMOLALITY (mOsm) (●)
303
300
297
294
291
288
3-4 4-5 5-6 6-7 7-8 8-9 9-10 10-11 11-12
MINUTES POSTINJECTION

Neither mean latencies to drink for the first and the second injection treatments, nor the mean latencies to drink on different treatment days were reliably different. Latency to drink, following salt injection, was not different whether the rat was on ad lib conditions, or on Days 1, 2, 3, 5, or 10, of a 23.5 hr. water deprivation schedule. Thus, it is concluded that latencies to drink, following salt injection, while adapting to 23.5 hr. water deprivation are not different at various stages of the adaptation process.

The second question to be answered is: "How does post-injection latency to drink while adapting (0-10 days on the schedule) to 23.5 hr. water deprivation compare with rats fully adapted (over 20 days on the schedule) to the schedule?"

Due to the similarities in procedure, the results of the present experiment can best be compared with the results obtained by Hatton and Thornton (1968). In both experiments, the rats were habituated to the drinking situation, handling and injection procedures were similar, site and concentration of the injected solution were the same, and injections were administered to satiated animals. The fact that Hatton and Thornton (1968) carried out their manipulations on rats fully adapted to 23.5 hr. water deprivation, whereas in the present experiment the manipulations were carried out on rats during the adaptation process, lends itself to meaningful comparisons.

Hatton and Thornton (1968) found that the median latency to drink following a 16% saline injection was

5 min. 22 sec. for fully adapted rats. This compares quite favorably with the results obtained here for rats during the adaptation process. Here, the median latencies to drink for the two injection treatments were 5 min. 28 sec. and 5 min. 44 sec. The range of the latencies to drink for the Hatton and Thornton experiment were 1 min. 59 sec. to 10 min. 47 sec., while in the present experiment the range of the latencies to drink were 2 min. 51 sec. to 11 min. 26 sec.

Thus, it seems that when certain variables are controlled (e.g., habituation to the drinking situation, handling procedures, lighting, temperature, and site of injection), latency to drink following a 16% saline injection is highly similar whether administered to rats under ad lib conditions, while adapting to 23.5 hr. water deprivation, or to rats fully adapted to this schedule.

The third question put forth was: "How reliable is latency to drink, in satiated rats following 16% saline injection, over two separate but similar occasions?"

It has been demonstrated in Experiment 1, that when deprivation periods are interrupted by ad lib recovery periods, rats must readapt to each ensuing deprivation period. Water intake and body weight require the same adjustment process during each adaptation. Due to this recurrent adaptation process, it was possible to inject a rat on two separate occasions and have the animal at approximately the same state of adaptation each time.

In the present experiment it was found that only three out of a possible thirty-five rats were more than one minute apart in latency to drink following the two injection treatments. A mean shift of 16.53 sec., in latency to drink, from 5 min. 45 sec. to 6 min. 1 sec. was not statistically significant. Rats that were above the mean in latency to drink following the first injection, tended to be above the mean in latency to drink following the second injection, and vice versa. This relationship is described by the high correlation coefficient ($r=0.711$) obtained between the two injection treatments. Rats that initiated drinking extremely fast following the first injection regressed towards the mean on the second injection. On the other hand, rats that were extremely slow to initiate drinking following the first injection were also extremely slow to initiate drinking following the second injection.

It is concluded from the above that during adaptation to 23.5 hr. water deprivation, postinjection latencies to drink are highly consistent and reliable.

From the answers to the preceding questions it becomes apparent that injection of hypertonic saline solutions have a profound effect on the rat, that of producing the behavioral act of drinking. The osmotic stress experienced by the rat, following injection, becomes the controlling influence in his behavior. Whether the animal is on ad lib conditions, adapting to 23.5 hr. water deprivation, or fully adapted to 23.5 hr. water deprivation, the variables which normally control the initiation of drinking behavior are

washed-out by the injection of hypertonic saline solutions.

Further support for the predominant influence of hypertonic saline injections comes from experiments in which water intake postinjection was measured. Hatton and Thornton (1968) found that the amount of water drunk increased monotonically with increased saline concentrations. Oatley (1967b) found that when hypertonic saline injections were administered at different times of the day, there was no potentiation or inhibition of the amount drunk at any of the times when tests were made. The osmotic stimulus swamped the rather small diurnal differences in drinking (found by Oatley, 1967a), and the amount of water drunk was systematically related only to the salt injections.

In order to attain an understanding of the mechanism by which the body detects a state of dehydration, it is necessary to determine bodily conditions when detection and initiation of drinking behavior occur. As has been previously indicated, blood plasma conditions have been considered to be critically involved in the detection process. This brings us to the forth, and most important question: "What are the plasma conditions (osmolality and protein concentration) at the initiation of drinking behavior, while on ad lib conditions and while adapting to 23.5 hr. water deprivation?"

In an earlier attempt to define some aspects of this problem, Hatton and Thornton (1968) found that 86% of their salt injected rats initiated drinking within 11 min. Later,

using another group of rats, they withdrew blood samples 2, 5, 8, and 11 min. postinjection. Here again, they used rats fully adapted to 23.5 hr. water deprivation and satiated immediately before injection. They found that in response to a 16% saline injection, plasma protein concentration decreased, and plasma osmolality increased, as a function of minutes postinjection.

In the present experiment, by using the mean of the two postinjection latencies to drink, which were shown to be highly consistent, it was possible to calculate a close approximation of a given rats predicted latency to drink. Thus, on a given rats treatment day, the injection was administered and at the calculated mean latency to drink, the blood sample was drawn.

Using this procedure, the results of the present experiment do not carry the same relationship reported by Hatton and Thornton (1968). Here, both plasma protein concentration and osmolality were irregular, with no clear-cut increase or decrease as a function of minutes postinjection (Figure 10).

The difference between the present experiment and that of Hatton and Thornton was to be anticipated. In the present experiment, time postinjection and blood conditions were dependent upon the initiation of drinking behavior, whereas in the Hatton and Thornton experiment the major independent variable was time postinjection.

Fitzsimons (1963) attempted to determine the percentage increase in osmotic pressure of body fluids at which rats would start to drink following slow intravenous infusion of

hypertonic solutions. The mean percent increase in osmotic pressure at which normal rats, infused with 1-M. sodium chloride, started to drink was 1.6±0.11%. Special emphasis was placed on slow infusion (3.5 to 14.2 micro l./100 grams body weight/min.) because the increase in osmotic pressure produced by slow infusion is similar to the gradual rise that occurs naturally due to a continuing loss of body water. The percentage increase (1.6%) reported by Fitzsimons is well in accord with Verney's (1947) estimation of 1-2% increase in plasma osmolality being sufficient to trigger central detector cells.

In the present experiment, the dilution of the salt pocket produced by the subcutaneous injection and the subsequent elevation in plasma osmolality is probably similar in rate to the osmotic pressure elevations produced by slow intravenous infusion used by Fitzsimons (1963).

The results of the present experiment depict plasma conditions at the initiation of drinking behavior while rats adapt to 23.5 hr. water deprivation. The plasma conditions reported here represent threshold values for the initiation of drinking behavior, and can best be appreciated when compared with the results of other related experiments conducted in our laboratory.

Hatton and Dittrich (1967) withdrew blood samples from rats, while adapting to 23.5 hr water deprivation, at the time when they normally would begin their daily drinking period. These 'predrink' blood samples were drawn on Days 0, 1, 2, 3, 5, and 10, of the adaptation to deprivation period,

and represent plasma conditions after 23.5 hrs. of water deprivation for successive days of the deprivation period. Mean predrink plasma osmolality is plotted as a function of days of adaptation to 23.5 hr. water deprivation in Figure 11, and mean predrink plasma protein concentration is plotted as a function of days of adaptation to 23.5 hr. water deprivation in Figure 12.

Using a similar procedure, Hatton and Almli (1967b) withdrew blood samples on Days 0, 1, 2, 3, 5, and 10, of adaptation to 23.5 hr. water deprivation. The blood samples, in this study, were withdrawn immediately following the daily drinking period. These 'postdrink' blood samples represent plasma conditions after 23.5 hrs. of water deprivation and drinking to satiation for successive days of the deprivation period. Mean postdrink plasma osmolality (Figure 11) and protein concentration (Figure 12), are plotted as a function of days of adaptation to 23.5 hr. water deprivation.

Also in Figures 11 and 12, are the plasma conditions obtained in the present experiment, plotted as a function of days of adaptation to 23.5 hr. water deprivation. These 'postinjection' blood samples represent plasma conditions when rats re-initiate drinking behavior, following drinking to satiation and a salt injection, for successive days of the deprivation period.

The combined results of these three experiments define the blood plasma conditions at three points of adaptation to 23.5 hr. water deprivation. They indicate plasma

Figure 11

Mean plasma osmolality obtained predrink, postdrink, and postinjection at the initiation of drinking behavior, plotted as a function of days of adaptation to 23.5 hr. water deprivation. Standard error indicated by flags. 'Predrink' data from Hatton and Dittrich (1967), N=36. 'Postdrink' data from Hatton and Almli (1967b), N=36. 'Postinjection' data from the present experiment, N=35.

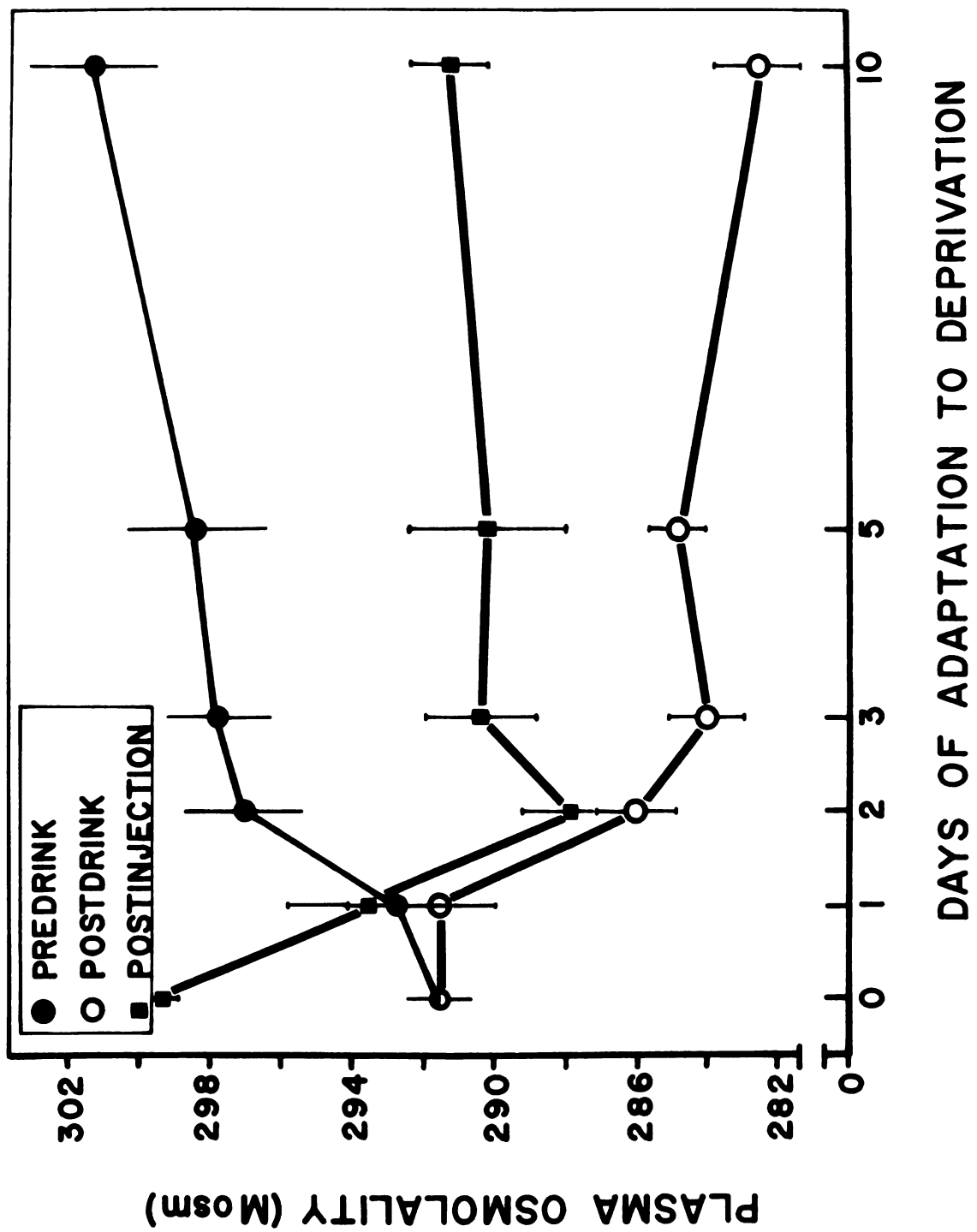
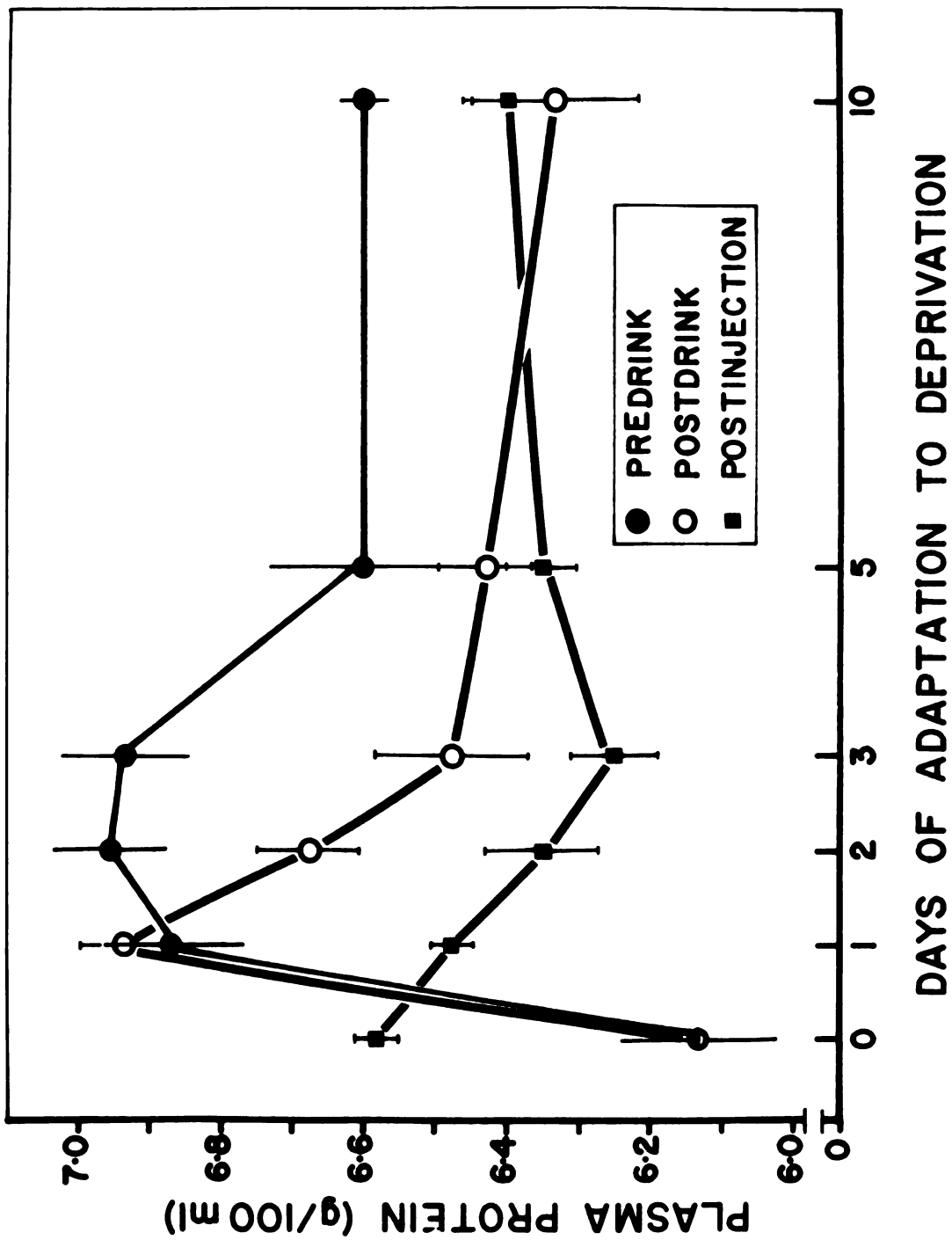

PREDRINK
POSTDRINK
POSTINJECTION
302
298
294
290
286
282
0
1
2
3
5
10
PLASMA OSMOLALITY (Mosm)
DAYS OF ADAPTATION TO DEPRIVATION

Figure 12

Mean plasma protein concentration obtained predrink, postdrink, and postinjection at the initiation of drinking behavior, plotted as a function of days of adaptation to 23.5 hr. water deprivation. Standard error indicated by flags.
'Predrink' data from Hatton and Dittrich (1967), N=36.
'Postdrink' data from Hatton and Almli (1967b), N=36.
'Postinjection' data from the present experiment, N=35.


PLASMA PROTEIN (g/100 ml)
7·0
6·8
6·6
6·4
6·2
6·0
0
1
2
3
5
10
DAYS OF ADAPTATION TO DEPRIVATION
PREDRINK
POSTDRINK
POSTINJECTION

conditions before drinking, after drinking, and when drinking is re-initiated following salt injection, for Days 0, 1, 2, 3, 5, and 10, of adaptation to 23.5 hr. water deprivation.

As can be seen from a comparison of Figures 11 and 12, plasma osmolality reflects a distinct separation of the three conditions (i.e., predrink, postdrink, and postinjection) from the third day of deprivation onward. However, this is not the case for plasma protein concentration. Due to the relative independence of the two variables (Figure 9), they will be discussed separately.

The threshold relationship between plasma osmolality following the drinking period (postdrink) and plasma osmolality at the re-initiation of drinking behavior (postinjection), becomes clearer if Verney's (1947) estimation of 1-2% increase in plasma osmolality being sufficient to trigger central detector cells, is applied to the data of the present study.

The percentage increase in plasma osmolality from 'postdrink' to plasma osmolality at the re-initiation of drinking behavior are presented in Table 3. As can be seen, these percentage increases are within the range, or slightly higher than Verney's estimation.

Of specific interest are the percentage increases in plasma osmolality on Days 0, 3, 5, and 10, which are 2.75, 2.02, 1.90, and 3.08%, respectively. While on ad lib conditions, and by the third day of deprivation, the rats drink sufficient amounts of water and regulate food intake such

Table 3

Mean threshold values of plasma osmolality at the postinjection initiation of drinking behavior, and mean latencies to drink for 'postinjection' rats, as a function of days of adaptation to 23.5 hr. water deprivation.*

	Day of adaptation to deprivation					
	0	1	2	3	5	10
Mean increase in mOsm/l. ± std. error, from 'postdrink' to 'postinjection'.	8.0 ±0.6	2.0 ±2.4	1.8 ±1.4	6.0 ±1.6	5.4 ±2.2	8.7 ±1.1
% increase (mOsm) from 'postdrink' to 'postinjection'.	2.75	0.69	0.63	2.02	1.90	3.08
Mean latency to drink in min:sec ± std. error in sec.	6:25 36	5:49 17	6:15 46	6:28 17	5:13 20	4:54 15

*Note: Postdrink data obtained from Hatton and Almli (1967b).

that body weight increases are now seen (Experiment 1). On these days, the initiation of drinking behavior occurs when plasma osmolality increases approximately 2-3% from the 'postdrink' level.

The smaller percentage increases in plasma osmolality necessary to produce drinking on Days 1 and 2 are probably due to the fact that the rats are still adjusting to the deprivation. Water intake is not sufficient to maintain body weight (Experiment 1), or to decrease plasma osmolality to the levels found on the days later in the schedule. Thus, the osmotic stress imposed by the salt injection produces drinking with a smaller percentage increase from the 'postdrink' level. However, this phase is highly complex and requires further study.

As shown in Figure 12, plasma protein concentration does not reflect a 'threshold' for the initiation of drinking behavior as is indicated by plasma osmolality. If changes in plasma protein concentration are interpreted in terms of plasma volume, (e.g., increases in protein concentration indicating decrease in plasma volume, and vice versa) on Days 3, 5, and 10, plasma volume at the re-initiation of drinking (postinjection) and following the drinking period (postdrink) are relatively stable with no large differences separating the two conditions. In terms of plasma osmolality on these same days, however, the two conditions are distinctly separated. Here, there is a 2-3% increase in osmolality from the postdrink condition to the re-initiation of drinking behavior.

In contrast, plasma volume at the re-initiation of drinking on Days 1 and 2 has increased considerably from the plasma volume following the drinking period. But, the plasma osmolality threshold for these two days represents an increase of less than 1% over the 'postdrink' plasma osmolality.

The ad lib rat, Day 0, does not fit into either of the schemes presented above. At the re-initiation of drinking there is a rather large decrease in plasma volume, with a threshold increase in plasma osmolality of 2.75% over the 'postdrink' condition.

Thus, it seems that during adaptation to water deprivation there is a process changeover at about the third day of the schedule in regard to the re-initiation of drinking following hypertonic injection. Early in the schedule (Day 1 and 2), there are large changes in plasma volume with small changes in plasma osmolality between the 'postdrink' condition and the re-initiation of drinking behavior. Later, (Days 3, 5, and 10), the situation is reversed. Small changes in plasma volume are associated with large changes in plasma osmolality between the 'postdrink' condition and the re-initiation of drinking behavior.

This seems to indicate that during the early days of adaptation to deprivation, especially Day 1, the rat is not drinking enough water to replenish both the cells and the plasma. When the rat is then injected with hypertonic saline, water is drawn from the cells into the plasma thereby increasing plasma volume. Here, it seems likely that the

re-initiation of drinking is triggered by dehydration at the cellular level, rather than by changes in plasma osmolality. This interpretation is based on the fact that Day 1 plasma osmolality 'postdrink' and 'postinjection' are not different from plasma osmolality when on ad lib conditions.

By Day 3 and beyond, rats drink sufficient amounts of water to rehydrate both the cells and the plasma. At the re-initiation of drinking, plasma volume is not different from plasma volume following the drinking period. Thus, it seems that the injection procedure did not change plasma volume, whereas plasma osmolality at the re-initiation of drinking reflects a distinct threshold, a 2-3% increase in plasma osmolality over the 'postdrink' condition.

The ad lib rat (Day 0) is similar to the rats with more than three days of experience on the deprivation schedule. Although plasma volume at the re-initiation of drinking is decreased from the 'postdrink' condition, it is not different from the plasma volume at the re-initiation of drinking on Day 10. So here again, the threshold increase in plasma osmolality of 2-3% is indicated at the re-initiation of drinking behavior.

In sum, blood conditions at the re-initiation of drinking behavior, following saline injection, seem to be dependent on the total degree of hydration experienced by the rat, i.e., at the plasma and cellular level. During the early days on the schedule, when drinking is not sufficient to bring the degree of hydration up to some required level,

the rats seem to be responding to cellular dehydration rather than elevations in plasma osmolality per se. Later in the schedule, sufficient amounts of water are ingested, and the re-initiation of drinking following injection, seems to be in response to changes in plasma osmolality. Thus, plasma osmolality seems to be a critical variable for the detection of states of dehydration under the conditions of this experiment. When satiated rats were injected with a hypertonic saline solution on Days 0, 3, 5, and 10, an increase in plasma osmolality of approximately 2-3% was sufficient to instigate corrective behavior in the form of drinking. This result corresponds with Verney's (1947) estimation of a 1-2% increase in plasma concentration being sufficient to trigger central detector cells.

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APPENDIX A

Apparatus

Description of the drinking boxes

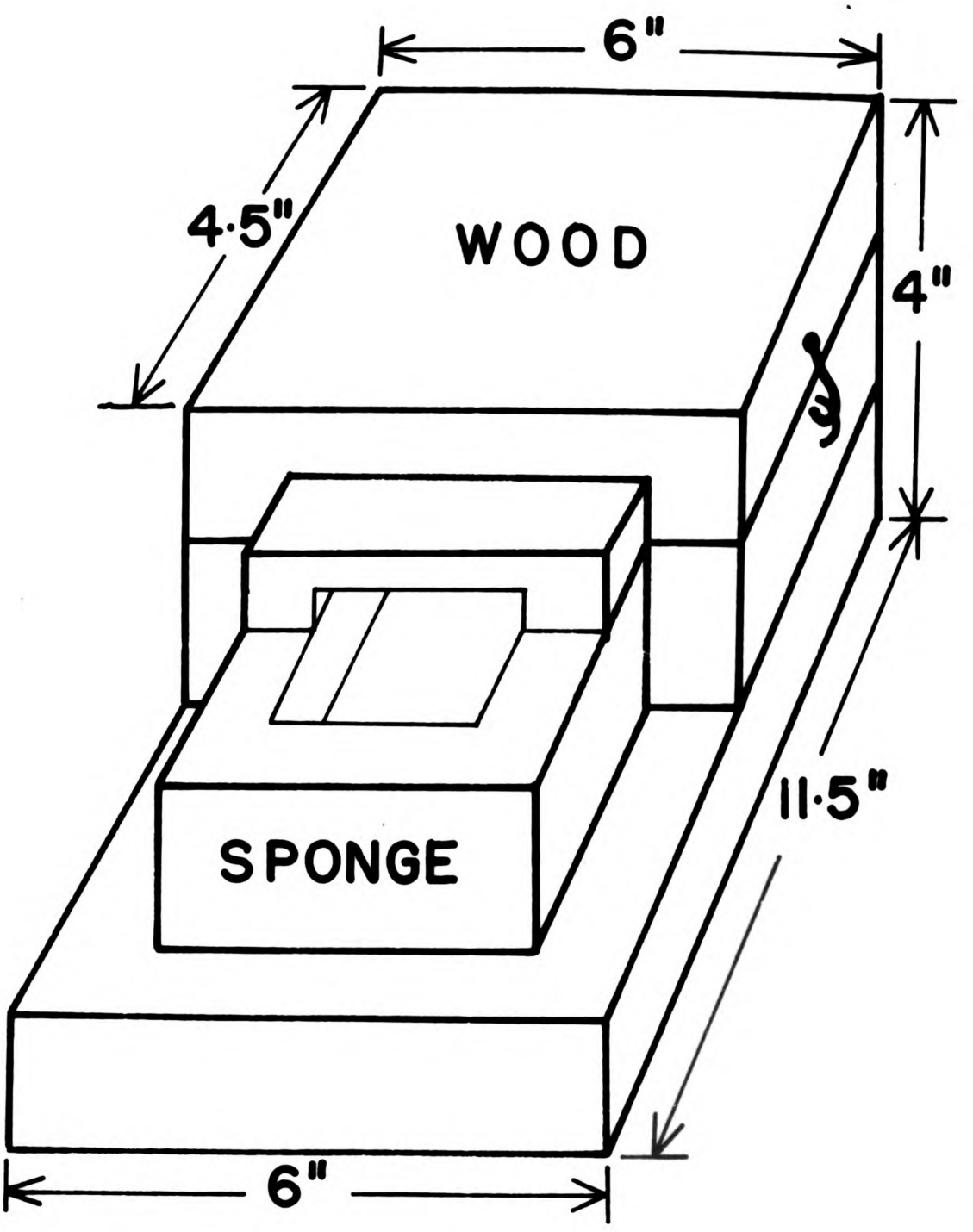
The drinking box was made up of six individual drinking compartments. Six glass gas collecting tubes were attached to the drinking box, the tubes were 27 in. long and were calibrated in 0.2 ml. The drinking compartments were 1 3/4 in. above a layer of corn-cob chips. The bottom of each drinking compartment consisted of 1/2 in. hardware cloth. Each drinking compartment was 11 3/4 in. long, 5 1/2 in. wide, and 7 3/4 in. deep. The drinking spout extended into the compartment 1 in., through a hole that was 2 1/2 in. from the bottom and 2 1/4 in. from the sides of the compartment. The metal drinking spout was coupled to the gas collecting tube by rubber tubing. Each compartment was equiped with an individual Plexiglas cover, which was hinged at one end and had magnetic locks on the other end in which to secure the compartment.

Description of the carrying-cage

The carrying-cage was made of wood with hardware cloth backing and fiber board doors in front. The carrying-cage consisted of six individual compartments which were 9 in. long, 4 1/8 in. high, and 6 1/4 in. deep. One whole side of the carrying-cage was 1/2 in. hardware cloth, the opposite side was made up of six individual sliding fiber board doors. The doors were equiped with magnetic locks. The overall dimensions of the carrying-cage were 19 in. long, 14 in. high, and 6 1/2 in. deep. A drawer-pull was attached to the top for a handle.

Description of the animal restrainer

The animal restrainer consisted of two wood sections which were hinged together. The base was made of wood and was 11 in. long, 6 in. wide, and 2 in. high. The top section was shorter than the bottom section; 4 1/2 in. long, 6 in. wide, and 2 in. high. Thick sponges were glued to the bottom of the top section and to the top of the bottom section. The rat was placed on the bottom section and the top section was pulled down over the rat and secured to the bottom section by a hook. The hind quarters of the rat were exposed, which permitted access to the rat for subcutaneous injection. A diagram of the restrainer is on the following page.



ANIMAL RESTRAINER

APPENDIX B

Procedure

Description of the heart puncture procedure

Each rat, on his treatment day, was taken to an adjoining room in a carrying-cage immediately following the drinking period. The rat was etherized in a battery jar, which had wood shavings in the bottom and a hardware cloth platform. When the rat was sufficiently immobilized, it was removed from the ether jar and placed on its stomach on a table. The rat was then injected in the hind quarters area and a stop-watch was started at the time of injection. The degree of anesthesia was regulated by means of an ether-filled nose cone.

Following the injection the rat was turned on its back. It took approximately 30 sec. to do the heart puncture and draw the blood, therefore, the heart puncture was started 15 sec. before the rat's mean latency to drink. The location of the heart on the rat's left side was determined by touch, the heart was punctured, and approximately 1 1/2 cc. of blood was drawn. At the completion of the heart puncture and drawing of the blood, the whole blood was carefully injected into a centrifuge tube and centrifuged for about 3 min. During this time the rat was returned to the home cage.

When the blood was sufficiently spun down, the plasma was drawn off. A small drop was analyzed for protein concentration in a refractometer. The remaining plasma was put into 6 dram vials, which were sealed and immediately frozen. These samples were later analyzed for osmolality

in a freezing-point osmometer.

The blood samples were drawn in heparinized syringes, the heparin was drawn into the syringe until the barrel was coated and then it was squirted out. The Panheprin (1000 U.S.P. units/ml.) was manufactured by Abbott Laboratories; Chicago, Ill.

The blood samples were spun down for approximately 3 min. in a centrifuge (Model CL) manufactured by International Equipment Co.; Needham Heights, Mass.

Protein concentration (grams/100 ml.) was determined in a Total Solids Meter (American Optical Co., Model 10401), which measures the refractive index of solutions. The value is read where the sharp boundary between the dark and light fields crosses the scale. The prism was cleaned with distilled water, and dried, after each determination.

Osmolality (mOsm) was determined in a freezing-point osmometer (manufactured by Precision Systems; Framingham, Mass., under the brand name of Osmette). Each sample was run through two determinations and the mean was computed as the sample value. If the first two determinations were more than 5 milliosmols apart, a third determination was performed and the mean computed on the three determinations.

APPENDIX C

Raw Data

Experiment I

Water intake (in ml.) on Days 1, 2, 3, and 10, of 23.5 hr. water deprivation, during adaptation periods I, II, and III. N=11.

	ADAPTATION PERIODS											
	I				II				III			
S No.	Day 1	Day 2	Day 3	Day 10	Day 1	Day 2	Day 3	Day 10	Day 1	Day 2	Day 3	Day 10
1	14.2	18.0	19.4	21.0	15.4	18.0	21.8	19.8	15.4	19.8	20.8	21.0
2	10.6	12.4	16.0	18.0	14.4	16.0	17.6	18.0	14.6	16.8	16.0	20.0
3	12.0	15.2	15.6	18.6	12.8	17.6	17.0	17.4	14.6	15.0	16.2	14.2
4	11.4	15.8	16.8	20.8	13.6	21.6	22.6	17.4	18.8	22.4	21.0	19.0
5	11.4	16.6	18.4	18.2	15.2	19.8	19.2	20.0	15.0	16.8	21.8	21.2
6	13.4	13.7	18.8	19.8	13.8	17.8	21.4	20.2	15.4	21.0	19.0	21.8
7	12.6	19.6	20.6	21.0	20.6	23.2	23.0	21.2	18.6	20.2	19.4	20.6
8	10.4	16.1	17.2	19.2	15.2	17.0	18.6	21.8	14.4	17.8	18.8	16.8
9	15.0	21.0	19.8	23.0	18.6	20.6	23.6	23.6	20.0	19.2	21.8	19.2
10	9.8	13.4	15.4	20.6	15.4	16.0	20.8	17.0	15.4	16.2	18.4	18.4
11	12.2	19.6	20.0	26.8	23.2	22.6	25.8	25.6	21.0	22.4	26.8	24.0

Experiment I

Latency to drink and reciprocal latency to drink (in sec.) on Days 1 and 10 of adaptation to 23.5 hr. water deprivation, for adaptation periods I, II, and III. N=11.

	ADAPTATION PERIODS											
	I				II				III			
	Day 1		Day 10		Day 1		Day 10		Day 1		Day 10	
S No.	Lat	1/Lat	Lat	1/Lat	Lat	1/Lat	Lat	1/Lat	Lat	1/Lat	Lat	1/Lat
1	350.61	.002	1.82	.549	1.84	.543	.70	1.428	.72	1.388	.72	1.388
2	117.11	.008	1.74	.574	2.40	.416	.71	1.408	.99	1.010	.63	1.587
3	111.65	.008	4.42	.226	.70	1.428	.72	1.388	.72	1.388	.81	1.234
4	352.35	.002	1.93	.518	.99	1.010	.72	1.388	.89	1.123	.70	1.428
5	27.45	.036	1.74	.574	1.00	1.000	.75	1.333	1.02	.980	.73	1.369
6	167.39	.005	2.61	.383	.97	1.030	.81	1.234	.98	1.020	.68	1.470
7	64.72	.015	2.91	.343	5.82	.171	.99	1.010	.92	1.086	1.36	.735
8	75.15	.013	3.41	.293	.94	1.063	.84	1.190	.91	1.098	.97	1.030
9	10.12	.098	1.76	.568	.97	1.030	.72	1.388	4.11	.243	.93	1.075
10	150.20	.006	1.92	.520	2.54	.393	.71	1.408	5.02	.199	.92	1.086
11	65.27	.015	2.58	.387	7.75	.129	.98	1.020	.98	1.020	.63	1.587

Experiment I

Body weight (in grams) on Days 0, 1, 2, 3, and 10, of adaptation to 23.5 hr. water deprivation, for adaptation periods I, II, and III. N=11.

	ADAPTATION PERIODS														
	I					II					III				
S No.	Day 0	Day 1	Day 2	Day 3	Day 10	Day 0	Day 1	Day 2	Day 3	Day 10	Day 0	Day 1	Day 2	Day 3	Day 10
1	367	351	347	348	365	415	390	385	386	395	434	413	406	414	421
2	328	314	316	305	317	378	356	350	352	361	402	380	375	374	390
3	358	348	344	342	353	404	383	378	382	387	414	396	393	393	400
4	340	327	320	319	331	379	359	352	357	368	411	382	382	387	399
5	358	346	340	339	355	408	381	375	378	383	430	405	395	395	409
6	379	360	352	351	379	435	417	397	398	411	461	436	428	434	442
7	368	343	339	345	366	409	384	383	390	396	432	408	404	405	419
8	363	347	341	342	357	410	382	378	379	388	433	404	399	401	409
9	364	345	341	343	363	405	379	375	379	397	429	409	410	412	417
10	359	342	335	333	354	410	374	372	372	376	418	396	389	392	402
11	378	356	353	357	384	430	400	399	402	415	461	433	433	438	457

Experiment II

Postinjection latency to drink, in min. and sec. and reciprocals (in sec.), for injection 1 and injection 2, for days of adaptation to 23.5 hr. water deprivation. (16% sodium chloride).

		Lat. (Min:Sec)			$\frac{1}{\text{Lat.}}$ (in sec.)		
Day Group	S No.	Inj. I	Inj. II	$\bar{X}$	Inj. I	Inj. II	$\bar{X}$
Day 0:	1	4:27	5:19	4:53	.0037	.0031	.0034
	2	7:36	6:38	7:07	.0021	.0025	.0023
	3	6:23	5:57	6:10	.0026	.0027	.0027
	4	10:16	10:05	10:10	.0016	.0016	.0016
	5	4:20	3:52	4:06	.0038	.0042	.0040
	6	6:21	6:02	6:11	.0026	.0027	.0027
Day 1:	1	4:54	5:07	5:00	.0033	.0032	.0033
	2	7:39	6:59	7:19	.0021	.0023	.0022
	3	6:01	6:19	6:10	.0027	.0026	.0027
	4	6:10	6:31	6:20	.0027	.0025	.0026
	5	5:18	5:44	5:31	.0031	.0028	.0030
	6	4:22	5:00	4:41	.0038	.0033	.0036
Day 2:	1	2:51	5:27	4:09	.0058	.0032	.0045
	2	3:28	4:14	3:51	.0048	.0039	.0044
	3	7:07	6:31	6:49	.0023	.0025	.0024
	4	6:38	5:56	6:17	.0025	.0028	.0027
	5	5:08	5:18	5:13	.0032	.0031	.0032
	6	11:26	11:06	11:16	.0014	.0015	.0015
Day 3:	1	5:25	6:22	5:53	.0030	.0026	.0028
	2	6:01	5:21	5:41	.0027	.0031	.0029
	3	7:04	6:41	6:52	.0023	.0024	.0024
	4	5:50	6:49	6:19	.0028	.0024	.0026
	5	7:35	8:41	8:08	.0021	.0019	.0020
	6	5:28	6:25	5:56	.0030	.0025	.0028
Day 5:	1	----	----	----	-----	-----	-----
	2	5:41	6:20	6:00	.0029	.0026	.0028
	3	6:07	6:58	6:32	.0027	.0023	.0025
	4	5:28	4:54	5:11	.0030	.0034	.0032
	5	3:41	3:57	3:49	.0045	.0042	.0044
	6	4:20	5:01	4:40	.0038	.0033	.0036
Day 10:	1	4:19	4:49	4:34	.0038	.0034	.0036
	2	4:39	5:26	5:02	.0035	.0030	.0033
	3	3:00	4:58	3:59	.0055	.0033	.0044
	4	6:29	5:45	6:07	.0025	.0028	.0027
	5	4:49	4:31	4:40	.0034	.0036	.0035
	6	4:43	5:37	5:10	.0035	.0029	.0032

Experiment II

Plasma osmolality and protein concentration at the postinjection initiation of drinking behavior, for successive days of adaptation to 23.5 hr. water deprivation. N=35.

Day Group:	S No.	Mean Postinjection lat. to drink Min:Sec	Protein (g/100 ml.)	Osmolality (mOsm.)
Day 0:	1	4:53	6.5	299.3
	2	7:07	6.6	300.0
	3	6:10	6.6	302.0
	4	10:10	6.5	299.5
	5	4:06	6.6	298.5
	6	6:11	6.7	298.0
Day 1:	1	5:00	6.6	286.7
	2	7:19	6.4	291.5
	3	6:10	6.4	290.5
	4	6:20	6.4	301.5
	5	5:31	6.5	299.0
	6	4:41	6.5	291.0
Day 2:	1	4:09	6.4	286.5
	2	3:51	6.6	289.0
	3	6:49	6.3	283.5
	4	6:17	6.5	286.0
	5	5:13	6.2	287.5
	6	11:16	6.1	293.5
Day 3:	1	5:53	6.1	286.5
	2	5:41	6.4	286.5
	3	6:52	6.3	296.0
	4	6:19	6.4	291.0
	5	8:08	6.2	291.0
	6	5:56	6.1	291.5
Day 5:	1	----	---	-----
	2	6:00	6.3	288.5
	3	6:32	6.4	291.5
	4	5:11	6.4	296.5
	5	3:49	6.2	283.0
	6	4:40	6.5	291.5
Day 10:	1	4:34	6.5	293.5
	2	5:02	6.5	292.5
	3	3:59	6.6	286.0
	4	6:07	6.3	292.5
	5	4:40	6.2	290.7
	6	5:10	6.3	292.3

Experiment II

Water intake (in ml.) for successive days of adaptation to 23.5 hr. water deprivation during Adaptation IV. N=35.

	TREATMENT DAYS					
S No.	0	1	2	3	5	10
1	4.2	14.2	17.2	20.2	----	21.2
2	2.8	14.6	17.4	18.4	20.6	19.4
3	3.6	17.8	15.0	17.6	16.6	22.8
4	4.8	15.8	17.8	20.6	18.8	15.4
5	1.2	15.2	18.0	19.2	24.8	24.4
6	3.8	16.8	22.2	19.6	19.2	18.8

Experiment II

Latency and reciprocal latency to drink (in sec.), for successive days of adaptation to 23.5 hr. water deprivation during Adaptation IV. N=35.

	TREATMENT DAYS									
	1		2		3		5		10	
S No.	Lat	$\frac{1}{Lat}$	Lat	$\frac{1}{Lat}$	Lat	$\frac{1}{Lat}$	Lat	$\frac{1}{Lat}$	Lat	$\frac{1}{Lat}$
1	0.93	1.075	0.86	1.153	0.93	1.075	----	-----	0.98	1.020
2	1.02	0.980	1.07	0.934	0.87	1.149	1.07	0.934	0.74	1.351
3	0.59	1.694	1.00	1.000	0.72	1.388	0.98	1.020	0.58	1.724
4	0.83	1.204	0.78	1.282	0.93	1.075	0.97	1.030	0.58	1.724
5	1.06	0.944	0.74	1.351	1.08	0.925	0.62	1.612	0.64	1.562
6	0.98	1.020	0.98	1.20	0.83	1.204	0.96	1.041	0.92	1.086

Experiment II

Body weight (in grams), for successive days of adaptation to 23.5 hr. water deprivation during Adaptation IV. N=35.

	TREATMENT DAYS					
S No.	0	1	2	3	5	10
1	469	431	433	426	---	462
2	486	434	428	458	441	452
3	478	452	439	453	421	425
4	465	450	462	454	464	455
5	446	438	421	424	477	444
6	450	465	472	452	478	431