WATER ABSORPTION AND CHANGES IN PLASMA OSMOTIC PRESSURE AS DETERMINANTS OF THE SATIATION OF THIRST

Thesis for the Degree of M. A.
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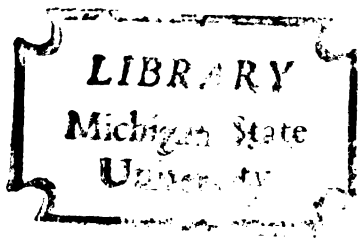
CHARLES THOMAS BENNETT
1969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ABSTRACT

WATER ABSORPTION AND CHANGES IN PLASMA OSMOTIC PRESSURE AS DETERMINANTS OF THE SATIATION THIRST

by

Charles Thomas Bennett

Historically, the cessation of drinking was thought to be brought about primarily by stomach distension. It was believed that absorption of water into the blood from the gut was too slow to effect humoral changes by the time an animal stopped drinking. It was the purpose of this thesis to measure the amount of water absorbed and the changes in plasma osmotic pressure that actually occurred by the time an animal stopped drinking.

It was necessary to first establish a criterion of the satiation of drinking. In Experiment I of this thesis, 12 albino rats were placed on a 23.5 hr water deprivation schedule for 10 days. After their intake rates during the 0.5 hr access to water were monitored, the following behavioral definition of satiety was offered: A rat was considered to have stopped drinking when its intake rate was equal to, or less than, 0.2 ml/min for three minutes.

In Experiment II, water absorption from the gut into the blood and changes in plasma osmotic pressure when animals reached the satiety criterion were measured. To monitor these changes, 78 albino rats were divided into Predrink groups, rats which had no access to water on a given day of

deprivation; Stopdrink groups, animals which were permitted to drink to the satiety criterion; and, Postdrink groups, animals which were permitted to drink for 0.5 hr. The amount of water absorbed into the blood from the small intestine and plasma osmolality were measured on Day 0, 1, 2, 5, and 10 of a 23.5 hr water deprivation schedule. It was found that (a) by the time rats stop drinking, approximately 4.5 ml of water had been absorbed, and (b) that their elevated plasma osmotic pressure had lowered to approximately ad libitum levels. The coincidence of the reduction in plasma osmotic pressure to ad libitum levels and the cessation of drinking support a cellular rehydration explanation of satiety.

Approved: Glenn I. Hatton Date: 11-19-69

Glenn I. Hatton, Chairman
John I. Johnson
Lawrence I. O'Kelly

WATER ABSORPTION AND CHANGES IN PLASMA OSMOTIC PRESSURE AS DETERMINANTS OF THE SATIATION OF THIRST

By

Charles Thomas Bennett

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But, especially, I would like to thank Dr. G.I. Hatton who patiently endured while I learned some of the fundamentals of water regulation.

I want to also thank C.R. Almli, who as a senior graduate student, helped clarify the goals of this research.

And, lastly, I wish to thank my wife, Diane, who endured these last few months better than I.

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GENERAL INTRODUCTION

As recently as 1967, the satiation of thirst has been explained in terms of an "early" and a "permanent" component (Adolph, 1967; Holmes, 1967). The "early" component, mediated by gastric and oral factors, was considered to effect the actual cessation of drinking; whereas, the "permanent" component, mediated by humoral factors, effected the cessation of thirst.

Oral and gastric factors have been most thoroughly studied. It was assumed by Cannon (1934) that the degree of dryness, or wetness, of the mouth and throat determined whether or not an animal would drink.

In 1961, Gregerson and Cizek concluded, after reviewing studies comparing thirst and salivation, that a decrease in salivation was a concomitant of thirst. However, Bellows and Van Wagenen (1939) reported that dogs drank normal amounts of water after denervation of the mouth and throat. And, in 1965, Vance reported that desalivated rats drank normal amounts of water when fed hydrated food. Apparently, then, dryness or wetness of the mouth is not a primary determinant of the cessation of thirst.

Gastric factors (primarily stomach distention) are not believed to be a primary determiner of satiety (Adolph, 1967; Holmes, 1967). However, they have been demonstrated to at least modulate the rate of ingestion. Montgomery and

Holmes (1955) inflated balloons in the stomachs of dogs, which received hypertonic saline injections. These animals did not drink for 20-40 minutes, after which time they would consume normal amounts of water, albeit over longer periods of time. On the other hand, after denervation of the stomach, by either vagotomy or total sympathectomy (Holmes and Gregersen, 1950; Towbin, 1955), dogs consumed normal amounts of water.

Since animals could adequately regulate their fluid balance without oral or gastric cues, it was believed that humoral factors were the ultimate determiner of satiety (Adolph, 1967; Holmes, 1967; and, Towbin, 1964). There are, however, two humoral factors, a volumetric and osmometric one, which could presumably effect satiety.

Volumetric factors might "quench" thirst as a result of an increase in blood volume resulting from the absorption of water. This increased blood volume would be detected by vascular stretch receptors which would "signal" the animal to stop drinking. In 1968, Corbit increased blood volume by intravenous injections of hypotonic solutions, while rats were drinking. Reportedly, these rats stopped drinking. However, other work by Corbit (1967, 1968) disputes the role volume might play. While rats were drinking, he injected serum and isotonic Ringer's solution intravenously. And, although he reportedly increased blood volume 20%, these rats continued to drink. Apparently, then, it is not merely

an increase in blood volume, *per se*, that will effect the cessation of thirst.

Hypothetically, osmometric factors would effect satiety as the result of absorbed water reducing the osmotic pressure of the surround of cells in the brain, which "signal" the satiation of thirst.

There is evidence to indicate that ionic concentration of body fluids do decrease, as a function of ingested water being absorbed into the vascular system. In 1934, Baldes and Smirk reported that after man ingested water, osmotic pressure of the blood decreased. In 1962, Novin reported decreases in electrical conductivity of the brain (indicating a decrease in ionic concentration), while rats were actually drinking. However, he believed that absorption of water was too slow, and that this decrease in ion concentration in the brain resulted from "...electrolytes moving into the gastrointestinal tract to maintain osmotic constancy" (p. 151). In 1969, Hatton and Almli suggested that humoral explanation of satiety was possible. They also inferred from their data that it was possible that a rat actually stopped drinking when its plasma osmolality reached approximately *ad libitum* levels: "...indeed, this is evident for Day 1 when rats stop drinking shortly before the 0.5 hr access period ends...," (p. 212) and, when their plasma osmolality had actually reached an *ad libitum* level.

It appears, then, that osmotic pressure does decrease following ingestion of water. However, as recently as 1967,

many believed, as did Adolph (1967), that water absorption was too slow to effect changes in body fluids by the time animals actually stopped drinking. But, as Holmes and Montgomery (1960) stated, "The mechanism by which this is accomplished or the time interval required need to be established"(p. 911). That is, the amount of water absorbed and the level of plasma osmotic pressure, when drinking ceases, need to be measured.

It was the purpose of this thesis, then, to examine more fully the osmometric component of satiety. However, it should be noted that a necessary condition for an osmometric explanation of satiety is that sufficient quantities of water must be absorbed in order to lower plasma osmotic pressure to ad libitum levels (or, at least some set point) by the time animals stop drinking.

Before this analysis could proceed, it was important to establish a reliable behavioral definition of satiety. This problem was the basis of Experiment I. Then, in Experiment II, changes in plasma conditions when rats reached the satiety criterion were measured.

EXPERIMENT I

Satiety is normally considered to have occurred when intake ceases. But, during its waking hours, an animal normally will drink for a short period, stop, drink again, stop, and so on. Given this, the question arises: How long does an animal have to stop drinking before satiety can reasonably be considered to have occurred?

However, apparently no reported studies have attempted to behavorially define satiety. It was the objective of this study, then, to establish a criterion by which satiety could be specified in rats.

Method

Subjects

Twelve naive, male, albino rats approximately 100 days old were housed in individual cages, under conditions of constant light. They were fed Wayne Mouse Breeder Blox and given water ad libitum for three days prior to the beginning of the experimental treatments.

Procedure

After being adapted to their home cages, the rats were placed on a 23.5 hr water deprivation schedule. During their 0.5 hr access period to water, they were placed in drinking boxes (Described in Appendix A) without food.

Beginning on Day 1 and continuing through Day 10 of deprivation, minute by minute water intake records were taken for the first twelve minutes of the period, and then

every three minutes thereafter. Immediately following the 0.5 hr access period, they were returned to their home cages.

Results

Figure 1 shows the mean amount of water drunk in three minute blocks, as they adapt to a water deprivation schedule. Figure 2 represents a cumulative plot of these data. Figure 3 shows the cumulative amount drunk as a function of the percent of total daily intake.

After these rats drank at least 80% of their total intake, their rate of intake dropped sharply. Prior to that point, their average rate of intake was 1.57 ml/min. In all cases, except on Day 1, this lowered intake rate was maintained for at least three consecutive minutes after 80% of a daily intake was reached.

Discussion

Ghent, (1957) indicated that rats on a deprivation schedule tend to spend more time at a water spout in the early part of an access period. And, it is apparent from these data that as rats adapt to a 23.5 hr water deprivation schedule that they tend to drink more and more water in the early portions of their access period.

Also, from these data a behavioral definition of satiety can be offered. This definition is based on the following two facts: a) coincident with a sharp drop in the rate of ingestion, the rats drank 80% of their total daily intake; b) except for Day 1, all rats reduced their intake

Figure 1

Mean amount of water drunk by 12 animals, in three minute blocks, as a function of days of adaptation to a 23.5 hr water deprivation schedule.

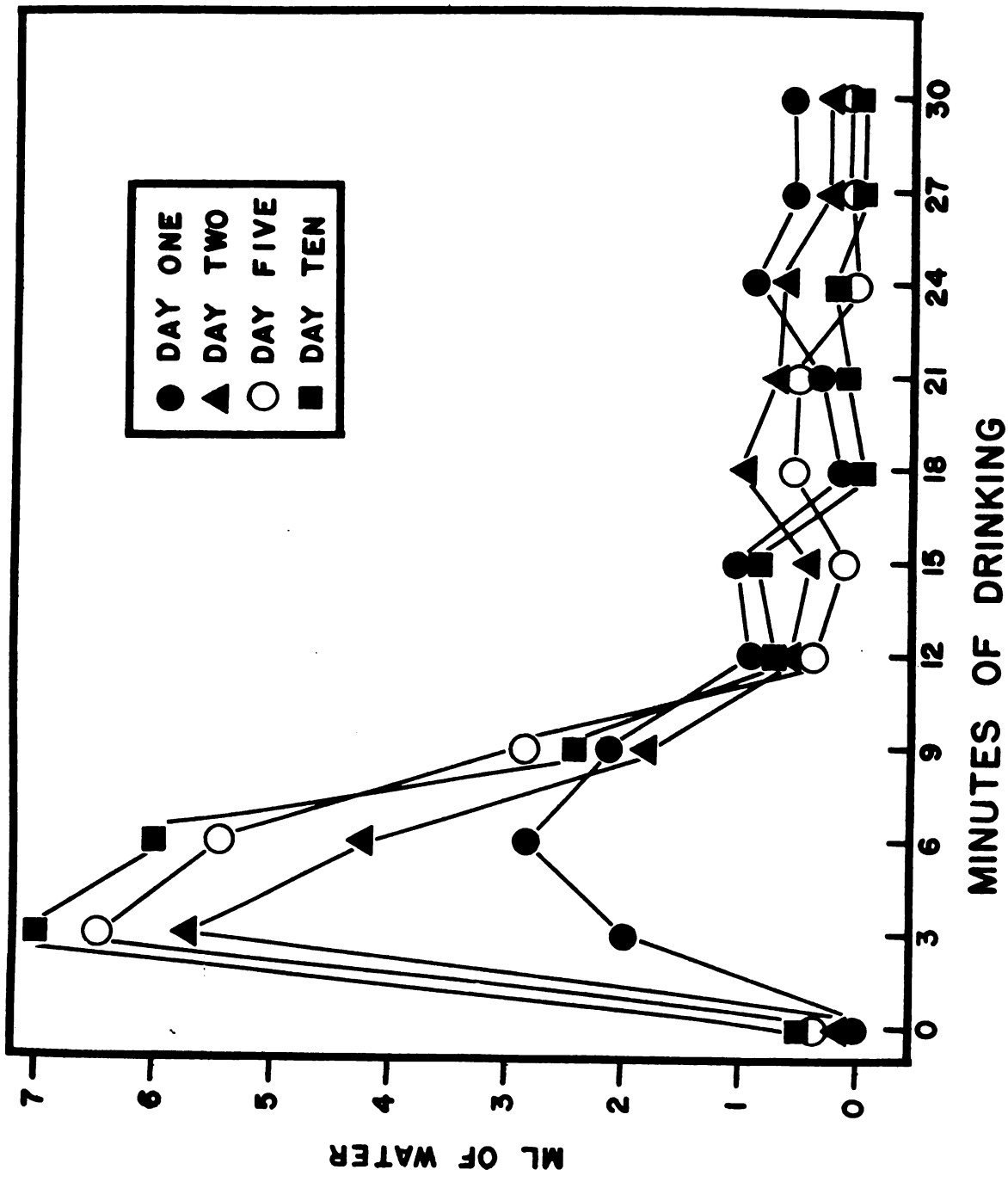

DAY ONE
DAY TWO
DAY FIVE
DAY TEN
ML OF WATER
0
1
2
3
4
5
6
7
0
3
6
9
12
15
18
21
24
27
30
MINUTES OF DRINKING

Figure 2

A cumulative plot of the mean amount of water drunk by 12 animals, in three minute blocks, as a function of days of adaptation to a 23.5 hr water deprivation schedule.

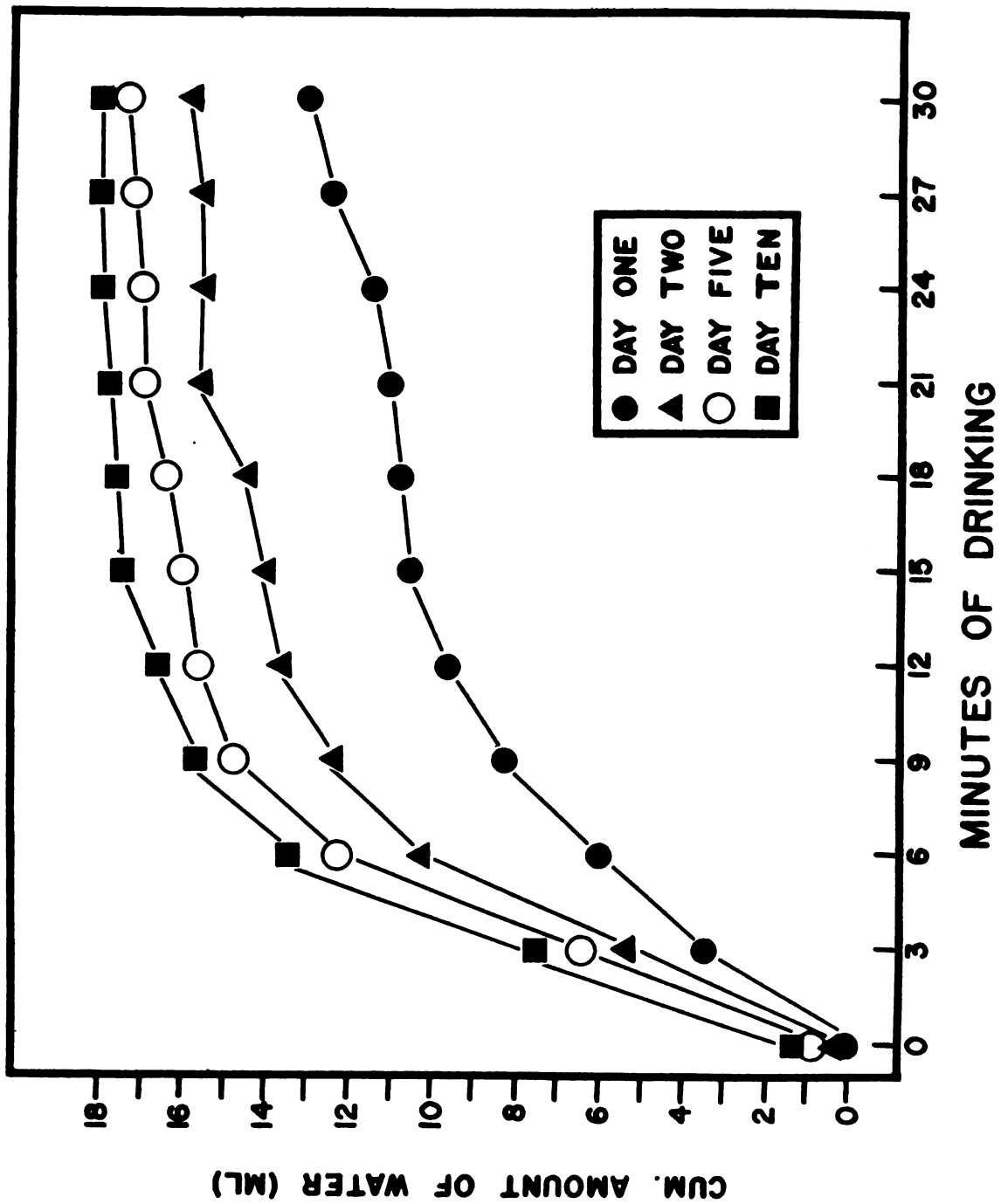
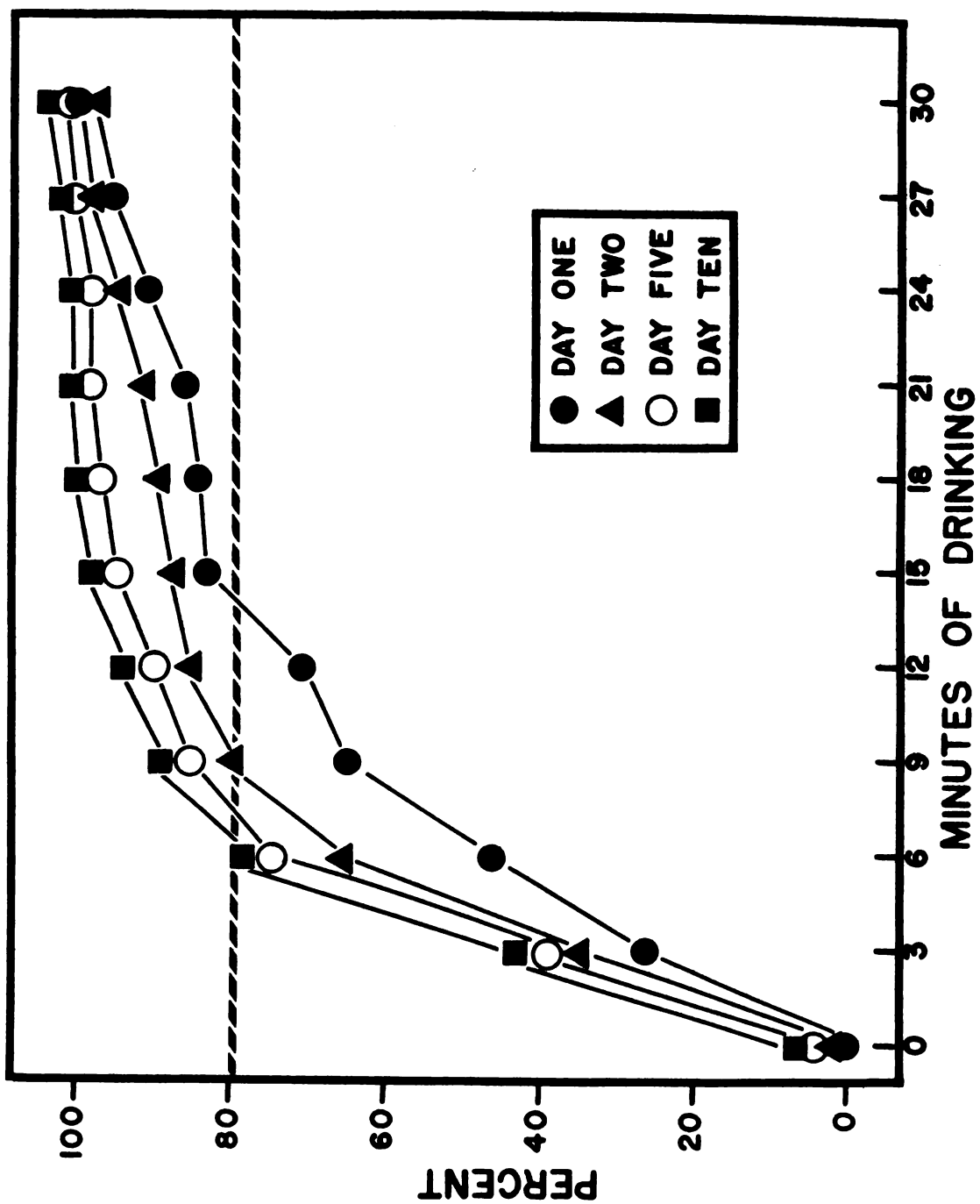

DAY ONE
DAY TWO
DAY FIVE
DAY TEN
0
3
6
9
12
15
18
21
24
27
30
MINUTES OF DRINKING
0
2
4
6
8
10
12
14
16
18
CUM. AMOUNT OF WATER (ML)

Figure 3

Cumulative amount drunk while 12 rats adapt to a 23.5 hr water deprivation schedule as a function of the percent of total daily intake.


DAY ONE
DAY TWO
DAY FIVE
DAY TEN
PERCENT
0
20
40
60
80
100
MINUTES OF DRINKING
0
3
6
9
12
15
18
21
24
27
30

rate to less than 0.2 ml/min for three consecutive minutes, after 80% of their intake for that day was ingested. This was true for only 50% of the animals on Day 1. Apparently, this resulted from the animals being differentially hydrated at the beginning of the deprivation schedule.

This significant behavioral change was the basis for the following definition of satiety. A rat is considered satiated when the intake rate is equal to, or less than, 0.2 ml/min for three consecutive minutes. In the discussion of Experiment II, the reliability of this criterion is supported.

EXPERIMENT II

Having established a satiety criterion in Experiment I, it was then possible to examine some of the physiological correlates of the cessation of thirst. In this study, then, measurements were made of amounts of water absorbed from the small intestine, plasma protein concentration, and plasma osmolality. This was done in order to determine the approximate changes in these variables at or near satiation.

Method

Subjects

The animals were 78 male, albino rats, 100-110 days old at the beginning of the deprivation conditions. They were housed under conditions of constant light in individual cages and allowed access to water and Wayne Mouse Breeder Blox *ad libitum*.

Procedure

Half of each group was treated at one time. The other half was treated a month later. This was done partly to lend greater credence to the reliability measures employed later.

Treatment groups. At the beginning of the experiment, six animals were randomly assigned to each of the following groups:

Day 0	Day 1	Day 2	Day 5	Day 10
	Predrink	Pre	Pre	Pre
Ad Libitum	Stopdrink	Stop	Stop	Stop
	Postdrink	Post	Post	Post

Day 0 represents ad libitum conditions. The other days represent days of adaptation to a 23.5 hr water deprivation schedule. Predrink groups refer to animals that have not had an opportunity to drink on a given day of deprivation. Stop-drink groups consist of animals that were tested when they reached the satiety criterion. Postdrink animals are those which have had their 0.5 hr access to water on a given day of deprivation. Day 0 rats were merely placed in the drinking box for 0.5 hr to insure that they were in fact sated ad libitum animals.

Treatment procedure. After allowing the rats to adapt to their home cages for three days, a 23.5 hr water deprivation schedule was initiated. The animals were permitted free access to food in their home cages. During the 0.5 hr access period, the groups placed on deprivation were put in a drinking box (described earlier). Their weights were recorded prior to each access period.

Day 0 (ad libitum) rats were placed in the drinking boxes for 0.5 hr after the adaptation period. At the end of their access to water, they were removed, the amount of water ingested recorded, and a 2 cc blood sample taken from their surgically exposed hearts.

After 23.5 hr of deprivation, a blood sample was taken from the exposed hearts of Day 1 Predrink animals. Their stomachs, small intestines, and caecums and colons (taken as one) were removed to determine a wet weight, and then dried at 100° C for 24 hr. An earlier pilot study had shown that

this time period insured that all fluids had evaporated.

The Day 1 Stopdrink group was placed in the drinking boxes. Minute by minute records were taken of their ingestion of water. When the satiety criterion was reached, they were taken out of the boxes and treated in the same manner as the Predrink group.

The Day 1 Postdrink group was put in the drinking boxes. At the end of the 0.5 hr access period, the amount of water ingested was recorded. They were then treated in the same manner as the Pre- and Stopdrink groups.

The rats in the Pre-, Stop-, and Postdrink groups for Day 2, 5, and 10 of deprivation were treated in the same way as the Day 1 groups.

Satiety criterion. This was an intake rate equal to or lower than 0.2 ml/min for three consecutive minutes.

Surgical procedure. The animals were deeply etherized. A midline incision was made from just above the penis to the sternum. The heart was then exposed and a 2 cc sample of blood withdrawn from the left ventricle. Hemostats were then positioned in the following places, and in this order (except for the Day 0, ad libitum, group): 1) small intestine, at the level of the duodenum; 2) esophagus, at the level of the antrum; 3) ileum, at the level of the caecum; and, 4) descending colon, at the level of the rectum.

The stomach, small intestine, and caecum and colon (taken as one) were removed and stripped of excess lipomal

and mysenteric tissue. A wet weight was determined for each of the organs. They were then individually dried in an oven at 100° C for 24 hr. After wet weights were determined, the blood sample was centrifuged, the plasma drawn off, and the protein concentration determined by a refractometer. The remaining plasma was frozen in sealed glass vials. The plasma osmolality was determined by freezing point osmometer, after the experiment. Because the rats were allowed to drink to criterion, 4 or 5 minutes elapsed between when the animal actually stopped drinking and when the blood was sampled.

Determination of gastrointestinal absorption of water. The amount of total fluid loss from each organ was determined by comparing dry and wet weights. The amount of water ingested that remained in each of the organs was determined by subtracting from an organ's total fluid loss the mean amount of fluid loss for the corresponding organ from the Predrink animals (on that day of deprivation).

The amount of water absorbed into the system was determined by subtracting the total amount of water remaining in the stomach, small intestine and large intestine of one rat from the amount it ingested. The corresponding formula would be:

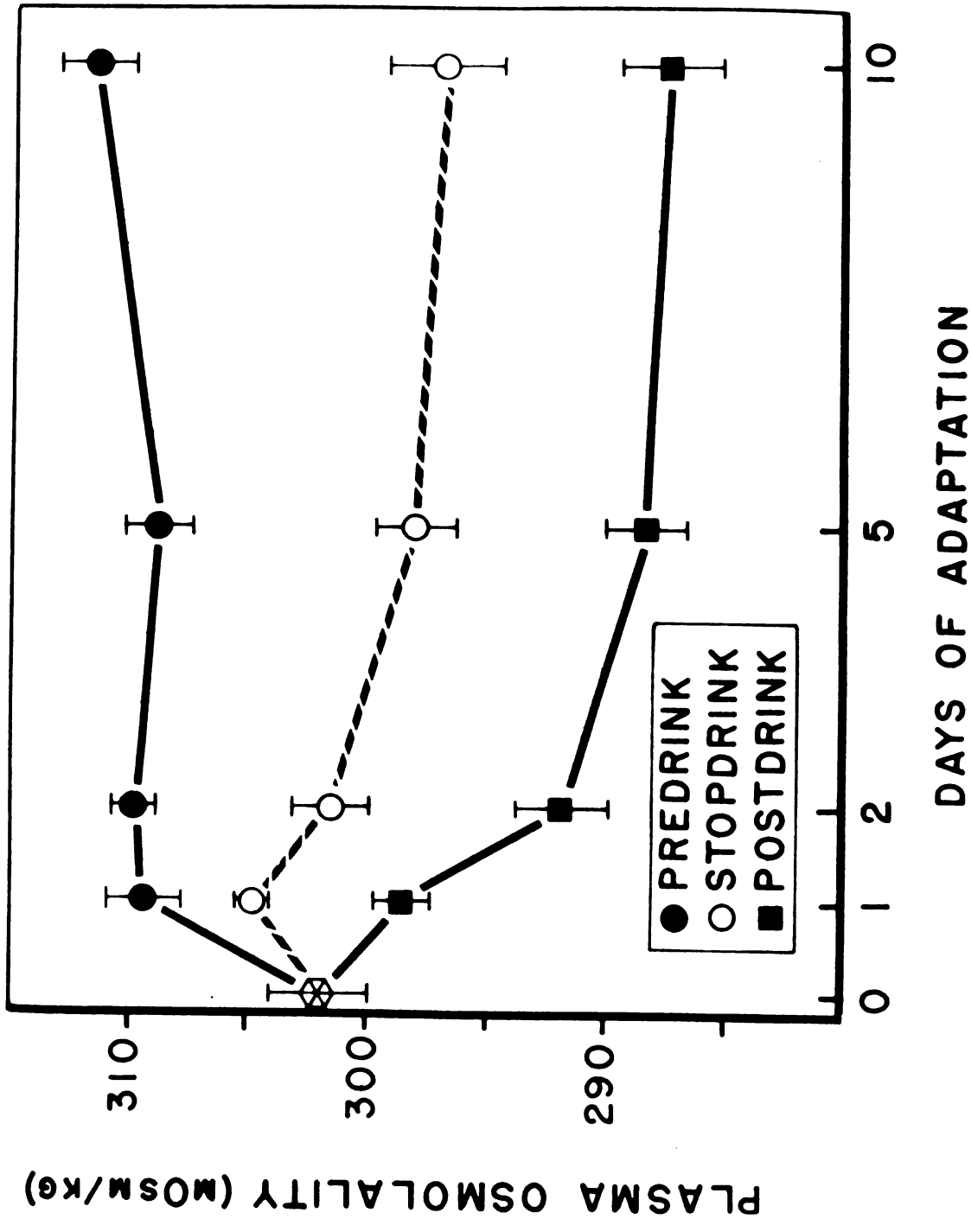
Total Water Absorbed = Intake - (Total Organ Water Loss-Mean Water Loss of Predrink Group Organs)

Results

Figure 4 shows the Pre-, Stop-, and Postdrink mean plasma osmolality as a function of adaptation to deprivation.

Figure 4

The Pre-, Stop-, and Postdrink mean plasma osmolality as a function of the days of adaptation to a 23.5 hr water deprivation schedule. Ad Libitum (starred hexagon), N=6; Predrink, N=24; Stopdrink, N=24; Postdrink, N=24. Standard errors are indicated by the flags.


PLASMA OSMOLALITY (MOSM/KG)
310
300
290
0
1
2
5
10
DAYS OF ADAPTATION
PREDRINK
STOPDRINK
POSTDRINK

A 3 x 4 factorial analysis of variance was computed on these conditions. Mean plasma osmolality differences were significant as a function of time of sampling, i.e., whether blood was sampled from a Pre-, Stop-, or Postdrink animal, ($F = 105.671$, $df = 2/60$, $p < 0.001$). Also significant were the differences among the days of adaptation to the deprivation schedule ($F = 7.002$, $df = 3/60$, $p < 0.001$). The magnitude of the mean differences among Pre-, Stop-, and Postdrink groups changed as a function of days of adaptation to the schedule; as indicated by a significant interaction effect ($F = 2.471$, $df = 6/60$, $p < 0.05$).

A 2 x 4 factorial analysis of variance was computed on the mean plasma osmolality of the Pre- and Stopdrink groups. While the mean plasma osmolality differences were significant as a function of time of sampling ($F = 64.872$, $df = 1/40$, $p < 0.01$), they did not differ significantly as a function of days of adaptation of the schedule ($F = 1.868$, $df = 3/40$, $p > 0.5$). However, though the mean plasma osmolality levels of these two groups remain fairly stable, the magnitude of the difference among the means change as a function of days of adaptation, as indicated by a significant interaction effect ($F = 3.216$, $df = 3/40$, $p < 0.025$).

To determine whether the Stopdrink mean plasma osmolality differed from that of the ad libitum group, as a function of days of adaptation, a one-way analysis of variance was computed on these groups. The analysis yielded $F = 2.677$, $df = 4/25$, $p > 0.05$.

During the adaptation to deprivation, then, Predrink plasma osmolality stabilizes at an elevated level, while Stopdrink plasma osmotic pressure remains around ad libitum levels. In contrast to this, Postdrink plasma osmolality tends to become lower over subsequent days of adaptation.

Table 1 shows the decreases in plasma osmolality in mOsm from Predrink levels to Stopdrink and Postdrink levels. Also represented in this table are the percent decreases in plasma osmotic pressure from Predrink levels to Stopdrink and Postdrink levels.

The mean amount of water ingested in ml/100 g of body weight is reported in Table 2, as a function of days of deprivation. One-way analyses of variance were computed on the means of the Stop- and Postdrink groups, across days of adaptation. The differences among the means of the Postdrink group were significant ($F = 8.143$, $df = 3/20$, $p<0.001$), as were the differences among those of the Stopdrink condition ($F = 0.966$, $df = 3/20$, $p<0.001$).

In Table 2, the mean time to reach the satiety criterion is also reported. It is interesting to note here, that although they had drunk different amounts when they stopped, the times at which they stopped did not differ significantly ($F = 1.194$, $df = 3/20$, $p>0.025$).

In Figure 5, the amounts of water absorbed in ml/100 g of body weight are also graphed. One-way analyses of variance computed on these groups, across days of adaptation, yielded $F = 0.135$, $df = 3/20$, $p>0.25$ for the Stopdrink treatment; and, $F = 3.324$, $df = 3/20$, $p<0.05$ for the Postdrink

Table 1

Mean decreases in plasma osmolality in mOsm from Predrink to Stopdrink and Postdrink levels. Also represented are the percent decreases in plasma osmotic pressure from Predrink levels to Stopdrink and Postdrink levels. Predrink, $N=24$; Stopdrink, $N=24$; and, Postdrink, $N=24$. Plus and minus one standard error is indicated.

Table 1

Actual decrease of plasma osmolality in mOsm.

	Day 1	Day 2	Day 5	Day 10
Pre to Stopdrink	4.2±0.8	8.5±1.8	11.3±1.6	15.2±2.6
Pre to Postdrink	10.5±1.3	18.2±1.9	20.7±1.9	23.7±2.3

Percent decrease of plasma osmolality.

	Day 1	Day 2	Day 5	Day 10
Pre to Stopdrink	1.4±0.3	2.6±0.6	3.7±0.5	4.9±0.8
Pre to Postdrink	3.4±0.4	5.9±0.7	6.7±0.6	7.6±0.7

Table 2

Mean amount of water ingested by the Stopdrink groups (N=24) and the Postdrink groups (N=24) as a function of days on a 23.5 hr water deprivation schedule. Plus and minus one standard error of the mean is indicated.

Mean time to reach the satiety criterion for the Stopdrink groups as a function of days on a 23.5 hr water deprivation schedule. Plus and minus one standard error of the mean is indicated. Stopdrink, N=24.

Table 2

Mean amount of water ingested.

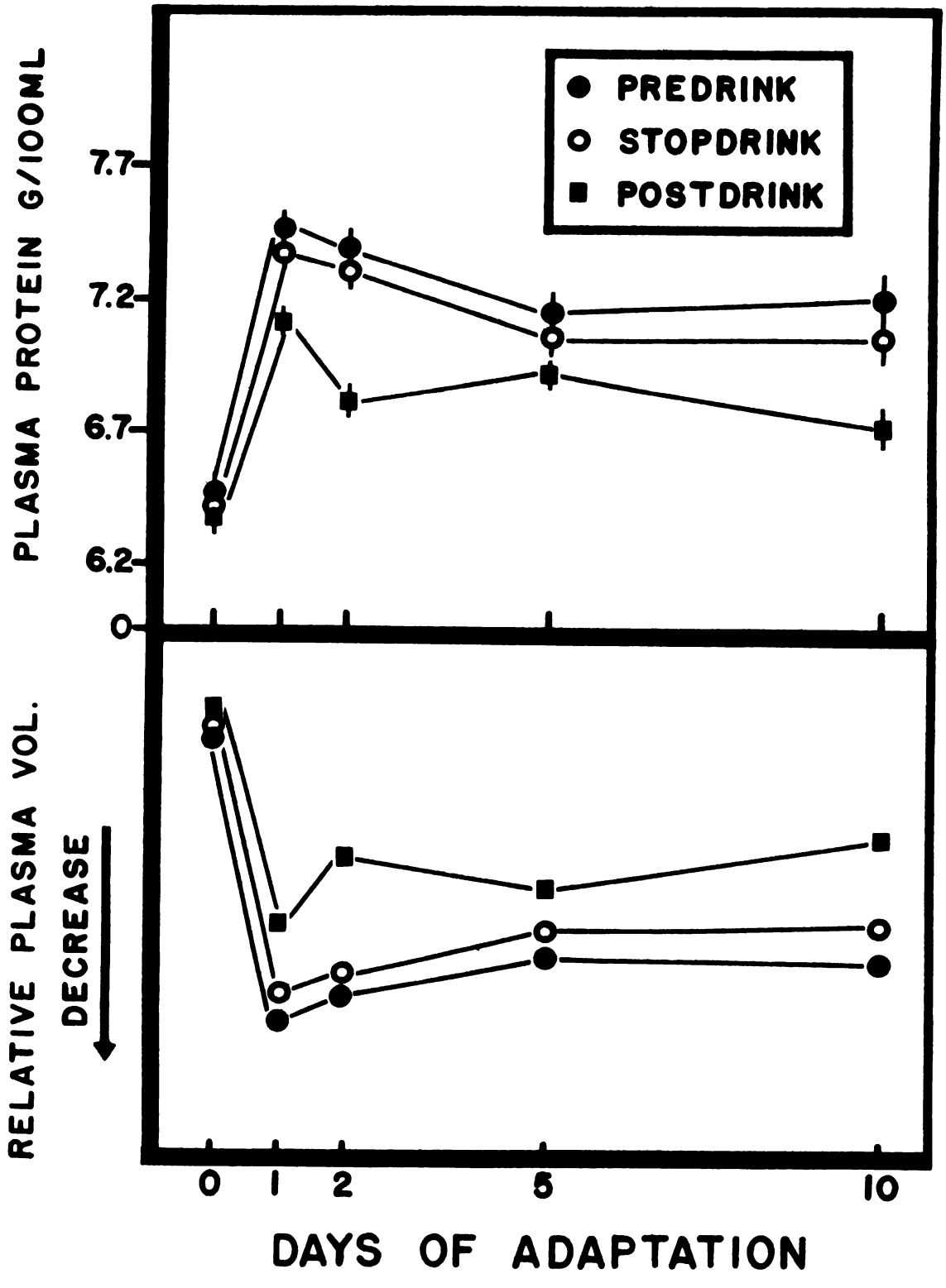
Day	Stopdrink	Postdrink
1	12.1±1.0	16.2±0.6
2	16.0±0.5	17.0±0.7
5	17.1±1.1	21.4±0.6
10	18.9±0.9	20.7±0.6

Mean time to reach the satiety criterion.

Day 1	Day 2	Day 5	Day 10
10.3±1.0	10.5±0.6	8.7±0.5	9.6±0.6

Figure 5

The mean amount of water remaining in the stomach and small intestine, and mean amount of water absorbed by the Stopdrink groups (N=24) and Postdrink groups (N=24) as a function of the days on a 23.5 hr water deprivation schedule. Standard errors of the mean are indicated by the flags.


PREDRINK
STOPDRINK
POSTDRINK
PLASMA PROTEIN G/100ML
7.7
7.2
6.7
6.2
0
RELATIVE PLASMA VOL.
DECREASE
0
1
2
5
10
DAYS OF ADAPTATION

group. Also graphed in Figure 5, is the mean amount of water remaining in the stomach and small intestine as a function of days of adaptation to deprivation.

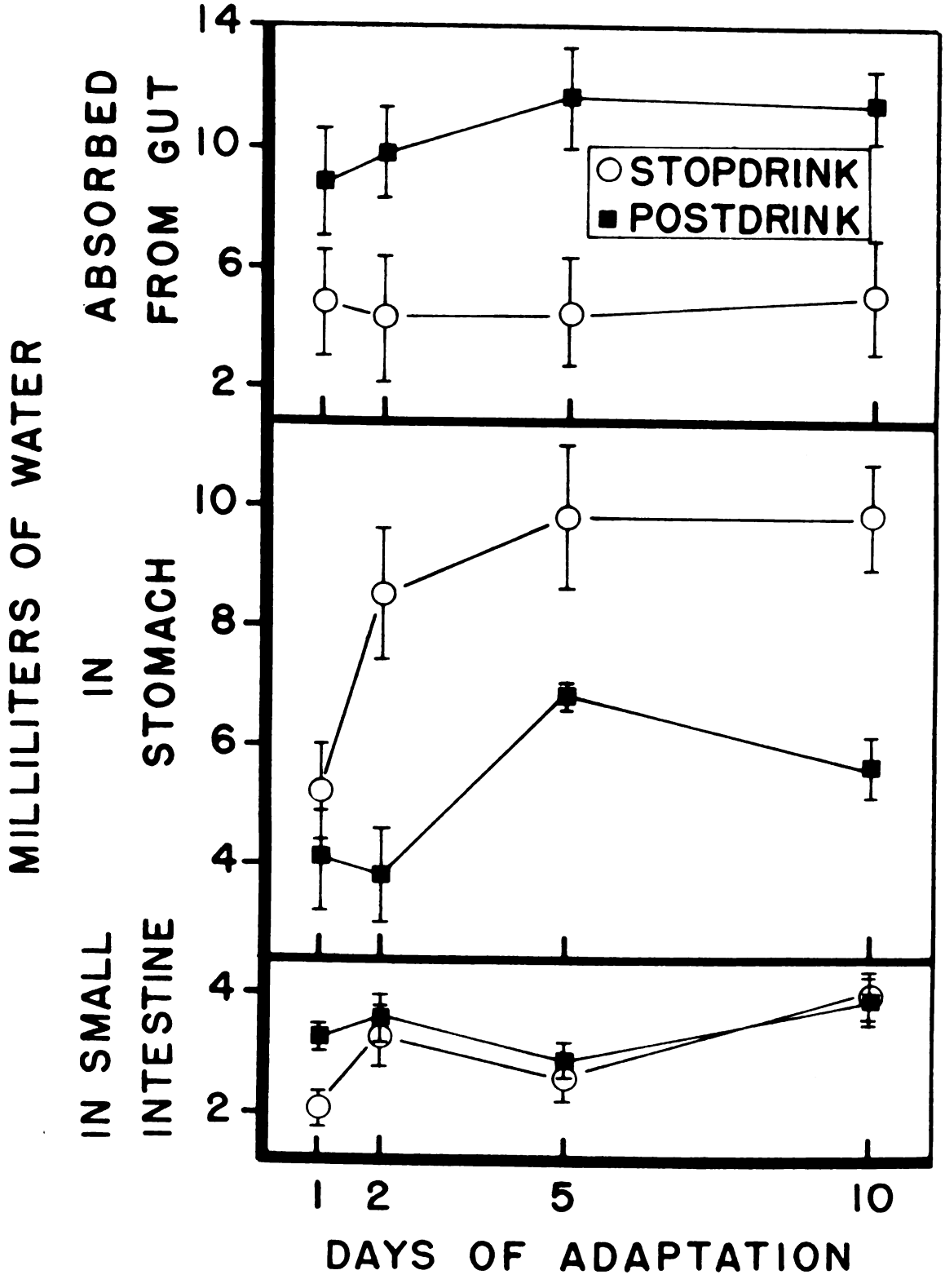
Caecal and colonic weights were measured to determine if, in fact, an appreciable amount of water enters the vascular system from these organs. It appears, however, as O'Kelly, Falk, and Flint (1958) indicated, that no appreciable amounts are absorbed from the caecum and colon during the time periods examined. A 3 x 4 factorial analysis of variance was computed on the total fluid loss from both these organs (taken as one) of the different groups. The mean total weight losses were not significantly different as a function of time of sampling ($F = 1.025$, $df = 2/60$, $p > 0.25$); but, they were significantly different as a function of days of adaptation ($F = 3.970$, $df = 3/60$, $p < 0.025$). Further, there was no significant change in the magnitude of the differences among the groups as a function of adaptation, as indicated by the interaction effect ($F = 0.531$, $df = 6/60$, $p > 0.25$).

Figure 6 shows the plasma protein concentration levels for the Pre-, Stop-, and Postdrink conditions, as a function of days of adaptation. A 3 x 4 factorial analysis of variance was computed on these treatment conditions. The means for plasma protein concentration differed significantly not only as a function of time of sampling ($F = 20.000$, $df = 2/60$, $p < 0.01$), but, also, as a function of adaptation of ($F = 7.923$, $df = 3/60$, $p < 0.01$). And, the magnitude of the

Figure 6

Top: The Pre-, Stop-, and Postdrink mean protein concentrations as a function of the days of adaptation to a 23.5 hr water deprivation schedule. Ad Libitum (stacked point), N=6; Predrink, N=24; Stopdrink, N=24; and, Postdrink, N=24. Standard errors are indicated by the flags.

Bottom: Relative plasma volumes of the Ad Libitum, Pre-, Stop-, and Postdrink groups as a function of days of adaptation to a 23.5 hr water deprivation schedule. This panel is the inverse of the top panel, indicating that as plasma protein concentration increases, plasma volume decreases.


MILLILITERS OF WATER
ABSORBED FROM GUT
IN STOMACH
IN SMALL INTESTINE
STOPDRINK
POSTDRINK
14
10
6
2
10
8
6
4
4
2
1
2
5
10
DAYS OF ADAPTATION

differences of the Pre-, Stop-, and Postdrink groups did not change significantly as a function of adaptation to deprivation, as indicated by the interaction effect ($F = 0.584$, $df = 6/60$, $p < 0.25$).

To determine whether the differences among the means for plasma protein concentration of the Pre- and Stopdrink groups differed significantly, a 2 x 4 factorial analysis of variance was computed on these conditions. The mean plasma protein concentration levels were significantly different as a function of adaptation ($F = 7.471$, $df = 3/40$, $p < 0.01$); but, they were not significantly different as a function of time of sampling ($F = 0.773$, $df = 1/40$, $p < 0.25$). And, there was no significant change in the magnitude of the differences of the Pre- and Stopdrink groups as a function of adaptation, as indicated by the interaction effect ($F = 0.056$, $df = 3/40$, $p > 0.25$).

It is apparent, then, that while there are rather large decreases in plasma protein concentration from Predrink to Postdrink levels, there is little, if any, real change from Predrink to Stopdrink levels.

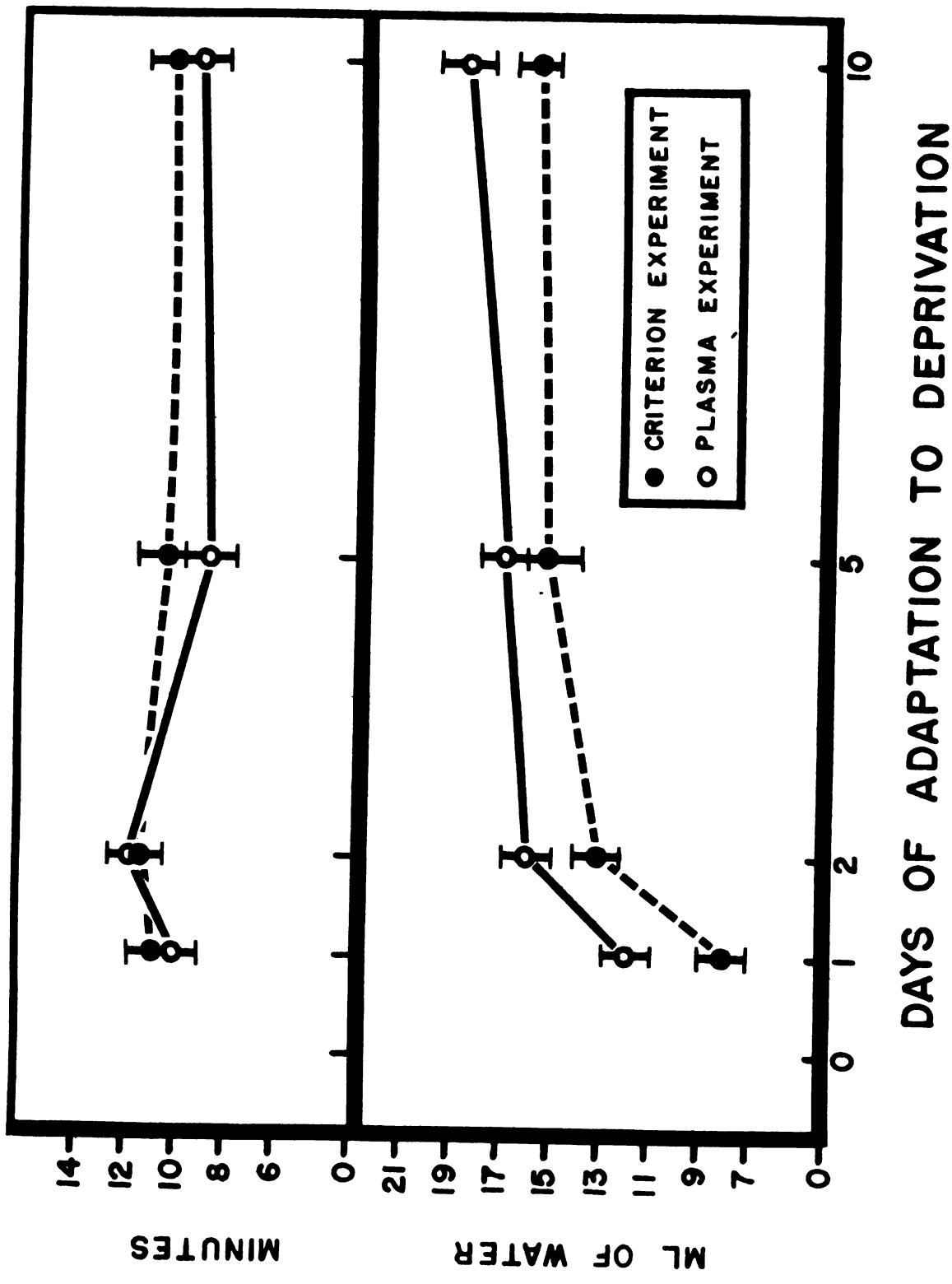
Figure 7 shows when the rats in the present experiment stopped drinking and how much they ingested on the different days of adaptation. Also, graphed in this figure are the data for when the animals in the Criterion Experiment would have stopped drinking and how much they would have drunk, if the satiety criterion had been applied to them. Because the

Figure 7

Top. Mean time when rats in the plasma experiment and criterion experiment stopped drinking as a function of adaptation to a 23.5 hr water deprivation schedule.

Bottom. Mean amount drunk when rats in the plasma and criterion experiments stopped drinking as a function of adaptation to a 23.5 hr water deprivation schedule.

Plasma experiment, N=24, and criterion experiment, N=12. Standard errors are indicated by the flags.


MINUTES
14
12
10
8
6
0
ML OF WATER
21
19
17
15
13
11
9
7
0
CRITERION EXPERIMENT
PLASMA EXPERIMENT
0
1
2
5
10
DAYS OF ADAPTATION TO DEPRIVATION

data from the Criterion Experiment were not collected from independent groups, the two curves in these figures could not be statistically compared. However, the similarity of the curves, in relative terms in the bottom of the figure and absolute terms in the top, is evident.

Discussion

Satiety criterion. As stated earlier, rats consistently stop drinking after a relatively constant time and after drinking similar amounts of water. Although the absolute amount ingested was different between Experiment I and II, it appears that by using the criterion for satiety that was offered, an adequate specification about when a rat will stop drinking can be made.

Plasma osmolality. Predrink plasma osmotic pressure levels of deprived rats rises approximately 2-3% over ad libitum levels (See Figure 4). And, presumably, soon after the deprived animal starts drinking, water begins to be absorbed and affects the ionic concentration of the plasma. As rats drink, the drop in ionic concentration of the blood continues, and, when plasma osmotic pressure reaches ad libitum levels, rats will stop drinking. However, because water still remains in the stomach, water continues to be absorbed. This will result in a further fall in osmotic pressure of the blood, as indicated by the Postdrink levels.

The temporal coincidence of plasma osmotic pressure reduction and the cessation of drinking is a necessary condition for an osmometric explanation of satiety. As stated

earlier, osomometric factors would effect satiety "as the result of absorbed water reducing the effective osmotic pressure of the surround of cells in the brain, which "signal" the satiation of thirst" (p. 3).

Since intra- and extracellular spaces are in a steady state (Darrow and Yannet, 1935), plasma osmotic pressure is an indication of the effective osmotic pressure exerted on cellular membranes. Presumably, then, because of the rapid equilibration between vascular and extravascular fluid compartments, the level of effective osmotic pressure that is maintained while an animal has free access to water is apparently re-established by the time the rat stops drinking.

In the past, it has been indicated that an animal stops drinking well before ionic concentration of body fluids could significantly change (Adolph, 1967; Holmes, 1967; and, Holmes and Montgomery, 1960). The conflict between this belief and the results of this study can perhaps be resolved by the following: a) There are apparently no reported data which would support the assumed lag between the cessation of drinking and significant changes in osmotic pressure of the blood. b) Most of the observations that led to this hypothesis were made on dogs. Perhaps, dogs do quickly consume sufficient quantities of water so that gastric factors could be the primary inhibitor of drinking. However, this is obviously mere speculation until the plasma conditions of dogs, at the cessation of drinking, is measured.

It should be pointed out here that the plasma osmolality values that were reported should not be considered threshold values. It might be recalled that there was a five minute delay between when the animal actually stopped drinking and when the blood was sampled. However, recent data (Hatton and Bennett, unpublished data) indicate that plasma osmotic pressure, when a rat actually stops drinking, is also within the range of ad libitum levels.

Plasma protein concentration. Short term changes in concentration of plasma protein are considered to be inversely related to blood volume, i.e., as protein concentration increases, blood volume decreases. Since the time span that was examined in this experiment was so short, actual amount of proteins in the plasma could not appreciably change. Therefore, plasma protein concentration is used here as an indication of relative changes in plasma volume. The bottom of Figure 5 represents the curves of Figure 5 inverted, and therefore, indicate the relative changes in plasma volume during the course of the access period.

Corbit (1968) concluded that there appeared to be no direct relationship between satiety and plasma volume, In an earlier experiment (1967), he injected isotonic Ringer's solution. In these studies, Corbit increased vascular volume up to 20%. However, these rats ingested normal amounts of water. But, when he injected distilled water intravenously, these rats stopped drinking. It appears, then, though not

concluded by Corbit, that it is not an increase in volume, *per se*, but a decrease in osmotic pressure of the blood which is perhaps the stimulus for the satiation of thirst. Furthermore, in the present study, plasma volume increased very little. However, there was a relatively large decrease in plasma osmolality.

As is indicated in Figure 6, plasma volume does not appreciably change by five minutes after satiety occurred. However, at the end of the access period, there is a relatively large increase. It appears that in the initial part of the access period, the water that is absorbed into the vascular space from the small intestine passes almost immediately into the extravascular space to equilibrate the two fluid compartments. However, once the osmotic steady state is lowered to *ad libitum* levels, or, some prior set point, the rate of intestinal absorption of water into the blood exceeds the flow of water into the extravascular space. As a result, plasma protein concentration (reflecting plasma volume) and plasma osmotic pressure are decreased substantially before a new steady state is established between these two compartments.

Intestinal absorption of water. Water passing into the rat's stomach becomes quickly concentrated (Follansbee, 1945). Though still hypotonic, Follansbee reports that it will then pass into the small intestine where it will become further concentrated to isotonicity. Apparently, the concentration

of water (by addition of ions) facilitates its absorption. O'Kelly, et al. (1958) reported that slightly concentrated solutions (0.5% NaCl) will be absorbed more quickly than distilled water. However, if solutions are hypertonic, absorption is slowed as a result of the flow of water into the intestinal lumen.

The process of water passing from the stomach into the small intestine can take place within ten seconds (Ivey, 1918). And, substantial quantities of water can be absorbed in relatively short periods. O'Kelly, et al. (1958) reported that 30% (3.5 ml) of a stomach load of water is absorbed in 13 minutes. In the present study, approximately 4.5 ml of water was absorbed in the 10 minute period before the cessation of drinking occurred. The discrepancy between these results and those reported by O'Kelly, et al may be accounted for by the following: a) During "normal" drinking, water reaches the stomach at a slower rate than during stomach loading. b) Because there are smaller quantities of water in the stomach at a given time, the increase in ion concentration might occur at a faster rate following drinking than following a stomach load of water. c) This would result in quicker release of water to the small intestine and a greater amount of water absorbed in a shorter time period. The data of Figure 5 indicates that rats on a water deprivation schedule absorb the same amount of water by the time they stop drinking, irrespective of the day of deprivation.

This is true, even though on Day 1 an average of 12 ml had been ingested, as compared with almost 19 ml of water that had been drunk on Day 10. And, it should be recalled that though these animals drank different amounts of water, they all stopped drinking in roughly the same amount of time, around 4.5 ml. At the end of thirty minutes, however, it appears that more water is absorbed by the Day 10 than the Day 1 animals. This could be the result of an increased hydrostatic pressure in the lumen of the small intestine, for more water is found in the intestine of the Day 10 animal than in the Day 1 rat.

It may be recalled from Table 1 that there is a larger decrease in plasma osmolality from Predrink to Stopdrink conditions of the Day 5 and 10 animal than the Day 1 rat. This could result because there is a greater amount of water in the vascular compartment on Day 5 and 10 than on Day 1. This hypothesis would be correct, if more water would have been drawn out of the plasma into the interstitial and intracellular compartments on Day 1. If this is true, it means that there is a higher gradient between the intracellular compartments on Day 1 than on Day 5 and 10, i.e., the intracellular fluid compartment on Day 1 is more concentrated (or, more dehydrated) than on Day 5 and 10.

There is some evidence to indicate that a difference in concentration gradient between Day 10 and Day 1 might exist. McDowell, Wolf, and Steer (1954) report evidence to indicate

a celluar (i.e., idiogenic) production of osmotically active substance in response to osmotic stress. Apparently this substance maintains cellular volume. Water loss on Day 1 of deprivation is the greatest of any day of an adaptation schedule, as indicated by weight loss. It would appear, then, that cellular volume is stressed more on the first day of deprivation than on any other day. If this is true, then the intracellular fluid of a Day 1 deprived animal is more concentrated (perhaps as a result of an idiogenic production of an organic cation within the cells, McDowell, et al., 1954). This, of course, would result in a greater amount of water that would flow, osmotically, into the cellular fluid compartment.

As noted earlier, Novin (1962) hypothesized that the decrease in electrolyte concentration that he found was not the result of absorption of water from the small intestine. He believed, as did many others (e.g., Adolph, 1967; Wolf, 1958), that these processes were too slow to be part of a satiety mechanism. However, because of the change in plasma osmotic pressure and intestinal absorption reported in the present study, these assumptions are particularly disputed here.

In summary, four points can be made about satiety: a) As rats adapt to water deprivation schedule, they will tend to drink more and more water on subsequent days of deprivation (until approximately Day 5), but, the amount absorbed

by the time satiety occurs remains relatively constant across days of adaptation. b) It appears that plasma volume, per se, is not a primary determinant of satiety. c) Enough water can be absorbed to lower plasma osmotic pressure to ad libitum levels by the time the rat stops drinking. d) In contradistinction to earlier hypotheses, these data indicate that an osmometric mechanism might effect the cessation of drinking.

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APPENDIX A

Apparatus

Description of the drinking box.

The drinking box was made up of six individual compartments. Each compartment was 11 3/4 in long, 5 1/2 in wide, and 7 3/4 in deep. The floor was 1/2 in hardware cloth. Six 100 ml gas collecting tubes, graduated in 0.2 ml were attached to each compartment. Metal spouts affixed to each tube protruded approximately 1 in into the compartment through a hole 2 1/2 in from the floor. Each compartment had a Plexiglas cover for a door.

Description of the freezing point osmometer.

The freezing point osmometer was manufactured by Precision Instruments, Inc., Framingham, Mass., under the brand name Osmette.

Description of the centrifuge.

It was model CL, manufactured by International Equipment Co., Needham Heights, Mass.

Description of refractometer.

It was model 10401, manufactured by American Optical Co., Instrument Division, Buffalo, N.Y.

APPENDIX B

Raw Data

Experiment 1

Minute by minute intake of water (in ml) for animals on Day 1 of a 23.5 hr deprivation schedule.

Min \ Subject	1	2	3	4	5	6
1	0.0	0.0	0.0	0.0	0.0	0.0
2	2.0	2.6	1.6	0.6	0.0	1.4
3	1.8	0.2	0.4	0.2	1.8	2.0
4	2.4	2.0	0.4	0.0	0.6	2.4
5	2.2	0.8	0.0	0.0	1.0	1.2
6	0.8	0.0	0.0	0.6	0.4	1.2
7	0.0	0.4	1.2	2.2	0.4	0.6
8	0.0	0.0	2.4	2.2	0.0	1.4
9	0.0	1.4	0.4	1.0	0.0	0.0
10	1.8	0.4	0.0	0.6	0.0	0.6
11	1.2	0.0	0.0	1.6	1.4	0.2
12	0.0	0.0	0.0	0.4	0.0	0.0
15	0.8	1.8	3.8	2.2	0.0	0.0
18	0.4	1.2	0.0	0.4	0.0	0.0
21	0.2	0.4	0.0	0.8	0.0	0.0
24	2.0	0.0	3.8	0.8	0.0	0.0
27	2.4	0.4	0.0	1.0	0.6	0.0
30	0.0	0.0	2.6	0.0	1.6	0.0

Min \ Subject	7	8	9	10	11	12
1	0.0	1.6	0.2	0.6	0.4	4.2
2	0.0	1.6	1.6	0.6	0.8	0.6
3	2.4	1.2	2.2	1.2	2.0	1.8
4	2.8	1.6	2.8	0.6	0.8	0.0
5	1.2	0.4	0.6	0.2	0.0	1.0
6	1.6	0.6	0.8	0.4	0.8	2.0
7	0.6	0.8	1.4	0.8	0.0	0.4
8	1.6	0.4	0.2	0.8	1.2	0.0
9	1.6	0.4	0.2	0.4	1.8	1.2
10	1.0	0.0	0.0	0.2	0.0	0.0
11	0.4	0.0	0.0	0.4	0.0	0.0
12	0.0	0.0	0.0	1.0	0.8	0.0
15	1.8	0.4	1.2	1.4	1.2	1.0
18	0.2	0.0	0.0	0.0	0.6	0.6
21	0.8	0.4	0.0	0.6	0.0	0.2
24	0.2	0.4	2.0	0.2	0.0	0.2
27	0.8	0.0	0.2	0.0	0.0	0.0
30	0.8	0.0	0.0	0.0	0.0	0.0

Experiment 1

Minute by minute intake of water (in ml) for animals on Day 2 of a 23.5 hr deprivation schedule.

Min	Subject 1	2	3	4	5	6
1	1.6	1.0	1.0	1.4	1.8	0.8
2	2.2	1.4	1.8	2.0	2.0	2.0
3	2.6	1.6	2.0	2.2	2.4	2.0
4	2.6	2.0	1.6	1.8	2.2	2.6
5	2.2	1.0	1.6	0.2	2.2	2.0
6	1.0	0.6	0.6	1.2	0.8	0.8
7	0.0	0.0	0.4	0.8	0.4	0.4
8	1.4	1.2	0.8	1.4	0.2	0.8
9	1.2	1.8	1.0	0.4	1.2	0.4
10	0.0	0.4	0.4	0.0	0.0	1.8
11	0.8	0.6	0.0	0.2	0.0	0.2
12	0.8	0.2	0.0	0.0	0.0	0.0
15	0.0	0.2	0.0	0.4	0.6	0.2
18	1.6	1.4	2.8	1.4	0.0	1.2
21	0.0	1.2	0.2	0.0	0.0	0.0
24	1.4	0.0	0.2	0.0	0.0	0.0
27	1.4	0.6	0.0	0.0	0.0	0.0
30	0.8	0.4	0.0	0.0	0.0	0.0

Min	Subject 7	8	9	10	11	12
1	1.8	1.8	2.2	1.2	2.4	2.8
2	2.4	2.0	2.0	0.8	2.8	2.0
3	2.0	2.4	1.8	1.0	2.0	2.8
4	2.6	2.0	0.4	1.4	2.6	0.6
5	2.4	1.8	3.4	2.0	0.4	1.0
6	2.4	1.6	0.8	0.6	0.0	0.6
7	1.4	1.4	0.2	1.2	1.4	1.2
8	1.0	1.2	0.6	0.4	0.0	0.4
9	1.2	0.0	0.4	0.4	0.0	1.0
10	0.0	0.0	0.4	0.4	0.0	0.2
11	0.2	0.0	0.4	0.0	0.2	0.0
12	1.2	0.0	0.4	1.2	0.2	0.0
15	0.6	0.4	0.0	1.2	0.4	0.0
18	0.8	0.4	0.4	0.4	0.6	1.2
21	1.8	0.0	0.0	0.2	1.0	0.6
24	0.0	0.0	0.0	0.2	0.2	0.0
27	0.0	0.0	0.0	3.0	0.0	0.0
30	0.0	0.0	0.0	0.8	0.2	0.0

Experiment 1

Minute by minute intake of water (in ml) for animals on Day 5 of a 23.5 hr water deprivation schedule.

Min	Subject 1	2	3	4	5	6
1	1.8	1.6	1.4	1.0	2.4	1.4
2	2.2	1.8	2.2	2.0	2.8	2.2
3	2.4	2.0	2.6	0.4	2.4	2.6
4	3.0	2.4	2.4	1.6	3.2	2.0
5	2.8	1.8	2.2	0.6	1.4	2.0
6	2.6	1.8	1.4	1.6	1.4	1.6
7	1.6	1.4	1.0	2.0	1.0	1.8
8	2.0	0.8	0.0	1.4	0.0	1.0
9	0.0	0.8	2.8	0.6	1.6	0.0
10	2.0	0.6	1.4	0.0	0.2	1.6
11	0.0	0.2	0.0	0.4	0.2	0.6
12	0.0	0.0	0.0	0.6	0.0	0.0
15	0.0	0.4	0.0	0.4	1.0	0.0
18	2.0	1.0	0.4	0.4	0.0	0.0
21	0.4	0.0	0.0	1.8	1.0	0.8
24	0.2	0.0	0.2	0.0	0.8	0.0
27	0.0	0.0	0.0	0.0	0.4	0.0
30	0.0	0.0	0.0	0.6	0.0	0.0

Min	Subject 7	8	9	10	11	12
1	1.6	2.4	2.0	1.2	3.4	2.6
2	2.2	2.4	2.2	1.2	3.6	2.0
3	2.4	2.6	2.2	1.4	3.4	3.4
4	3.0	1.8	0.2	2.2	1.8	2.6
5	1.8	1.6	4.0	2.2	1.2	1.6
6	2.6	1.4	1.0	1.4	0.4	0.6
7	2.0	0.6	1.4	0.8	0.2	0.8
8	1.0	0.0	1.2	2.0	0.0	0.0
9	1.0	0.0	0.2	0.4	0.2	1.0
10	0.0	0.0	0.0	0.6	0.0	0.2
11	0.0	0.0	0.0	0.2	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.8	0.4	0.0
18	0.0	0.0	0.0	1.6	0.2	1.4
21	0.0	0.8	0.0	0.6	0.4	0.0
24	0.0	0.2	0.0	0.0	0.0	0.0
27	0.0	0.2	0.0	0.8	0.0	0.0
30	0.0	0.0	0.2	0.8	0.0	0.0

Experiment 1

Minute by minute intake of water (in ml) for animals on Day 10 of a 23.5 hr water deprivation schedule.

Subject	1	2	3	4	5	6
Min						
1	2.2	2.0	2.0	1.4	2.8	2.6
2	2.2	1.6	2.0	1.2	2.6	2.2
3	3.0	2.2	2.6	2.2	2.4	2.4
4	3.6	2.2	2.4	2.0	2.6	2.4
5	3.6	2.6	2.8	2.0	2.0	1.2
6	1.6	1.8	0.6	2.2	1.4	1.4
7	2.2	1.6	2.0	2.0	1.2	0.6
8	0.6	0.4	0.0	1.2	0.2	1.4
9	0.2	0.2	1.4	1.8	1.8	0.0
10	0.2	1.6	0.0	2.0	0.2	0.0
11	0.0	0.0	0.0	1.0	0.4	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0
15	2.0	0.6	3.2	2.4	0.4	1.0
18	1.0	0.4	0.0	0.0	0.0	0.0
21	0.0	1.0	1.2	0.4	0.0	0.0
24	0.0	0.6	0.0	0.8	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0

Subject	7	8	9	10	11	12
Min						
1	2.4	3.0	3.2	1.6	4.4	3.8
2	2.6	2.2	2.4	1.0	3.8	2.4
3	3.4	2.6	1.4	1.6	3.2	3.0
4	4.2	1.8	2.4	2.6	1.6	2.6
5	3.2	2.0	1.6	1.8	1.0	2.0
6	2.0	1.4	2.0	1.6	0.6	1.0
7	0.6	1.0	0.0	1.4	1.0	1.2
8	0.0	1.0	0.4	2.2	0.2	0.4
9	1.2	0.2	0.0	0.2	0.0	0.0
10	1.8	0.2	0.2	0.2	0.2	1.2
11	0.0	0.0	0.8	0.2	0.2	0.2
12	0.0	0.0	0.0	0.0	0.0	0.4
15	0.0	0.0	0.6	1.4	0.0	1.0
18	0.0	0.2	0.0	0.2	0.0	0.0
21	0.0	0.2	0.0	0.4	0.6	0.0
24	0.0	0.8	0.0	0.8	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0

Experiment 2

Plasma osmolality (mOsm/kg) of the Ad Libitum, and Predrink, Stopdrink, and Postdrink groups on the various days of the deprivation schedule.

Ad Libitum — Day 1

Subject	Predrink	Stopdrink	Postdrink
1 307	312	306	298
2 308	307	305	299
3 306	316	301	295
4 299	306	306	303
5 298	307	306	295
6 294	308	305	301

Day 2

	Predrink	Stopdrink	Postdrink
1	308	299	289
2	311	297	287
3	309	297	287
4	308	305	291
5	314	307	299
6	309	304	298

Day 5

	Predrink	Stopdrink	Postdrink
1	312	298	287
2	311	298	286
3	303	296	297
4	307	306	283
5	313	292	296
6	307	298	291

Day 10

	Predrink	Stopdrink	Postdrink
1	311	288	287
2	306	297	283
3	307	298	279
4	314	307	292
5	316	293	295
6	315	298	288

Experiment 2

Plasma protein concentration (g/100 ml) of the Ad Libitum, and Predrink, Stopdrink, and Postdrink groups of the various days of deprivation

Ad Libitum — Day 1

Subject		Predrink	Stopdrink	Postdrink
1	6.6	7.8	7.6	7.6
2	6.7	7.3	7.4	7.2
3	6.4	7.7	7.5	7.4
4	6.4	7.3	7.7	7.1
5	6.3	7.4	7.5	6.8
6	6.2	7.5	7.0	6.7

Day 2

	Predrink	Stopdrink	Postdrink
1	7.7	7.5	6.8
2	7.1	7.4	7.0
3	7.5	7.2	6.8
4	7.2	7.4	7.0
5	7.3	7.3	6.6
6	7.6	7.5	6.7

Day 5

	Predrink	Stopdrink	Postdrink
1	7.1	7.3	7.1
2	7.3	7.1	7.0
3	7.5	7.2	7.0
4	6.9	7.0	6.2
5	7.4	6.7	6.5
6	6.7	7.1	7.3

Day 10

	Predrink	Stopdrink	Postdrink
1	7.1	6.9	6.8
2	7.4	7.4	6.4
3	7.3	7.4	6.8
4	7.1	7.3	6.5
5	7.2	6.7	6.9
6	7.0	6.9	6.9

Experiment 2

Amount of fluid lost from the stomachs of the Ad Libitum and Predrink, Stopdrink and Postdrink groups on the various days of the deprivation schedule.

Ad Libitum — Day 1

Subject		Predrink	Stopdrink	Postdrink
1	5.1	2.5	7.7	7.4
2	2.9	2.5	5.0	9.2
3	2.1	1.6	6.4	3.6
4	2.2	2.6	10.1	6.9
5	2.2	2.[illegible]	9.5	4.6
6	4.3	2.[illegible]	6.7	7.1

Day 2

	Predrink	Stopdrink	Postdrink
1	1.7	8.0	3.7
2	1.5	10.7	3.2
3	2.6	7.4	5.5
4	2.2	14.5	8.1
5	2.0	12.4	7.8
6	1.8	9.9	6.5

Day 5

	Predrink	Stopdrink	Postdrink
1	1.8	10.3	9.0
2	1.8	16.2	7.8
3	1.7	8.1	8.8
4	2.2	9.9	9.0
5	2.5	12.6	9.4
6	1.9	13.6	8.5

Day 10

	Predrink	Stopdrink	Postdrink
1	2.8	9.8	6.5
2	1.6	13.9	6.7
3	2.2	15.2	8.1
4	2.3	11.9	7.4
5	2.3	11.5	9.5
6	1.9	9.5	8.3

Experiment 2

Amount of fluid lost from the small intestines of the Ad Libitum, and Predrink, Stopdrink, and Postdrink groups on the various days of the deprivation schedule.

Ad Libitum — Day 1

Subject	Predrink	Stopdrink	Postdrink
1 8.1	6.9	8.4	10.1
2 8.7	7.9	9.4	10.4
3 10.8	6.6	8.8	9.0
4 10.5	7.8	6.4	9.9
5 8.6	6.8	8.4	10.3
6 6.6	7.1	8.9	9.2

Day 2

	Predrink	Stopdrink	Postdrink
1	5.8	8.6	11.1
2	5.6	11.3	8.7
3	6.5	9.3	9.9
4	7.9	8.1	9.2
5	6.4	10.9	10.9
6	6.0	9.9	10.1

Day 5

	Predrink	Stopdrink	Postdrink
1	6.7	10.0	12.0
2	5.7	11.6	9.9
3	6.8	9.0	10.6
4	10.6	10.1	10.5
5	8.4	10.8	10.2
6	7.9	10.0	10.0

Day 10

	Predrink	Stopdrink	Postdrink
1	6.0	10.3	9.7
2	5.8	10.8	10.9
3	6.5	10.4	11.3
4	8.3	14.2	10.6
5	6.5	11.7	9.9
6	7.7	9.5	12.3

Experiment 2

Amount of fluid lost from the colon of the Ad Libitum, and Predrink, Stopdrink, and Postdrink groups on the various days of the deprivation schedule.

Ad Libitum Subject		Day 1 Predrink	Stopdrink	Postdrink
1	4.9	4.3	4.2	4.5
2	5.6	5.2	3.8	4.7
3	5.4	4.7	3.8	4.9
4	5.6	5.0	7.9	4.9
5	6.9	5.3	4.7	5.6
6	4.1	5.1	5.6	5.0

Day 2

	Predrink	Stopdrink	Postdrink
1	3.5	3.6	3.6
2	3.6	4.4	4.3
3	4.5	4.3	4.1
4	5.2	2.0	5.4
5	3.7	5.8	5.4
6	3.8	4.1	4.7

Day 5

	Predrink	Stopdrink	Postdrink
1	6.6	5.6	5.2
2	3.7	4.8	5.6
3	4.1	4.5	4.1
4	4.3	4.5	6.3
5	5.0	5.1	5.6
6	5.8	5.5	4.8

Day 10

	Predrink	Stopdrink	Postdrink
1	5.0	4.4	5.4
2	5.0	4.8	4.8
3	4.1	4.7	4.5
4	5.0	4.9	5.3
5	4.7	4.2	6.0
6	6.5	4.4	6.2

Experiment 2

Amount of water consumed by the Stopdrink and Postdrink animals on the various days of the deprivation schedule.

	Day 1		Day 2	
	Stopdrink	Postdrink	Stopdrink	Postdrink
1	12.0	18.0	15.4	17.8
2	9.2	15.6	18.0	13.6
3	13.0	13.6	14.4	18.2
4	16.4	16.8	16.0	18.4
5	12.8	15.8	16.8	17.0
6	9.0	17.6	15.6	17.2

	Day 5		Day 10	
	Stopdrink	Postdrink	Stopdrink	Postdrink
1	16.6	22.2	17.4	18.2
2	18.8	21.8	21.2	20.2
3	13.0	21.6	20.2	22.8
4	14.0	23.6	17.0	21.4
5	19.8	19.0	21.8	21.2
6	20.0	19.4	15.2	20.6

Experiment 2

Time at which the Stopdrink animals stopped drinking on the various days of the deprivation schedule. Time is in minutes.

	Day 1	Day 2	Day 5	Day 10
1	10	13	10	11
2	9	12	8	8
3	15	10	9	8
4	11	9	7	10
5	7	9	8	12
6	10	10	10	9