ABSTRACT

THIRST MOTIVATED BEHAVIOR:
SPECIFICATION OF THE ADEQUATE INTERNAL STIMULUS

By

Charles Robert Almli

This study addressed itself to determining the nature of the adequate internal stimulus for thirst, and the psychophysical relationship between that stimulus and the drinking response. The determination of the adequate internal stimulus for the initiation of the drinking response is important for a complete understanding of how mammals regulate their water balance within homeostatic levels.

Many investigators have postulated that there may be two independent internal stimuli for effecting a drinking response. Both elevated effective osmotic pressure (increased concentration) of the extracellular fluids and decreased vascular volume have been given the status of adequate internal stimuli for drinking. Further, it is thought that these stimuli have their own separate sensing mechanisms. These postulates have arisen from the demonstration that both stimuli acting together (i.e., hyperosmolality and hypovolemia) result in greater drinking than either acting alone. This phenomena has been labeled 'additivity of stimuli for drinking', because the stimuli seem to add their effects on subsequent amounts consumed.

The purpose of the present series of experiments was twofold: (1) to test the within animal additivity of stimuli for drinking, i.e., measure drinking in response to hyperosmotic

and/or hypovolemic stimuli, and (2) to evaluate blood plasma conditions at the initiation of drinking in an effort to determine the degree of relationship between hyperosmolality and hypovolemia and the initiation of the drinking response.

Part A of Experiment 1 measured rats latencies to drink and hourly water intakes (I) for six hours following: (a) injection of hypertonic saline, (b) hemorrhage, and (c) injection of hypertonic saline plus hemorrhage. Water intakes were additive, i.e., Ia + Ib = Ic, for periods of three or more hours of access to water. Post-treatment latencies to drink did not show a similar additivity. In Part B, blood plasma conditions were measured at the initiation of drinking following injection of hypertonic saline, hemorrhage, and injection of hypertonic saline plus hemorrhage. Significant plasma osmotic pressure elevations were obtained under all conditions, even following hemorrhage which was previously thought to be free of plasma osmotic pressure changes.

Part A of Experiment 2 determined plasma conditions at various times following hemorrhage, while in Part B, intra-animal changes in plasma conditions at the initiation of drinking following hemorrhage were determined. Hemorrhage induced plasma hyperosmolality was found to be coincident with the onset of drinking only, with no changes from ad libitum levels at the other times tested. The intra-animal comparisons validated the finding that hemorrhage induced drinking is accompanied by significant plasma osmolality increase.

Experiment 3 evaluated plasma conditions following polyethylene glycol produced hypovolemia. Significant plasma osmotic pressure increase was found at the initiation of drinking, as was the case for hemorrhagic hypovolemia.

The results are discussed in terms of: (a) the hyperosmotic and hypovolemic additivity of water intakes, (b) the ubiquity of plasma osmotic pressure increase at the initiation of drinking, and (c) the adequate internal stimulus for drinking.

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DEDICATION

To Sheila,

She pointed the way,

And could foresee;

That long off day,

Of Hubby's Ph.D.

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INTRODUCTION

Restricting an animal's water supply is one of the most commonly used techniques for inducing thirst motivated behavior, i.e., behavior which is oriented towards obtaining and ingesting water. This technique of producing the onset of drinking is a relatively natural one, for water is not always readily available in the natural environment of most mammals. As a result of water deprivation, certain physiological changes signal the animal to seek water, and commence drinking in order to restore his bodily states back to within homeostatic levels (Hatton and Almli, 1969; Hatton and Bennett, 1970). The determination of these physiological states which signal the need for water was the purpose of this investigation. Stated more explicitly, the purpose of this study was to determine the adequate internal stimuli for thirst motivated behavior.

Thirst, and hence drinking, are often produced in animals by water deprivation. Two rather profound physiological changes which occur as a result of water deprivation are, an increase in the osmotic pressure (hyperosmolality) of the blood plasma, and decreased volume (hypovolemia) of this same plasma (Chew, 1965; Kutcher, 1965). Both of these states are the natural result of water loss from the body without equivalent water

input. The alteration in these two plasma characteristics in response to water deprivation has led certain investigators, e.g., Corbit (1968), and Fitzsimons and Oatley (1968), to consider either of these changes (hyperosmolality or hypovolemia) to be an adequate internal stimulus for effecting the drinking response.

Subsequent experimentation has, to some extent, borne out their contention. It has been found that water intake following hyperosmolality and hypovolemia was greater than the amounts of water consumed following either alone (Oatley, 1964; Corbit, 1968). Such meager results have led to the conclusion that hyperosmolality and hypovolemia exert an additive effect on amounts of water consumed. This phenomena has been labeled 'additivity of stimuli for drinking', and can be characterized by the following equation: combined hyperosmotic-hypovolemic intake > hyperosmotic intake or hypovolemic intake.

From the above, it has been further proposed that hyperosmolality and hypovolemia are independent modes of stimulation (Fitzsimons, 1961; Stricker, 1966; Stricker and Wolf, 1967), i.e., they can be experimentally produced independent of one another. In accord with both of these modes of stimulation being considered adequate internal stimuli for producing the onset of drinking, a dual detector system for the control of drinking has been proposed. One of the detector mechanisms is thought to transduce plasma osmotic pressure changes, while the other is thought to transduce plasma volume changes (Corbit, 1968).

While the logical progression of the above seems sound, there are enough unanswered questions to make such theorizing tenuous at best. First, investigators have not been able to demonstrate additivity of drinking on a number of occasions, e.g., Fitzsimons (1961) and Fitzsimons and Oatley (1968). Second, additivity of stimuli for drinking has never been demonstrated on the same animal, the investigators always resorting to the use of independent groups. And third, but most important, there is a lack of experimental verification for the assumption that hyperosmolality and hypovolemia are independent modes of stimulation, yet, this assumption has been perpetuated.

The present series of experiments were conducted to: (a) test the additivity model in its strictest form, i.e., hyperosmotically induced intake + intake induced by hypovolemia = intake induced by simultaneous hyperosmolality and hypovolemia; and (b) to measure blood plasma conditions at the initiation of drinking following these modes of stimulation, in an effort to account for such additivity of water intakes. These studies constitute Experiment 1.

Experiment 2 was based on the finding in Experiment 1, that hemorrhage resulted in plasma osmolality increase. This result was subjected to further test and validation in Parts A and B of Experiment 2.

Experiment 3 was conducted to test whether the use of polyethylene glycol, another technique often used to produce hypovolemia, resulted in plasma osmolality increase.

EXPERIMENT 1

PART A

The first part of this experiment was concerned with testing the additivity of hypertonic saline injections and hemorrhagic hypovolemia in the production of thirst, as measured by amounts of water consumed. Both intra- and inter-animal comparisons were made. A strictly additive model requires that water intake following a hyperosmotic stimulus (S_O), plus water intake following a hypovolemic stimulus (S_V), equal the water intake following both stimuli combined (S_{OV}), i.e., S_O intake + S_V intake = S_{OV} intake.

Method

Subjects

In the first part of this experiment, five male albino rats of the Holtzman strain were used. The rats were housed in individual wire cages in a temperature controlled room (22°-25° C.), under conditions of constant light. Wayne Mouse Breeder Blox and tap water were constantly available in the home cage throughout the experiment. The rats were approximately 90-100 days old and weighed 370 $\pm$ 26 grams at the start of the experiment.

Apparatus

One six-unit drinking box was used for the observations of 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. A stop watch was used to measure the latencies to drink, i.e., the time lapse from access to water until the initiation of drinking.

Subcutaneous 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. Hemorrhagic hypovolemia was produced by cutting off approximately 1 inch of tail and collecting 5 cc of whole blood in a graduated centrifuge tube.

Procedure

The five rats were adapted to the drinking boxes for 3 hours per day for six days. The rats were on ad libitum food and water at all times, except while in the drinking boxes where only water was present. Each rat received all three of the following treatments in a counter-balanced order: injection of 16% NaCl (I), 5 cc tail hemorrhage (H), and injection of 16% NaCl and 5 cc tail hemorrhage (IH). Even under the I treatment, a portion of the tail was cut off, but it was ligated so that no hemorrhage occurred. On treatment days, a rat was anesthetized with ether, and one of the treatments was administered. (All treatments were accompanied by 3 minutes of ether anesthesia.) The rat was then placed in the drinking box where its latency to initiate was measured, and hourly water intakes (for 6 hours) were recorded. Each

rat received its first, second, and third treatments at least 12 days apart.

It was necessary to establish an appropriate baseline for evaluating the potency of the treatments in the production of thirst. The following tail-cut control (T) was performed: 12 days after the last treatment, these same rats had a portion of their ligated tails cut off. They were placed in the drinking boxes as before, and their 6 hour water intakes were measured at hourly intervals.

Results

Between Parts A and B of this experiment, the latencies to drink following a given treatment (I, H, or IH) were not reliably different at the 0.05 level of statistical significance, and therefore were combined. For purposes of statistical analysis these data were transformed into reciprocals. Mean reciprocal latency to drink ± estimated standard error of the mean (S.E.) is plotted for the following treatments: injection alone (I), hemorrhage alone (H), and injection and hemorrhage combined (IH), in Figure 1-A. The latencies to drink were reliably different from one another where S.E. bars do not overlap. The mean latencies to drink following I, H, and IH, were 17.39, 53.12, and 30.08 minutes, respectively.

Presented in Figure 1-B are the mean 6 hr water intakes (±S.E.) plotted for each treatment condition. The different treatment conditions (tail-cut (T), I, H, and IH) resulted in reliably different 6 hr water intakes ($F=43.75$, $df=3/12$, $p<0.01$), non-overlapping S.E. bars indicating reliable differences

between means. Each of the treatments resulted in significantly greater drinking than the T control, indicating that even 5 cc hemorrhage was an adequate stimulus for drinking, in spite of the rather small intake. Following the T control, the rats drank a mean of 1.2 ml water, while following H they drink a mean of 3.4 ml water. These two conditions were also different in terms of the temporal drinking patterns, which is apparent in Figure 2, where water intakes are plotted as a function of hours of access to water. Following H, the rats drink most of their total water intake in the first three hours of access, while following T, they drink only in the final three hours of the access period.

The water intake data indicate that hemorrhagic hypovolemia results in significant drinking. The latency to drink and the temporal drinking patterns further indicate that hemorrhagic hypovolemia is a reliable stimulus for motivating drinking behavior. These results are contrary to Stricker's (1968) statement that: "The anemia and low blood pressure of such animals after large hemorrhage may well interfere with most behavioral tests of motivation and thus preclude an experimental demonstration of thirst." (p. 384).

On the left side of Figure 3 are presented the individual 6 hr water intakes following I, H, IH, and the arithmetic sum of the intakes for I plus those for H, i.e., I + H. The sum of the intakes for I + H are approximately equal to the intake following IH for each individual animal. On the right side of this figure, are the mean 6 hr water intakes for all rats across

FIGURE 1

A: Mean (±S.E.) reciprocal latency to drink following: injection of hypertonic saline solution (I), hemorrhage (H), and saline injection plus hemorrhage (IH), for Parts A and B of Experiment 1.

B: Mean (±S.E.) six hour water intakes (in ml) following tail-cut control (T), I, H, and IH. See text for explanation of treatments.

Figure 1

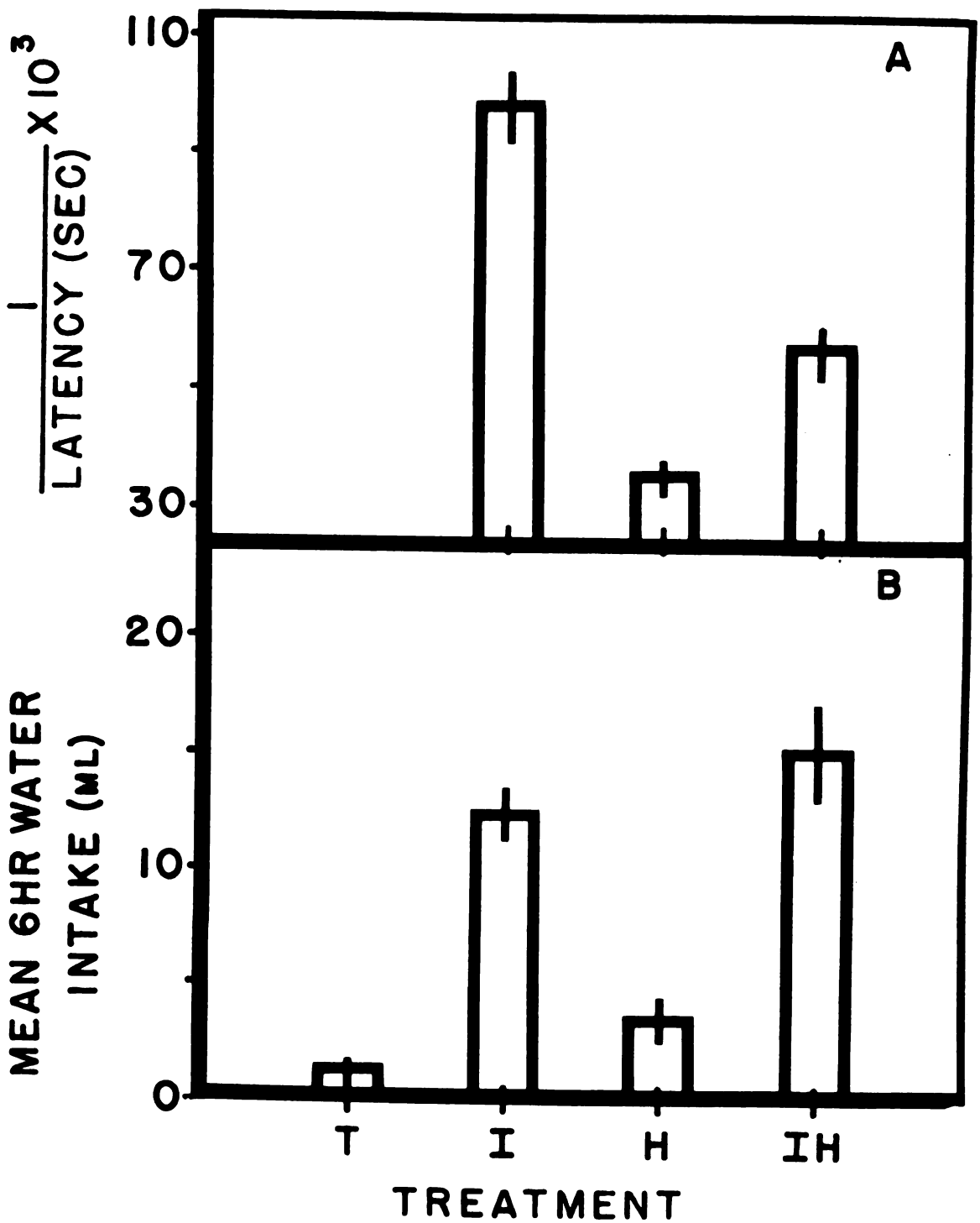

110
70
30
A
$\frac{1}{\text{LATENCY (SEC)}} \times 10^3$
20
10
0
B
MEAN 6HR WATER INTAKE (ML)
T
I
H
IH
TREATMENT

FIGURE 2

Mean hourly water intakes (in ml) following hemorrhage (H) and tail-cut control (T). See text for explanation of treatments.

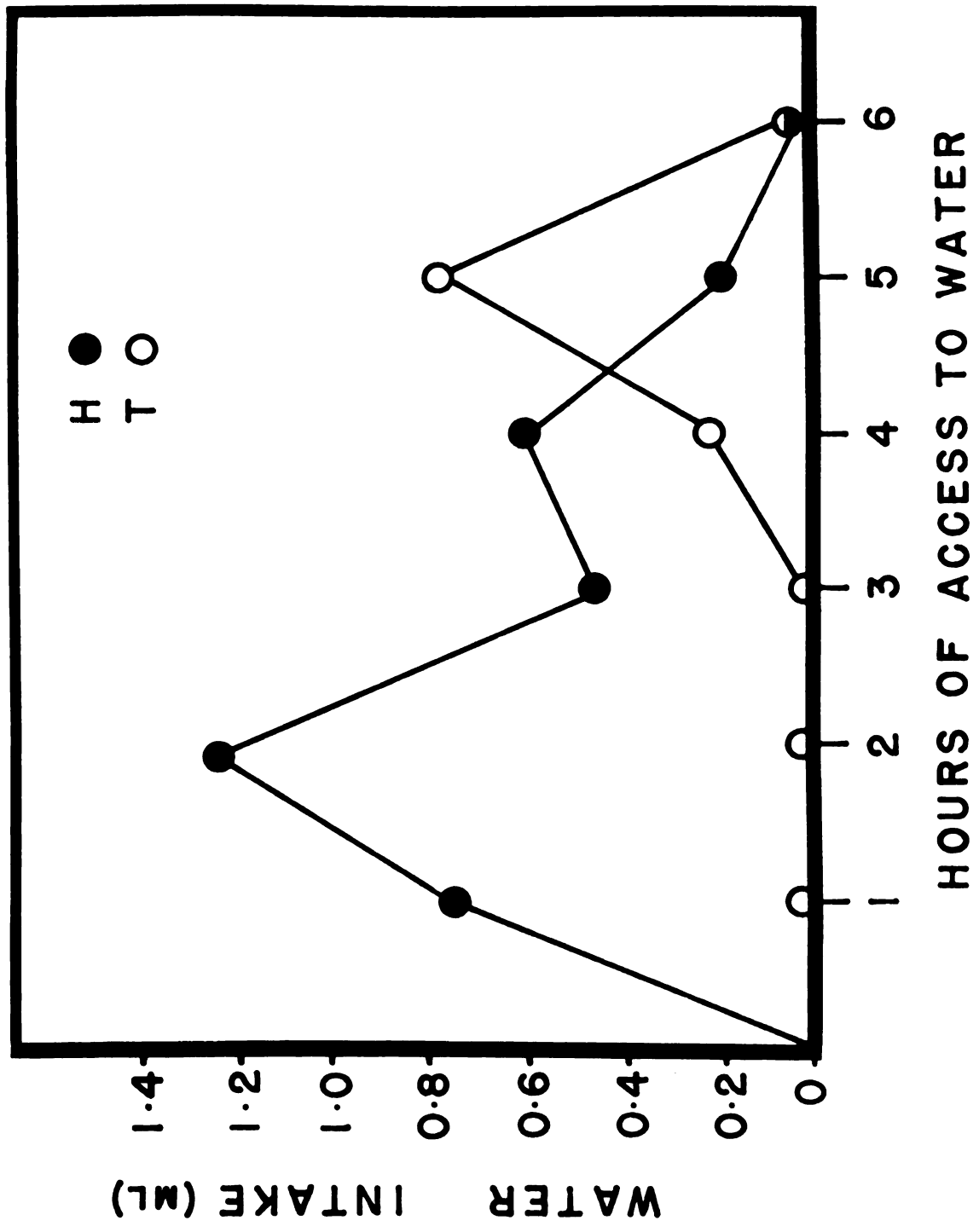

WATER INTAKE (ML)
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
H O
T O
1
2
3
4
5
6
HOURS OF ACCESS TO WATER

Figure 2

FIGURE 3

Individual 6 hr water intakes (in ml) following I, H, and IH, are presented on the left side. The two bars represent I + H and IH intakes. Mean 6 hr water intakes for I + H and IH are presented on the right side. See text for explanation of treatments.

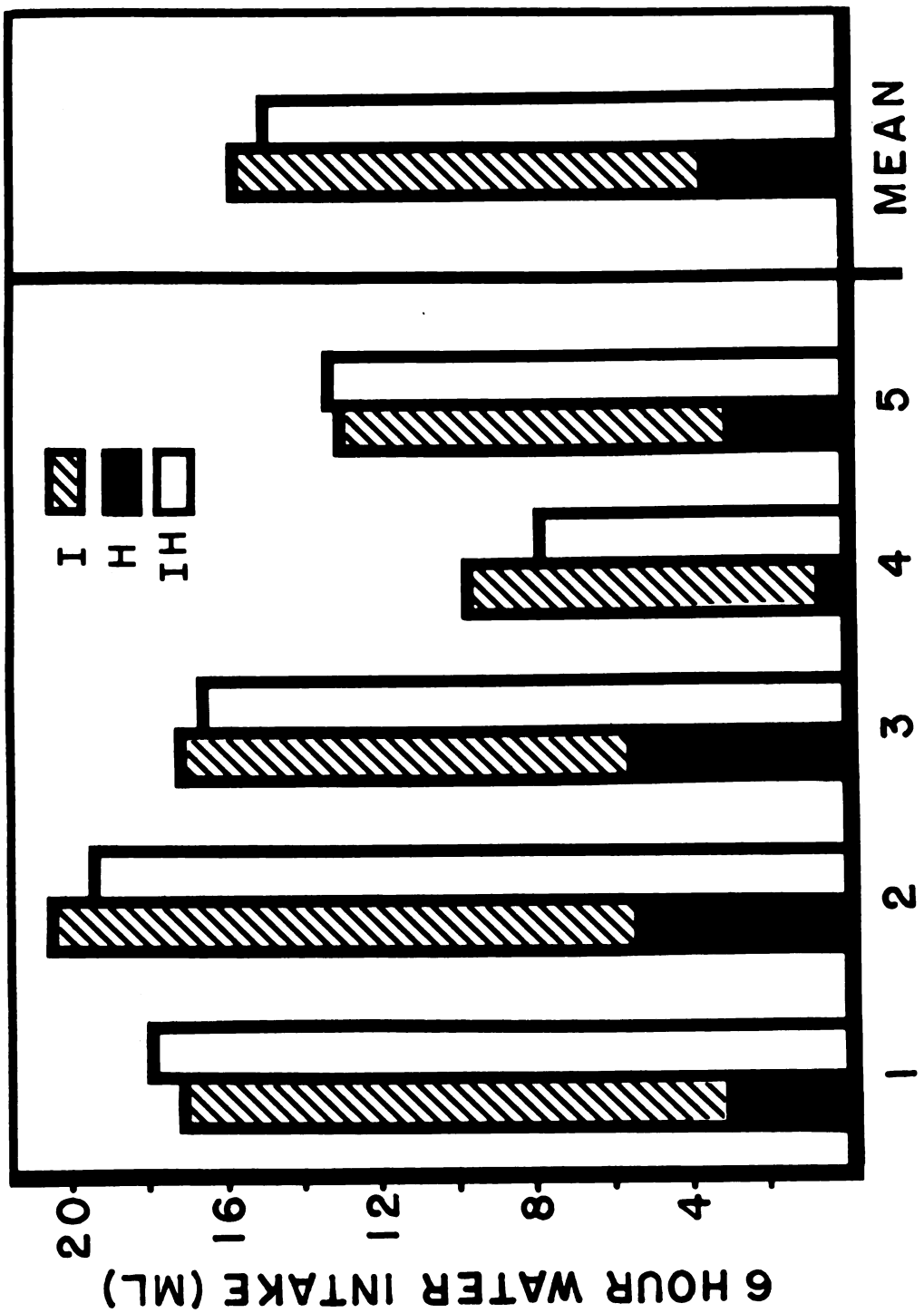


Figure 3

all conditions. Here again, the sum of the intakes following I plus those following H, are approximately equal to the intake following IH, i.e., I + H $\simeq$ IH. The mean intake for I + H (15.7 ml) was not reliably different at the 0.1 level of statistical significance from the mean intake for IH (15.0 ml), indicating that the effects of the treatments are additive in terms of water intakes. Pearson product moment correlation of these two measures yielded r=0.96; thus, the intra-animal reliability of the water intakes following I + H and IH being approximately equal was quite high. The relationship can be described by the following linear regression equation, Y=1.09 (X-15.7) + 15.0.

PART B

The second part of this experiment was conducted to determine if blood conditions (plasma osmolality and protein concentration) at the initiation of drinking, following I, H, and IH, could account for, or reflect, the additivity of water intakes following such treatments.

Method

Subjects

Eight male albino rats of the Holtzman strain were housed under the same conditions as those in Part A. They were 100-110 days old at the start of the experiment and weighed 386 ± 29 grams.

Apparatus

In addition to the apparatus used in Part A, 2 cc heparinized glass syringes were used for obtaining the blood samples. The blood was centrifuged for 3 min at 3300 revolutions per

minute. The plasma was analyzed in a refractometer (protein concentration). The remaining plasma was frozen in capped vials for later osmolality determination in a freezing-point osmometer.

Procedure

The rats were adapted to the drinking boxes, and treatments I, H, and IH, were administered in an identical manner as in the first part of this experiment. Following the treatments, the rats were placed in the drinking boxes where latency to drink was measured. At the initiation of drinking, the rats were immediately removed from the drinking boxes, quickly anesthetized with ether, and a 2 cc blood sample was withdrawn from the heart. The time lapse from the initiation of drinking to the completion of blood withdrawal was less than 90 seconds. Two rats had ad libitum blood samples withdrawn on two separate occasions. On one occasion, the rats had a portion of their ligated tails cut off, and a blood sample was immediately withdrawn via heart puncture. On a second occasion, separated from the first by 12 days, the rats again had a portion of their ligated tails cut off. This time they were delayed for 53 min (mean post-hemorrhage latency to drink) without food or water, then a blood sample was withdrawn. This ad libitum delay condition controlled for possible temporal effects due to the long latency to drink for the hemorrhage treatment. These ad libitum samples served as a baseline to which the treatment samples could be compared.

Each of the treated rats were given two of the three treatments, no two rats receiving the same treatments in the same order. At least 12 days elapsed between treatments, with habituation to the drinking boxes being maintained during the inter-treatment days.

Results

The procedures used in this experiment might sound somewhat traumatic or severe. However, body weights and mobility patterns of the treated rats indicate that this may not be the case. Upon arousal from the anesthetic the rats were quite active, none showing any signs of debilitation except for a short-lived (about 1 minute) pain reaction shown by some of the rats. Following this, they displayed the usual exploratory and grooming behaviors before the onset of drinking.

Body weights for each successive treatment were significantly elevated over the previous treatment. Mean body weight at the beginning of the experiment was 370 $\pm$ 26 grams, while at the completion of the experiment, mean body weight had increased to 472 $\pm$ 45 grams. This increase was reliable at the 0.01 level of statistical significance.

As presented earlier, the post-treatment latencies to drink obtained in Part B for I, H, and IH, were reliably different from each other. But, because these treatment latencies were not reliably different from those obtained in Part A, for the same treatments, the latencies to drink were combined and are presented in Figure 1-A.

The delayed and non-delayed ad libitum plasma samples were not reliably different from one another in either protein concentration or osmolality, i.e., the 53 min delay had no detectable effect on ad libitum plasma conditions. Therefore, the two ad libitum plasma determinations were combined, and are presented as a single ad libitum baseline group.

In Figure 4-A, means ± S.E. for plasma protein concentration at the initiation of drinking are plotted for each treatment condition and for the ad libitum baseline condition. Differences between means are reliable where S.E. bars do not overlap. There was a decrease in protein concentration from ad libitum levels to those levels found at the initiation of drinking following I, a further decrease at the initiation of drinking following H, and a still further decrease following IH, the differences being reliable at the 0.005 level of significance.

Presented in Figure 4-B are the means ± S.E. for plasma osmolality at the initiation of drinking following I, H, IH, and for the ad libitum baseline. Plasma osmolality at the initiation of drinking following all treatments was reliably higher than the osmolality under ad libitum conditions, this difference being reliable at the 0.025 level of significance.

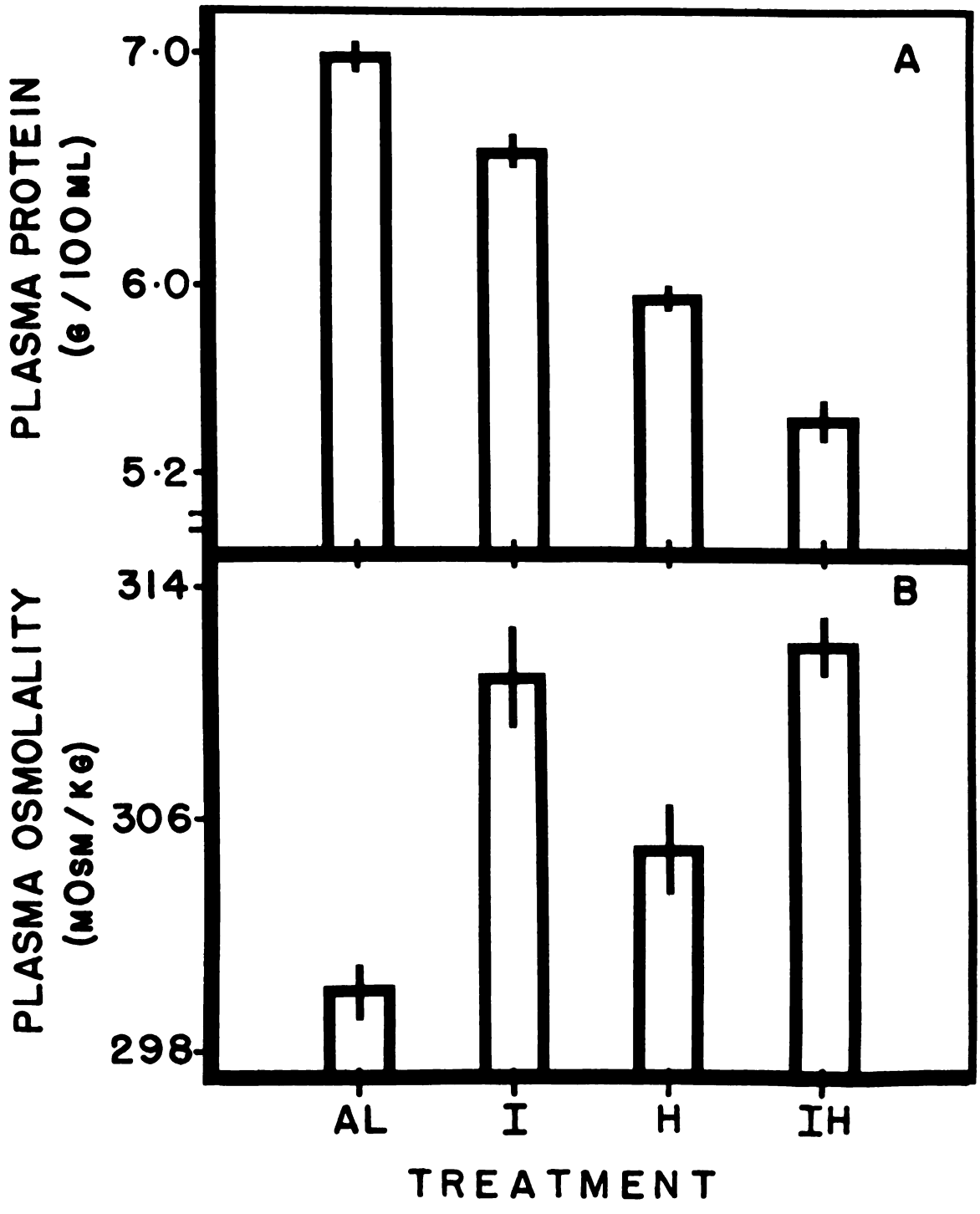
Since the blood samples of the treated rats were taken at the initiation of drinking, the difference between ad libitum osmolality and the osmolality at the onset of drinking following each treatment represent mean threshold values. The 3.7% mean threshold elevation at the initiation of

FIGURE 4

A: Mean (±S.E.) for plasma protein concentration for ad libitum baseline, and at the initiation of drinking following I, H, and IH.

B: Mean (±S.E.) for plasma osmolality for ad libitum baseline and at the initiation of drinking following I, H, and IH. See text for explanation of treatments.

Figure 4


PLASMA PROTEIN
(g/100ML)
7·0
6·0
5·2
A
PLASMA OSMOLALITY
(MOSM/KG)
314
306
298
B
AL
I
H
IH
TREATMENT

drinking following I was not reliably different from the 4.0% mean threshold elevation at the initiation of drinking following IH.

The hemorrhage treatment also resulted in an elevation in plasma osmolality at the initiation of drinking. The increase here representing a 1.7% mean threshold. This finding was unexpected, and therefore was subjected to further study in Experiment 2.

Discussion-Parts A and B

In Part A of this experiment, additivity of water intakes were demonstrated for ad libitum rats with treatments consisting of hypertonic saline injection and/or hemorrhage. Water intakes following hypertonic saline injection (I), hemorrhage (H), and hypertonic saline injection and hemorrhage combined (IH), fit an additivity model. A strictly additive model requires that water intake following I plus water intake following H (I+H), equal the intake following IH. The results of this experiment meet these requirements, for a 6 hr water access period.

Even for the first 3 hours of the access period, the additivity of water intakes was present (I+H=14.8 ml, IH=13.1 ml). However, for periods of less than 3 hours, intakes were not additive. Perhaps this lack of additivity was due to the differential latencies to drink following the treatments. As an example, the different treatments resulting in different latencies allowed, during the first hour, the I treatment rats to have a mean of 36 minutes more drinking time than the rats under the H treatment. As drinking time increases to

3 hours, the effect of the differential latencies to drink seems to be attenuated and the additivity becomes manifest.

While the amounts of water consumed fit the additivity model, the data concerning post-treatment latency to drink do not. A strictly additive model might lead to the prediction that the IH treatment would result in the shortest latency to drink. However, this would be the case only if the stimuli were additive in a temporal sense. This departure, of the latency measure, from the additivity model could possibly be explained by the considerations listed below.

First, the slower latency to drink for the IH treatment as opposed to the I treatment could be due to the debilitating effect of hemorrhage following IH. However, if hemorrhage has such a debilitating effect, one might expect the IH latency (30 min) to be biased closer to the H latency (53 min), instead of being closer to the I latency (17 min) as it was. A second possible explanation of the differential latencies to drink comes from the data concerning plasma conditions at the initiation of drinking following the treatments. This will be discussed below, after discussion of the plasma variables studied.

Changes in plasma protein concentration are typically used as an index of plasma volume changes, e.g., increases in protein concentration indicating decreases in plasma volume. While this may be justifiable for various manipulations (e.g., hypertonic saline injection) which result in altered vascular volume, it does not seem justifiable to use this index in the case of hemorrhage. For example, if hemorrhage results in a

decreased protein concentration when compared to ad libitum control levels, it would be unreasonable to infer from this data that hemorrhage (which depletes vascular volume) causes a volume increase. However, this protein concentration index can be used in conjunction with hemorrhage to indicate dilution of plasma proteins, if a decrease in protein concentration is measured following hemorrhage. Adolph, Gerbasi, and Lepore (1933) support this notion, having demonstrated that vascular dilution does occur in response to hemorrhage, presumably by intra-cellular and interstitial fluids entering the vascular compartment.

Plasma protein concentration at the initiation of drinking, if used as an index of plasma volume or protein dilution, does not seem to reflect or parallel either the water intakes or the latencies to drink following I, H, or IH. Protein concentration at the initiation of drinking following all treatments was depressed below ad libitum levels. The lowest protein concentration was obtained for the IH treatment, this level was at the same time, associated with the largest water intake and the second longest latency to drink. The highest protein concentration for treated rats was obtained at the initiation of drinking following the I treatment, and was associated with the shortest latency to drink and the second largest water intake.

At the initiation of drinking following all treatments, including hemorrhage, plasma osmolality was elevated over

ad libitum levels. While this result was expected for the I and the IH treatments because of the hypertonic saline injection, it was not expected in the case of hemorrhage alone. The importance of this finding is apparent, and was further analyzed in Experiment 2. Because of the elevation in plasma osmolality at the initiation of drinking following the I, H, and IH treatments, it was possible to compute osmolality thresholds for drinking. These thresholds are computed by determining the difference between the ad libitum control osmolality and the osmolality at the initiation of drinking following the individual treatments. These differences represent the sufficient elevations in plasma osmolality for the initiation of drinking. The mean threshold elevations for I, H, and IH, were 3.7, 1.7, and 4.0%, respectively.

The osmolality thresholds were not different for the I and IH treatments, in spite of the fact that the latencies to drink and water intakes were different. This state of affairs might be explained by the following two considerations. First, the differences in latency to drink following I and IH could be due to a differential time course in the rise of plasma osmolality, and second, the differences in amount consumed following I and IH could be due to an osmotic pressure summation in the case of IH.

The differences in latency to drink could be due to the dilution of the protein concentration produced by hemorrhage in the case of the IH treatment, which is not in common with the I treatment. The hemorrhage induced dilution may have

buffered the osmotic pressure increase produced by the hypertonic saline injection, thus slowing the osmotic pressure rise over time. This hypothesis is tenable because the IH treatment resulted in the lowest protein concentration, i.e., greatest dilution. This state of affairs could then result in a longer latency to drink for IH, even though the osmolality drinking thresholds are the same for the IH and I treatments.

The differences in water intake following the I and IH treatments can be explained in a similar manner. With the finding that hemorrhage results in an osmolality increase, the IH treatment may in fact be associated with two osmotic pressure elevations. The IH treatment results in elevated osmolality via the hypertonic saline injection, and may have a further osmolality elevation coming about later in response to the hemorrhage. The hemorrhage alone results in an osmolality increase approximately 23 minutes after the IH treatment produces the onset of drinking. Thus, the hemorrhage induced osmotic pressure increase does not occur in time to contribute to a greater osmolality threshold for the IH treatment as opposed to the I treatment, but it still can contribute later to an increased water intake for the IH treatment versus the I treatment. The hypothesis being put forth is that the dual treatment (IH) may result in greater water intake due to two osmotic pressure elevations summating over time. If these two osmotic pressure elevations are additive in their effects on water intake, then the IH treatment should result in

drinking equal to the drinking in response to the osmotic pressure elevations acting alone. This is exactly what the results of this study indicate, i.e., I + H $\simeq$ IH, 15.7 $\simeq$ 15.0.

The results of this experiment indicate that at a behavioral level, the effects of hypertonic saline injection and hemorrhage are additive in terms of water intakes. These results do not support the notion that hypertonic saline injection and hypovolemia, as produced by hemorrhage, are independent stimuli for the production of thirst and drinking. Both of these treatments result in elevated plasma osmolality, when measured at the initiation of drinking. This information suggests that the additivity model, as pertains to these data, may be nothing more than the temporal summation of plasma osmotic pressure elevations, and not two independent modes of stimulation (i.e., hyperosmolality and hypovolemia) as previously thought.

EXPERIMENT 2

PART A

The purpose of this experiment was to analyze more closely the effects of hemorrhage on blood plasma conditions, specifically osmolality and protein concentration. The first part of this experiment was concerned with determining plasma conditions at three different times following hemorrhage: 20 min, at the initiation of drinking, and 2 hr after hemorrhage.

Method

Subjects

The animals were 11 male albino rats of the Holtzman strain, weighing 410 ± 31 grams and between 110-120 days old, and were housed under the same conditions as those in Experiment 1.

Apparatus

The apparatus used in this experiment was the same as that used in Experiment 1.

Procedure

Following six days of adaptation to the drinking boxes, at 3 hours per day, 9 of the rats were hemorrhaged of 5 cc from the tail. The first group of 3 rats had a 2 cc heart puncture blood sample withdrawn 20 min post-hemorrhage (20 min group), and a second group of 3 rats had a heart blood sample withdrawn 2 hr post-hemorrhage (2 hr group). Neither of these groups had access to food or water during the delay period. A third group of 3 rats was given access to water following hemorrhage, and at the initiation of drinking a heart blood sample was withdrawn (Initiation group). The remaining 2 rats served as ad libitum baseline controls, having a portion of their ligated tails cut off, and a heart puncture blood sample withdrawn. With this procedure, a sample of the time course of hemorrhage induced blood changes was determined.

Results

In Figure 5-A are presented the means ± S.E. for plasma protein concentration plotted as a function of time following

hemorrhage and for the ad libitum baseline. Mean protein concentration was reliably different across sample times at the 0.01 level of significance, and all means are reliably different from the ad libitum mean at the 0.025 level of significance or better. Hemorrhage had the effect of decreasing protein concentration from ad libitum levels at all times tested.

Presented in Figure 5-B are the means ± S.E. for plasma osmolality plotted as a function of time following hemorrhage, and for the ad libitum baseline. Means for plasma osmolality were reliably different across times sampled at the 0.01 level of significance, but only the osmolality at the initiation of drinking was reliably elevated over the ad libitum level ($t=6.58$, $df=3$, $p<0.005$). The post-hemorrhage latencies to drink for the Initiation group were 44, 56, and 61 min. At 20 min and 2 hr following hemorrhage, plasma osmolalities were not reliably different from one another.

The mean increase in plasma osmolality at the initiation of drinking was 6 mOsm, which represents a 2.1% threshold elevation. This threshold compares closely with the threshold increase of 1.7% obtained under the same conditions in Part B of Experiment 1.

PART B

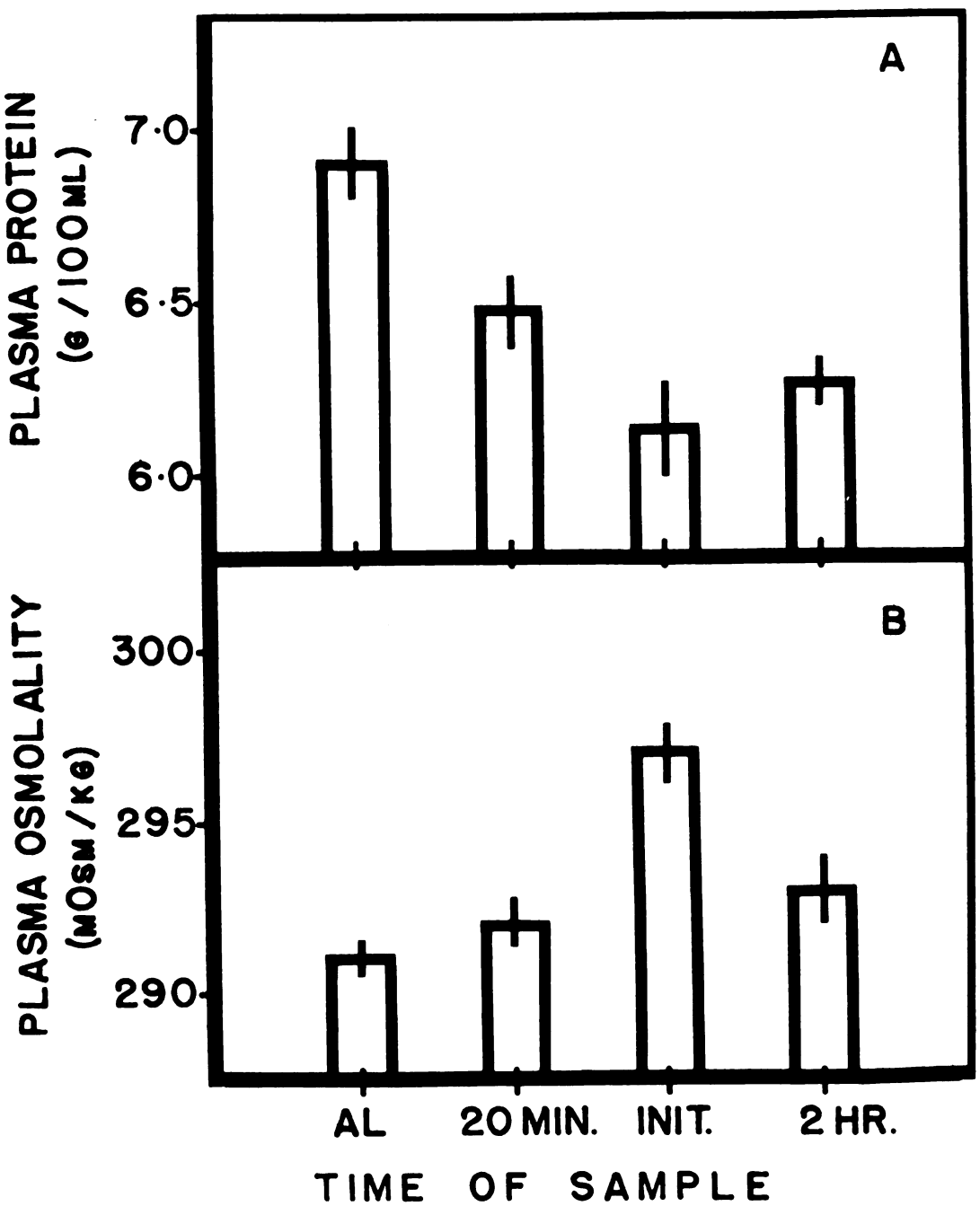
The second part of this experiment was conducted to determine as closely as possible, the actual threshold increase in plasma osmolality sufficient for the production of drinking following hemorrhage. This kind of analysis, of course, required that each rat serve as its own control.

FIGURE 5

A: Mean (±S.E.) for plasma protein concentration for ad libitum baseline, and, 20 min, at the initiation of drinking, and 2 hr following hemorrhage.

B: Mean (±S.E.) for plasma osmolality for ad libitum baseline, and, 20 min, at the initiation of drinking, and 2 hr following hemorrhage. See text for explanation of treatments.

Figure 5


A
B
PLASMA PROTEIN
(g / 100 ML)
7·0
6·5
6·0
PLASMA OSMOLALITY
(MOSM / Kg)
300
295
290
AL
20 MIN.
INIT.
2 HR.
TIME OF SAMPLE

Method

Subjects

In this part of the experiment, 6 of the 11 rats used in Part A were reused. Fifteen days of ad libitum food and water separated Parts A and B of this experiment. The three rats that already had post-hemorrhage latencies to drink determined (Initiation group), as well as 3 other rats randomly selected from the 20 min and 2 hr delay groups were used.

Apparatus

The apparatus used in this experiment was the same as that used in Experiment 1.

Procedure

The 6 rats received the following treatment: first, a 1 cc heart blood sample was withdrawn (which served as each animal's ad libitum baseline sample). This was immediately followed by 4 cc tail hemorrhage, for a total blood loss of 5 cc. The rats were delayed a length of time equal to their 'predicted latency to drink' then a 2 cc heart blood sample was withdrawn. The 3 rats from the Initiation group of Part A, had latencies to drink of 44, 56, and 61 min, so their heart blood samples were taken after these delays.

The other 3 rats were depleted of a total of 5 cc blood in an identical manner, i.e., 1 cc from the heart and 4 cc from the tail. Their second heart blood sample was withdrawn after a delay equal to the overall mean post-hemorrhage latency to drink, i.e., after 53 min delay.

In this part of the experiment, a predicted post-hemorrhage latency to drink was used rather than actually sampling the blood at the behavioral onset of drinking as in Experiment 1. A predicted latency was used because of the debilitating effect of the heart puncture technique used to obtain the ad libitum sample. This procedure made possible the determination of intra-animal changes in plasma conditions from their ad libitum levels to those at the initiation of drinking following hemorrhage. Also, with the use of intra-animal comparisons, more reliable estimates of the actual changes in plasma conditions were possible, although the necessity to use predicted latencies to drink may have introduced a source of variability which would somewhat attenuate the reliability of these estimates.

Results

Plotted on the left side of Figure 6 are the intra-animal changes in plasma protein concentration from ad libitum levels (A) to the predicted post-hemorrhage initiation of drinking (P). On the right side of this figure are the mean changes (± S.E.). Each rat shows a decrease in protein concentration from ad libitum levels to the levels at the predicted post-hemorrhage initiation of drinking, this decrease being reliable at the 0.001 level of significance ($t=7.51$, $df=5$).

In Figure 7, the intra-animal changes in plasma osmolality from ad libitum levels (A) to the levels at

FIGURE 6

Individual plasma protein concentration at the ad libitum baseline (A) and at the predicted post-hemorrhage initiation of drinking (P) for rats 1-6 is presented on the left side. On the right, the mean (±S.E.) for plasma protein concentration for A and P are presented. See text for explanation of treatments.

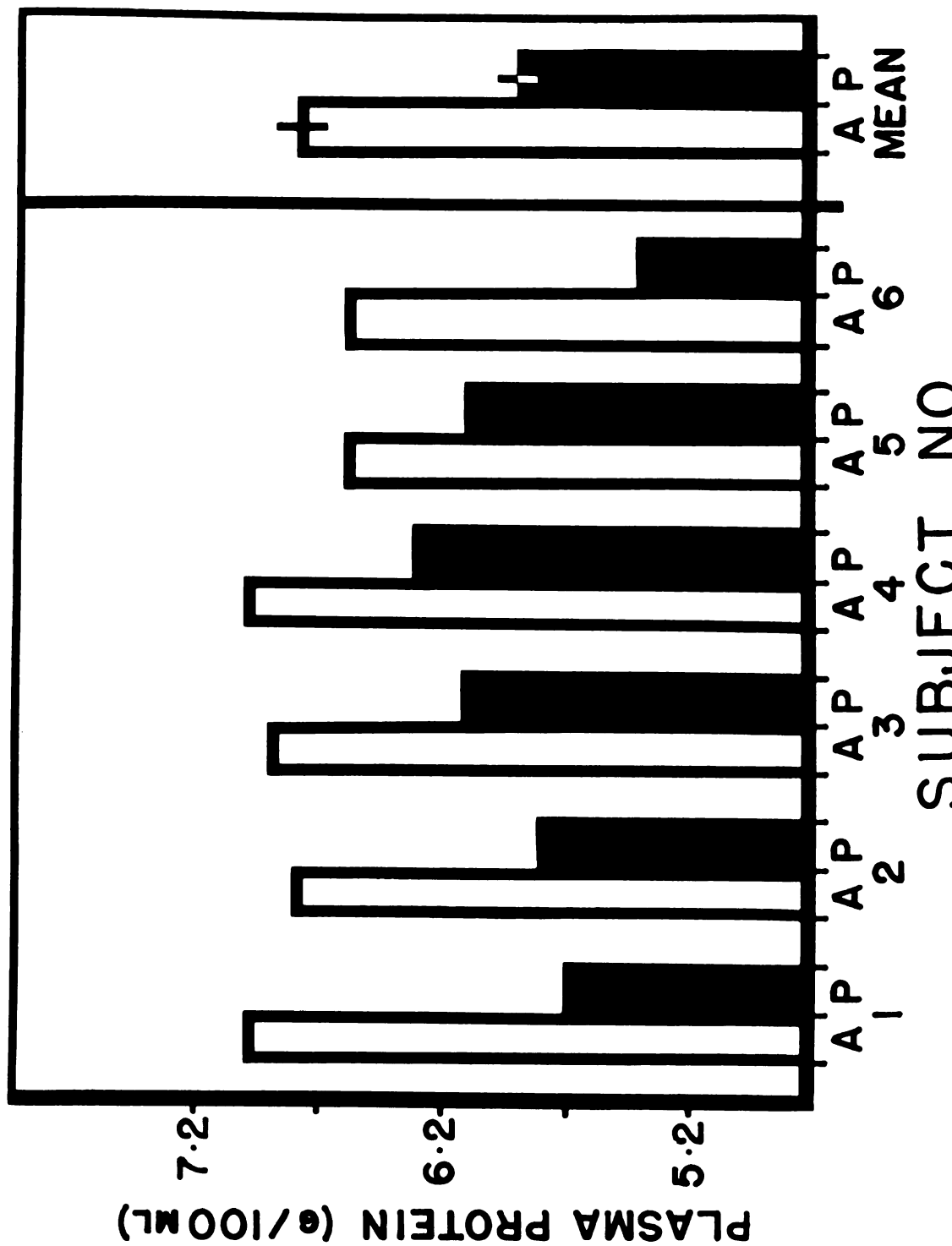

PLASMA PROTEIN (g/100ML)
7.2
6.2
5.2
A P
1
A P
2
A P
3
A P
4
A P
5
A P
6
A P
MEAN
SUBJECT NO.

Figure 6

FIGURE 7

Individual plasma osmolality levels of rats 1-6 at the ad libitum baseline (A) and at the predicted post-hemorrhage initiation of drinking (P) are presented on the left side. On the right, mean (±S.E.) for plasma osmolality is presented for A and P. See text for explanation of treatments.

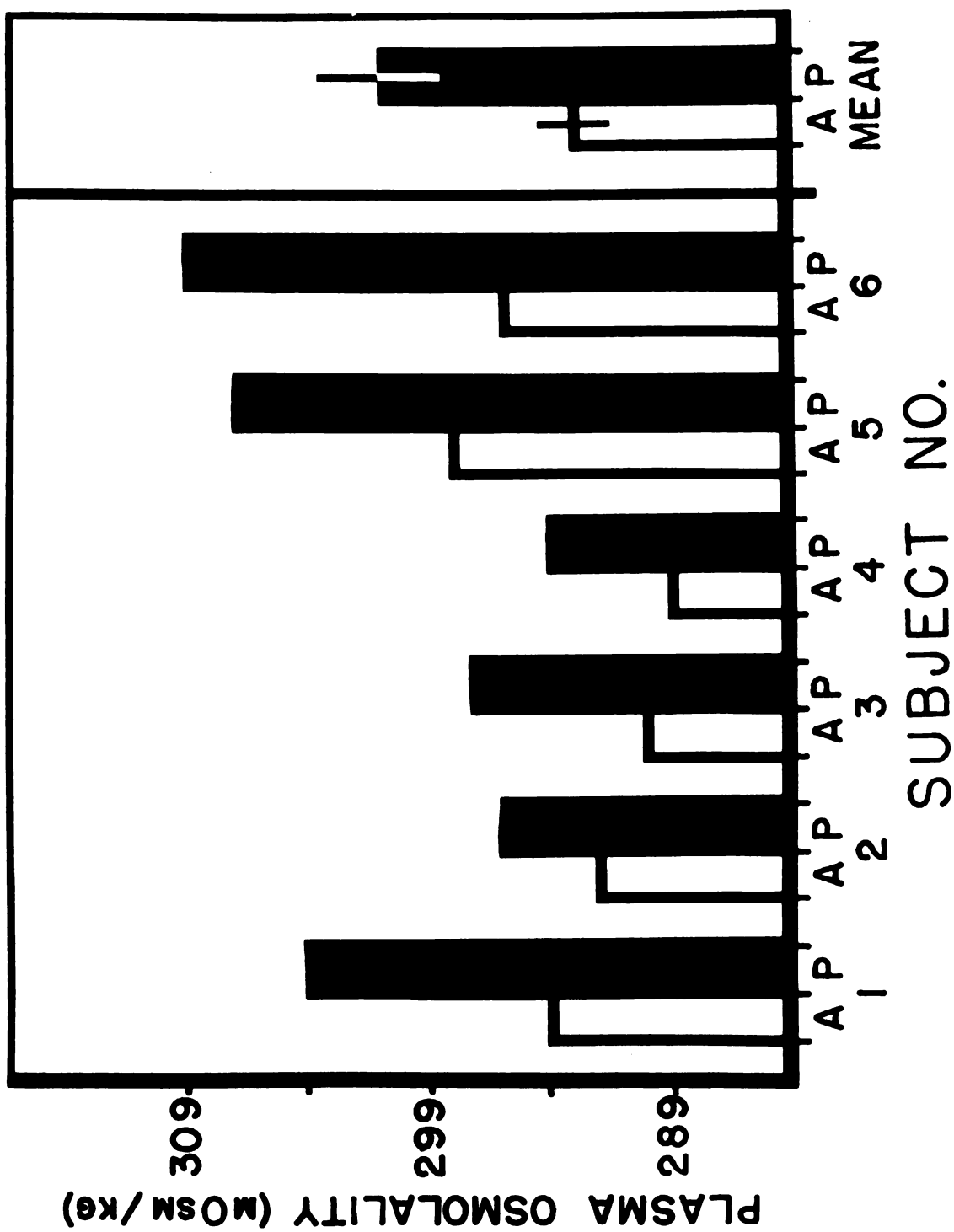

PLASMA OSMOLALITY (MOSM/KG)
309
299
289
A P
1
2
3
4
5
6
MEAN
SUBJECT NO.

Figure 7

the predicted post-hemorrhage initiation of drinking (P) are presented on the left side. On the right, the mean changes (± S.E.) in plasma osmolality are presented. Note that each rat showed an elevation in osmolality at the initiation of drinking, the mean change in this measure being reliable at the 0.01 level of significance ($t=5.86$, $df=5$). The mean increase in plasma osmolality was 8 mOsm, which represents a 2.7% threshold elevation.

Discussion-Parts A and B

In Part A of this experiment, plasma osmolality elevation was found to be coincident with the initiation of drinking following hemorrhage. When plasma was sampled at a time preceding or following the initiation of drinking, no change from ad libitum levels was found. This result seems to indicate that the increase in plasma osmolality takes time to develop, and if water is not available, other regulatory factors come into play. Kidney regulation and the osmotic flow of intracellular and interstitial fluids into the vascular compartment are most likely responsible for the plasma osmolality decrease after the time that drinking would have been initiated had water been available.

It has been assumed by other investigators (Fitzsimons, 1961; Oatley, 1964), that hemorrhage does not produce plasma osmolality changes. This assumption, unfortunately, has not been subjected to experimental verification until now. It is important to note that if post-hemorrhage plasma conditions

are not sampled at the critical time, i.e., at the initiation of drinking, no change from ad libitum levels may be found. If plasma is sampled only after arbitrarily imposed delays, erroneous conclusions could be drawn about the effect of hemorrhage on plasma osmolality changes.

If plasma osmolality returns to ad libitum levels at roughly 2 hr post-hemorrhage, what mechanism could account for drinking after such delays? The answer to this question seems to be cellular dehydration. The elevated plasma osmolality levels (associated with the initiation of drinking) pulls fluids from the cells due to the concentration gradient. This along with some kidney regulation results in decreased plasma osmolality, while increasing the degree of relative cellular dehydration. Thus, cellular dehydration, which is commonly caused by increased plasma effective osmotic pressure, can account for the rat's drinking in the absence of a concurrently measureable elevation in plasma osmolality.

The intra-animal comparisons in Part B of this experiment, further substantiate the finding that hemorrhage results in increased plasma osmolality. The individual threshold elevations ranged from 1.3% to 4.3%, the mean elevation being 2.7%. The size of this range is possibly due to the fact that these plasma samples were obtained at the predicted initiation of drinking, rather than at the actual initiation as was the case in the previous experiments. It is interesting to note, however, that the individual thresholds overlap the thresholds of 1.7% and 2.1% obtained from the inter-animal

comparisons of Experiments 1-B and 2-A, respectively. These data suggest that the 'true' threshold for the initiation of drinking following hemorrhage probably lies between 2% and 3% increase in plasma osmolality.

This 2-3% plasma osmolality threshold for hemorrhage induced drinking has theoretical importance. Using hypertonic saline as an osmotic stimulus, Wolf (1950) obtained a 2.6% threshold for the initiation of drinking for dogs, while Fitzsimons (1963) calculated a 1.6% threshold for rats. Hatton and Almli (1969) extended the generality of such a threshold by determining drinking thresholds for ad libitum rats as well as for rats with differential experience on a 23.5 hr water deprivation schedule. They also determined the drinking threshold after rats ate dry food. The plasma osmolality thresholds that they obtained ranged between 2-3% . For ad libitum rats injected with hypertonic saline the threshold was 2.8%, while a 2.1% threshold was obtained in response to eating dry food.

This 2-3% threshold value has been found for hypertonic saline injection, eating dry food, and now, for hemorrhage. The generality of such results is readily apparent, and strongly supports the notion put forth by Hatton and Almli (1969) that a 2-3% elevation in plasma osmolality "is a consistent and reliable stimulus for the onset of drinking; and that this threshold value is not sensitive to the mode of producing the increase" (p. 213).

One of the most perplexing questions brought about by this series of experiments is how hemorrhage results in plasma osmolality increase. While no simple explanation is presently available, certain physiological mechanisms activated by hemorrhage may be implicated.

Hemorrhage, due to its stressing effect or due to the decreased vascular volume, has been found to result in increased antidiuretic hormone (ADH) release (Ginsberg and Heller, 1953), and increased aldosterone liberation (Farell, Rosnagie, and Rauschkolb, 1956). Fine, Meiselas, and Auerback (1958) have speculated that ADH may be released in response to the rising tonicity of the plasma due to the primary kidney conservation of sodium by aldosterone release. If the rising tonicity of the plasma is sufficient to result in ADH release, then this same rising tonicity may also account for the onset of drinking. With the initiation of drinking occurring about 53 min after hemorrhage, and aldosterone being released within 15 min post-hemorrhage (Mulrow and Ganong, 1961), there seems to be adequate time for such an elevation in osmolality to occur and result in the onset of drinking.

The results of the experiment conducted here also have important implications for the study of hypovolemically induced drinking. Three techniques commonly employed for the production of hypovolemia are: hypertonic glucose dialysis, hemorrhage, and hyperoncotic colloid dialysis (e.g., polyethylene glycol). Previous research (Boyer, Gill, Epstein, 1967;

Share, 1961) indicates that hypertonic glucose dialysis results in plasma osmotic pressure increase, supposedly as a result of glucose entering the vascular compartment. This result, coupled with the plasma osmolality increase demonstrated here in response to hemorrhage, leaves only the hyperoncotic colloid method without a demonstrated plasma osmolality increase.

No detailed analysis of the effects of hyperoncotic colloid dialysis on plasma conditions is available. In most experiments where hyperoncotic colloids are used to produce hypovolemia and drinking, plasma conditions are either not evaluated (Corbit, 1968) or are tested hours after administration (Fitzsimons, 1961; Stricker, 1968; Stricker and Wolf, 1967), and even then are sometimes confounded with the effects of food and/or water deprivation (Stricker, 1966). This state of affairs has led to the perpetuation of an assumption: that hypovolemia is not accompanied by plasma osmotic pressure level changes.

EXPERIMENT 3

The purpose of this experiment was to determine plasma conditions at the initiation of drinking following polyethylene glycol treatment. This experiment was specifically concerned with determining whether or not the hypovolemia induced by polyethylene glycol resulted in plasma osmolality increase similar to that found for hemorrhage.

Method

Subjects

Twelve male albino rats of the Holtzman strain were used in this experiment. The rats were 95-100 days old and weighed 391 ± 22 grams at the start of the experiment. As in the previous experiments, the rats were on ad libitum food and water conditions except while in the drinking boxes where only water was available, and the rats were habituated to the drinking boxes before treatments were administered.

Apparatus

In addition to the apparatus previously described, hypovolemia was produced in this experiment using 5 ml of 20% polyethylene glycol solution (polyethylene glycol, 400 molecular weight), the solvent being physiological saline (0.87% or 0.15 M NaCl).

Procedure

On treatment days, 6 of the rats were injected, subcutaneously, in the back region with 5 ml of 20% polyethylene glycol (PG) solution. The rats were immediately placed in the drinking boxes following injection, and latency to initiate drinking was measured.

At the initiation of drinking, the rats were removed from the drinking boxes, were anesthetized with ether, and a 2 cc heart puncture blood sample was withdrawn. The remaining 6 rats had blood samples withdrawn, and served as

ad libitum baseline controls. The heart puncture blood sample technique and blood plasma analysis were identical to that used in the previous experiments.

Results

Table 1 contains the data (means±S.E.) concerning the effects of polyethylene glycol (PG) treatment on latency to drink, and plasma protein concentration and osmolality at the initiation of drinking. Also presented are ad libitum levels of plasma protein concentration and osmolality.

All rats, except one, initiated drinking within 6.9 to 13.9 min following PG treatment. One rat initiated drinking 21.3 min post-treatment, this longer latency probably being due to extraneous noises which noticeably distracted the rat.

At the PG induced initiation of drinking, plasma protein concentration was reliably elevated over ad libitum levels ($t=1.86$, $df=10$, $p<0.05$).

Plasma osmolality at the initiation of drinking, following PG treatment, was found to be reliably elevated over ad libitum levels ($t=4.89$, $df=10$, $p<0.001$). The increase represents a mean threshold elevation of 3.1%.

While the results presented here are obvious and consistent, a word of caution is necessary. The results of this PG study, and other studies using PG as a means of inducing hypovolemia are questionable, due to the considerations presented in the following discussion.

Table 1

Plasma osmolality and protein concentration of ad libitum rats and for those at the polyethylene glycol (PG) induced initiation of drinking. Latency to drink for PG treated rats also presented.

Condition	Mean (± S.E.)
Osmolality	
Initiation (PG)	300 ± 1.46 mOsm/kg
Ad libitum	291 ± 1.21 mOsm/kg
Protein concentration	
Initiation (PG)	7.02 ± 0.08 gm/100 ml
Ad libitum	6.80 ± 0.09 gm/100 ml
Latency to drink (PG)	12.3 ± 2.1 min

Discussion

A small, but reliable, plasma protein concentration increase was found at the initiation of drinking following PG treatment. This result, however, may not reflect a plasma volume decrease as previously thought. The measured increase in plasma protein concentration index may be the result of PG entering the vascular compartment. This hypothesis is tenable because molecules of a molecular weight of up to 5000 appear to pass readily across the capillary membrane (Ruch and Patton, 1965). The twenty per cent PG solution used in this experiment almost assuredly crosses the vascular membrane, and gives a protein concentration index of well over 13.0 gm/100 ml, which is the upper limit of normal plasma protein concentration. For physiological saline (0.87% NaCl solution) the protein concentration index was off the refractometer scale in the opposite direction, i.e., below 2.5 gm/100 ml. The total solids readings for 20% PG solution and physiological saline were 1.3632 and 1.3346, respectively. Thus, if PG molecules are entering the vascular compartment, the use of protein concentration as an index of plasma volume could be grossly misleading.

The increased protein concentration index obtained with the use of PG may lead to two contradictory, but equally plausible, interpretations. First, it may indicate

that the PG is in fact reducing plasma volume to the extent given by the protein concentration index, or second, it may merely be the result of PG crossing the vascular membrane and entering the vascular compartment. The inability to accurately determine the true state of affairs requires that the effects of PG on plasma volume levels be subject to further experimentation, lest further erroneous conclusions be drawn.

As with hemorrhage, PG treatment resulted in elevated plasma osmolality at the initiation of drinking. Other investigators (Fitzsimons, 1961; Stricker,1966) have not previously found elevated plasma osmolality in response to PG treatment. This state of affairs may be due to the fact that they used PG of sufficient molecular weight that no osmotic pressure changes were produced, or because they did not sample plasma conditions at the critical time, i.e., at the initiation of drinking. Typically, plasma determinations have been made one or more hours after PG administration, and have not been yoked to the animal's behavior as in the present experiment. If PG treatment results in plasma osmolality changes similar in time course to those found for hemorrhage, then temporal factors become of primary importance.

Hemorrhage resulted in plasma osmolality elevation at the initiation of drinking. Before or after that time, ad libitum levels were obtained. The decrease in plasma osmolality after the time that drinking would have first

occurred, had it been allowed, was probably accomplished by other regulatory mechanisms such as: kidney regulation and the osmotic flow of fluids from the intracellular and interstitial compartments into the vasculature. If PG treatment parallels this single transient increase in plasma osmolality, this may account for the failure of others to find it because they were sampling plasma conditions hours after PG administration. The drinking that they obtain after similar delays, and after the osmolality elevation has been decreased by other regulatory mechanisms, could be due to cellular dehydration. Cellular dehydration could be the result of the elevated plasma osmolality pulling fluids from the cells and interstitial compartment, thereby reducing plasma osmolality and increasing cellular dehydration. Such cellular dehydration could result in the animal drinking significant amounts of water without increased plasma osmolality.

While the above considerations are consistent with the data obtained here, and somewhat in agreement with the results of other studies, a caution is also in order here. If, as referred to in the protein concentration discussion, PG molecules cross the vascular membrane and enter the vascular compartment, then another interpretation is possible. Polyethylene glycol is essentially an antifreeze, being frequently used in car radiators in winter. If PG molecules enter the vasculature, the result could be enhanced freezing-point depression, and hence elevated plasma osmolality. This possibility was tested.

A 0.5 ml plasma sample was subjected to osmolality determination, and retested after 0.005 ml of 20% PG solution was added. The nontreated sample was found to be 301 mOsm. However, after the 20% PG solution was added, the determination was increased by 21 mOsm, to 322 mOsm. If PG molecules are entering the vascular compartment, the increased plasma osmolality found at the initiation of drinking may be due to the PG exerting its effect on freezing-point depression. Thus, the measured increase in plasma osmolality after PG treatment may not reflect a biological phenomenon, as was the case for hemorrhage, but may be the result of PG entering the vasculature and contaminating the plasma sampled.

Explanation of the water intake data easily follow from the above considerations. After PG treatment, the rats may be drinking in response to cellular dehydration. Cellular dehydration could be caused by PG molecules entering the vascular compartment, thereby increasing the oncotic pressure of the plasma. This increased oncotic pressure could draw fluids from the intracellular and interstitial compartment, leaving the cells in states of relative dehydration. Again, these hypotheses may not be accurate, if other investigators use PG of sufficient molecular weight that none crosses the vascular membrane. Future experimentation is required to accurately determine the effects of PG on blood plasma conditions.

In this series of experiments there seems to be one rather profound, and consistently obtained result: that the onset of drinking is accompanied by plasma osmolality increase. This was true whether drinking was induced by hypertonic saline injection, hemorrhage, or PG treatment. While this result was expected for hypertonic saline injection, it is contrary to the assumption that hypovolemia is unaccompanied by plasma osmotic pressure increase.

The assumption that hypovolemia does not result in plasma osmolality increase has been perpetuated due to the lack of importance given to the animal's behavior, and should point up the necessity of making physiological measurements pertinent to the behavior studied. If a given manipulation is thought to result in drinking, it would be logical to determine the physiological effects of such manipulations at the critical time, at the onset of drinking, rather than after some arbitrarily imposed time delay. Investigators may impose time delays, but because these time delays are not respected by physiological regulatory systems, misleading results may be obtained.

In Table 2 are presented the data concerning latency to drink and plasma osmolality conditions at the initiation of drinking for this series of experiments. (The hemorrhage data was combined; and the overall mean is presented.) It is easy to see from this table that the post-treatment latencies to drink are rather widely dispersed. This temporal measure was not very consistent across the different treatments. However,

Table 2

Mean (± S.E.) threshold values of plasma osmolality (mOsm and %) and mean (± S.E.) latencies to drink (min) for hypertonic saline injection, hypertonic saline injection plus hemorrhage, hemorrhage, and polyethylene glycol treatments.

Treatment Condition	mOsm	Percent	Latency
Hypertonic saline injection	11 ± 1.68	3.7%	17.39 ± 2.76
Injection plus hemorrhage	12 ± 0.87	4.0%	30.08 ± 6.92
Hemorrhage*	6 ± 1.64	2.2%	53.12 ± 9.35
Polyethylene glycol	9 ± 1.46	3.1%	12.33 ± 2.14

*Note: Hemorrhage data combined across experiments.

a high degree of consistency was obtained for the elevations in plasma osmolality at the initiation of drinking. The thresholds obtained for all treatments compare closely to the 2-3% thresholds obtained by Hatton and Almli (1969) using hypertonic saline injections and the eating of dry food as osmotic stimuli.

The consistency with which the onset of drinking is associated with 2, 3, and 4% increases in plasma osmotic pressure is becoming of primary importance. This threshold magnitude has been obtained across a variety of conditions, e.g., hypertonic saline injection, eating dry food, hemorrhage, and PG treatment. The ubiquity of such results suggests that the need for a dual detector system (one for increases in effective osmotic pressure and one for reduction in plasma volume) for the control of drinking behavior may be non-existent. While volume sensitive entities are probably necessary for such regulatory phenomena as blood pressure, vasomotor activities, etc., an osmosensitive neural detector may be all that is required for the control of drinking behavior. While the results of this series of experiments are strongly suggestive, much research is needed before the hypothetical dual detector system for the control of drinking behavior can be completely ruled out.

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APPENDIX

APPENDIX

Raw Data

Experiment 1

Part A

Hourly water intakes (ml) following; injection of hypertonic saline (I), hemorrhage (H), injection of hypertonic saline plus hemorrhage (IH), and tail-cut control (T), for rats 1-5.

S no.	Treatment	Hourly water intake						
		1	2	3	4	5	6	Total
1	I	9.0	1.2	3.0	1.0	0.0	0.0	14.2
	H	0.6	0.4	0.0	2.0	0.0	0.6	3.0
	IH	3.2	6.4	5.6	1.4	1.4	0.0	18.0
	T	0.0	0.0	0.0	1.0	1.4	0.2	2.6
2	I	10.4	3.4	1.0	0.6	0.0	0.0	15.4
	IH	9.8	2.2	0.4	3.6	3.4	0.0	19.4
	H	0.6	2.8	0.8	1.1	0.0	0.0	5.3
	T	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	H	0.6	2.2	1.4	0.0	1.0	0.2	5.4
	I	6.4	5.0	0.2	0.2	0.0	0.0	11.8
	IH	12.1	1.5	0.8	1.0	1.0	0.2	16.6
4	H	0.0	0.2	0.0	0.0	0.0	0.2	0.4
	IH	4.0	2.2	1.2	0.0	0.0	0.3	7.7
	I	7.6	0.0	2.2	0.0	0.0	0.0	9.8
	T	0.0	0.0	0.0	0.0	1.8	0.2	2.0
5	IH	7.6	3.5	1.1	0.0	0.7	0.6	13.5
	H	2.0	0.6	0.2	0.0	0.1	0.0	2.9
	I	7.4	2.0	0.8	0.0	0.0	0.0	10.2
	T	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Experiment 1

Part A

Latency to drink following; injection of hypertonic saline (I), hemorrhage (H), injection of hypertonic saline plus hemorrhage (IH), and tail-cut control (T), for rats 1-5.

S no.	Treatment	Latency to drink Minutes:Seconds	Latency to drink Seconds X 10^{-3}
1	I	19:35	0.00085
	H	34:15	0.00049
	IH	24:55	0.00067
	T	225:00	
2	I	14:15	0.00117
	IH	24:25	0.00068
	H	48:15	0.00035
	T	Never drank	
3	H	50:25	0.00033
	I	17:30	0.00095
	IH	30:03	0.00056
4	H	112:35	0.00015
	IH	47:20	0.00035
	I	24:15	0.00068
	T	285:00	
5	IH	29:20	0.00057
	H	54:40	0.00031
	I	18:05	0.00092
	T	Never drank	

Experiment 1

Part A

Body weights (grams) for rats 1-5 receiving treatments of; injection of hypertonic saline (I), hemorrhage (H), injection of hypertonic saline plus hemorrhage (IH), and tail-cut control (T).

S no.	Treatment			
	I	H	IH	T
1	452	485	505	508
2	463	493	494	498
3	480	460	492	
4	510	474	490	518
5	419	477	457	424

Experiment 1

Part B

Plasma osmolality (mOsm/kg) and protein concentration (grams/100 ml) for ad libitum rats, and for rats at the initiation of drinking following; injection of hypertonic saline (I), hemorrhage (H), and injection of hypertonic saline plus hemorrhage (IH). Subject number in parentheses.

Condition	Osmolality	Protein concentration
Ad libitum	301 (7)	6.9 (7)
	300 (8)	6.9 (8)
Ad libitum delay	300 (7)	7.0 (7)
	298 (8)	7.1 (8)
I	307 (1)	6.4 (1)
	315 (2)	6.6 (2)
	310 (4)	6.6 (4)
	312 (5)	6.7 (5)
H	302 (1)	6.0 (1)
	303 (3)	6.0 (3)
	305 (4)	5.8 (4)
	309 (6)	6.0 (6)
IH	312 (2)	5.5 (2)
	313 (3)	5.6 (3)
	312 (5)	5.2 (5)
	309 (6)	5.4 (6)

Experiment 1

Part B

Latency to drink and body weights (grams) for ad libitum rats, and for rats at the initiation of drinking following; injection of hypertonic saline (I), hemorrhage (H), and injection of hypertonic saline plus hemorrhage (IH). Subject number in parentheses.

Condition	Latency to drink Minutes:Seconds		Seconds X 10^{-3}	Body Wt.
Ad libitum				429 (7)
				413 (8)
Ad libitum delay				390 (7)
				446 (8)
I	18:07	(1)	0.00092	380 (1)
	14:09	(2)	0.00118	400 (2)
	13:54	(4)	0.00119	419 (4)
	17:46	(5)	0.00094	408 (5)
H	56:10	(1)	0.00029	411 (1)
	51:10	(3)	0.00033	405 (3)
	37:15	(4)	0.00045	398 (4)
	40:05	(6)	0.00042	431 (6)
IH	33:51	(2)	0.00049	446 (2)
	24:34	(3)	0.00068	432 (3)
	29:46	(5)	0.00056	378 (5)
	27:41	(6)	0.00060	390 (6)

Experiment 2

Part A

Plasma osmolality (mOsm/kg), protein concentration (grams/100 ml), and body weight (grams) for *ad libitum* rats, and for rats at 20 minutes, at the initiation of drinking, and 2 hours following hemorrhage.

Condition	Osmolality	Protein Conc.	Body Wt.
Ad libitum	291	7.0	484
	291	6.8	500
20 min. delay	291	6.6	478
	293	6.4	486
	293	6.4	494
Initiation	296	6.2	509
	298	6.3	460
	296	5.9	492
2 hour delay	295	6.3	471
	292	6.3	493
	292	6.2	489

Experiment 2

Part A

Plasma osmolality (mOsm/kg), protein concentration (grams/100 ml), and body weight (grams) for rats sampled while under ad libitum conditions and again sampled at the initiation of drinking following hemorrhage.

S no.	Condition	Osmolality	Protein Conc.	Body Wt.
1	Ad libitum sample	294	7.0	480
	Initiation sample	304	5.7	
2	Ad libitum "	292	6.8	504
	Initiation "	296	5.8	
3	Ad libitum "	290	6.9	493
	Initiation "	297	6.1	
4	Ad libitum "	289	7.0	514
	Initiation "	294	6.3	
5	Ad libitum "	298	6.6	482
	Initiation "	307	6.1	
6	Ad libitum "	296	6.6	501
	Initiation "	309	5.4	

Experiment 3

Plasma osmolality (mOsm/kg), protein concentration (grams/100 ml), and body weights (grams), for rats under *ad libitum* conditions, and for polyethylene glycol treated rats at the initiation of drinking. Latencies to drink (minutes:seconds) for polyethylene glycol treated rats.

Condition S no.	Body Wt.	Lat. to drink Min.:Sec.	Osmolality	Protein Conc.
Poly. gly.				
1	387	13:57	304	7.0
2	336	6:54	298	7.0
3	351	11:15	299	6.9
4	357	13:04	302	7.2
5	379	21:22	301	7.2
6	362	7:27	294	6.8
Ad lib.				
7	385		291	7.2
8	349		291	6.8
9	386		289	6.7
10	377		288	6.5
11	368		289	6.9
12	372		296	6.7