EFFECT OF RESTORATION OF BODY FLUID ON FOOD AND WATER INTAKE IN WATER DEPRIVED RATS

by

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STATEMENT BY AUTHOR

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ABSTRACT

After rats were deprived of water for 21-1/2 hours, one of the following 14 treatments was given to each subject: intraperitoneal injection of 2, 4, 6, or 8 cc of water, 2, 4, 6, or 8 cc of 0.45 per cent NaCl solution, or 2, 4, 6, or 8 cc of 0.9 per cent NaCl solution; or one of two control conditions which consisted of sham injection or a 30-minute period with free access to water. Thirty minutes following the treatment either food or water was presented for one hour to assess the effects of the treatment. This period was followed by a one-hour recovery period during which both food and water were provided. The results indicated that injection of isotonic saline failed to either reduce water intake or increase food intake beyond the sham control level, but injections of distilled water and hypotonic saline altered water and food intake in proportion to the amount injected. Overall, injection of 0.45 per cent saline was half as effective as water injection on both food and water intake. The complex thirst induced by water deprivation was not reduced by extracellular volume restoration. The conclusion was reached that intracellular hydration is necessary to reduce the complex thirst which subsequently induces appetite for food.

CHAPTER 1

INTRODUCTION

It has been shown that thirst inhibits food intake. Epstein and Teitelbaum (1964) found that lateral hypothalamic lesioned rats were adipsic, and therefore did not consume food. The amount of food eaten during 24 hours by water deprived rats was about 60 per cent of the amount eaten by those which were not water deprived (Bolles, 1961). Verplanck and Hayes (1953) reported a similar result and found that reduction in food intake resulted from the animals' consuming less food at a given time rather than eating less frequently. They explained this interaction between hunger and thirst by the fact that large quantities of water are used by the rat in digesting dry food, so that when its food supply is restricted, its water requirements are correspondingly reduced.

When water is made available after a period of water deprivation, rats first drink and then eat to make up the self-imposed food deficit (Verplanck and Hayes, 1953). Also, food related instrumental responses have been shown to increase after water deprived rats are given the opportunity to drink (Grice and Davis, 1957). Thus, it appears that a rat's appetite returns after hydration.

When a restricted amount of water is given, rats reduce food intake proportionally to maintain their water balance (Collier and Knarr, 1966; Collier and Levitsky, 1967). There is a reduction in body mass which maintains the ratio of body water to lean body mass. This ratio is approximately 0.7. Rats without water consume about two-thirds the amount of dry food during a two-hour meal as compared to rats eating a meal with water, but the stomach contents remain about 50 per cent water after either condition. This result suggests that rats regulate their food intake to match the amount of water that can be mobilized from body tissue to maintain the proper gastric water-food ratio. The water seems to be removed primarily from the skin and probably the adipose tissues. Similarly, the intestinal lumen contains about 75 per cent water, whether or not water is available with meals (Lepkovsky, Lyman, Fleming, Nagumo, and Dimick, 1957).

Water deprivation has been regarded as inducing a complex thirst which is a combination of both osmotic and volemic influences (Corbit, 1969). It was shown that hyperosmolarity and hypovolemia combine to increase thirst (Corbit, 1968; Fitzsimmons and Oatley, 1968; Oatley, 1964). The homeostatic maintenance of body fluids appears to be accomplished by two types of receptors, one type responsive to changes in the osmolarity of the extracellular fluid, or to concomitant changes in intracellular fluid volume, and the other type responsive to changes in intravascular fluid volume. Sodium and chloride ions occur mainly in the extracellular fluid. While these ions retain water, water by itself readily enters cells. Consequently, isotonic saline loads should result in expansion of the extracellular compartment without expansion of the intracellular compartment. On the other hand, water injected into the extracellular fluid compartment enters the intracellular fluid compartment because of osmotic pressure. In this way the intracellular compartment can become hydrated. Stricker and Wolf (1967) found that volemic thirst was reduced more effectively with isotonic saline loading than with water loading, whereas osmotic thirst was reduced more effectively with water loading. Their dependent variable measure was the amount of water intake. However, it is not clear whether the complex thirst which is a function of both of the above factors can be partially reduced by either saline or water alone.

The present study investigated (a) the nature of the thirst induced by water deprivation, (b) the quantitative relationship between restoration of body fluid osmolarity or volume after water deprivation and the return of appetite, and (c) the quantitative relationship between restoration of body fluid osmolarity or volume after water deprivation and the reduction of thirst.

CHAPTER 2

METHOD

Subjects and Apparatus

Twenty-eight female Wister albino rats approximately 110 days old, weighing between 241 and 298 gm., were individually housed in a constantly illuminated laboratory with temperature of 72°F and humidity about 45 per cent. The experiment was conducted using a standard bank of 6 by 5 cages which the animals occupied at all times. A 100 cc graduated tube with a metal tip was used to measure water intake. Food intake was measured by use of a 250 cc glass beaker fastened to a corner of the cage. Powdered Purina Lab Chow was introduced into the beaker for eating. Spillage was minimal since the subject always stood at the rim to eat with its head inside the beaker. Food intake was measured to the closest 0.1 gm. and water intake to the closest 1 cc.

Procedure

After the subjects were adapted to eating from the beaker for 10 days, they were deprived of water for 21-1/2 hours. After deprivation one of the following 14 treatments was given to each subject: there were 12 experimental conditions which consisted of intraperitoneal injection of 2, 4, 6, or 8 cc of distilled water, 2, 4, 6, or 8 cc of 0.45 per cent NaCl solution, or 2, 4, 6, or 8 cc of 0.9 per cent NaCl solution; in addition, there were two control conditions which consisted of sham injection with needle puncture only or a 30-minute period with free access to water. Thirty minutes after the injections and immediately after the 30-minute period with free access to water, either food or water was presented for a one-hour period to assess the effect of the treatments. This period was followed by a one-hour recovery period with both food and water presented before the subsequent water deprivation began.

There were a total of 28 conditions with 14 treatments and 2 intake measures. All 28 conditions were given to each subject in a sequence determined by a 28 by 28 completely balanced Latin square design (Cochran and Cox, 1957) which insured that every condition was preceded by all other conditions. This design balanced possible carry-over effects from preceding conditions.

Solution concentrations were in percentage per volume basis; e.g., 0.9 per cent saline solution was prepared by adding 0.9 gm. of NaCl (Analytical Reagent) to 100 cc distilled water.

One subject died of unknown cause one day before the end of the experiment, but its data were included in the results.

Intraperitoneal injection was used in the present study because Adolph, Barker, and Hoy (1954) reported that hypertonic intraperitoneal injection influenced water intake more effectively than stomach loading as late as six hours after injection. Also, intraperitoneal injection (differing from stomach loading) does not produce stomach distention which prevents eating or drinking (Moyer and Bunnell, 1962).

CHAPTER 3

RESULTS

Figure 1 shows the effects of injecting varying amounts of water or saline solutions on subsequent water intake. Analysis of variance indicated that the differences in mean water intake between the three solutions, between the amounts, and the interaction were all statistically significant ($\underline{P}<.01$). This analysis is summarized in Table 1. The mean intake after "nothing" did not differ significantly from 0.9 per cent saline means, though it differed from 0.45 per cent saline or water mean intake. During the 30-minute period with free access to water, the subjects drank a mean of 9.9 cc of water and subsequently drank 1.1 cc in one hour; this water intake was significantly lower than all other intake means.

Figure 2 shows the effects of injections of water or saline solutions on food intake. The differences in mean food intake between the solutions, between the amounts, and the interaction were all statistically significant. The results of this analysis of variance are summarized in Table 2. The mean food intake after "nothing" differed significantly from the food intake means following water and 0.45 per cent saline treatments but not from the 0.9 per cent saline means. During the 30-minute period with free access to water, the subjects drank a mean of 9.9 cc of water and subsequently ate a mean of 5.1 gm.; this differed significantly from all other food intake means.

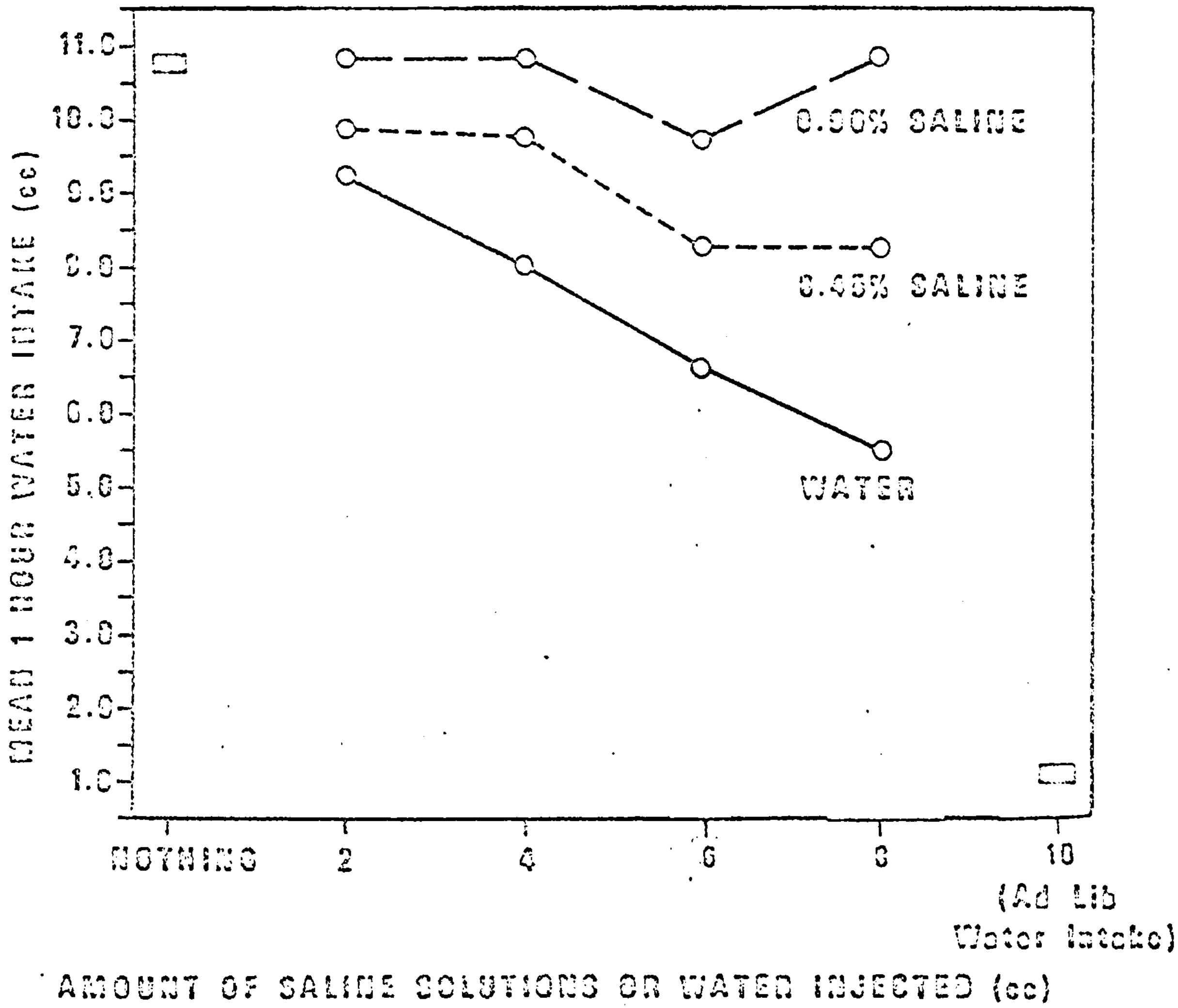

Fig. 1. Water Intake as a Function of Amount and Concentration of Saline Solutions Injected

"Nothing" indicates sham injection control. "Ad lib. water intake" indicates the amount of water drunk during a 30-minute period with a mean intake of 9.9 cc.

Table 1. Summary of Analysis of Variance:
Effects of Water and Saline Solution Injections on Water Intake

Source	df	MS	F	P
Solution Injected	2	298.46	71.57	<.01
Amount Injected	3	68.26	15.20	<.01
Solution x Amount	6	16.41	4.66	<.01
Subjects	27			
Subjects x Solution	54	4.17		
Subjects x Amount	81	4.49		
Subjects x Solution x Amount	158	3.52		
Total	331			

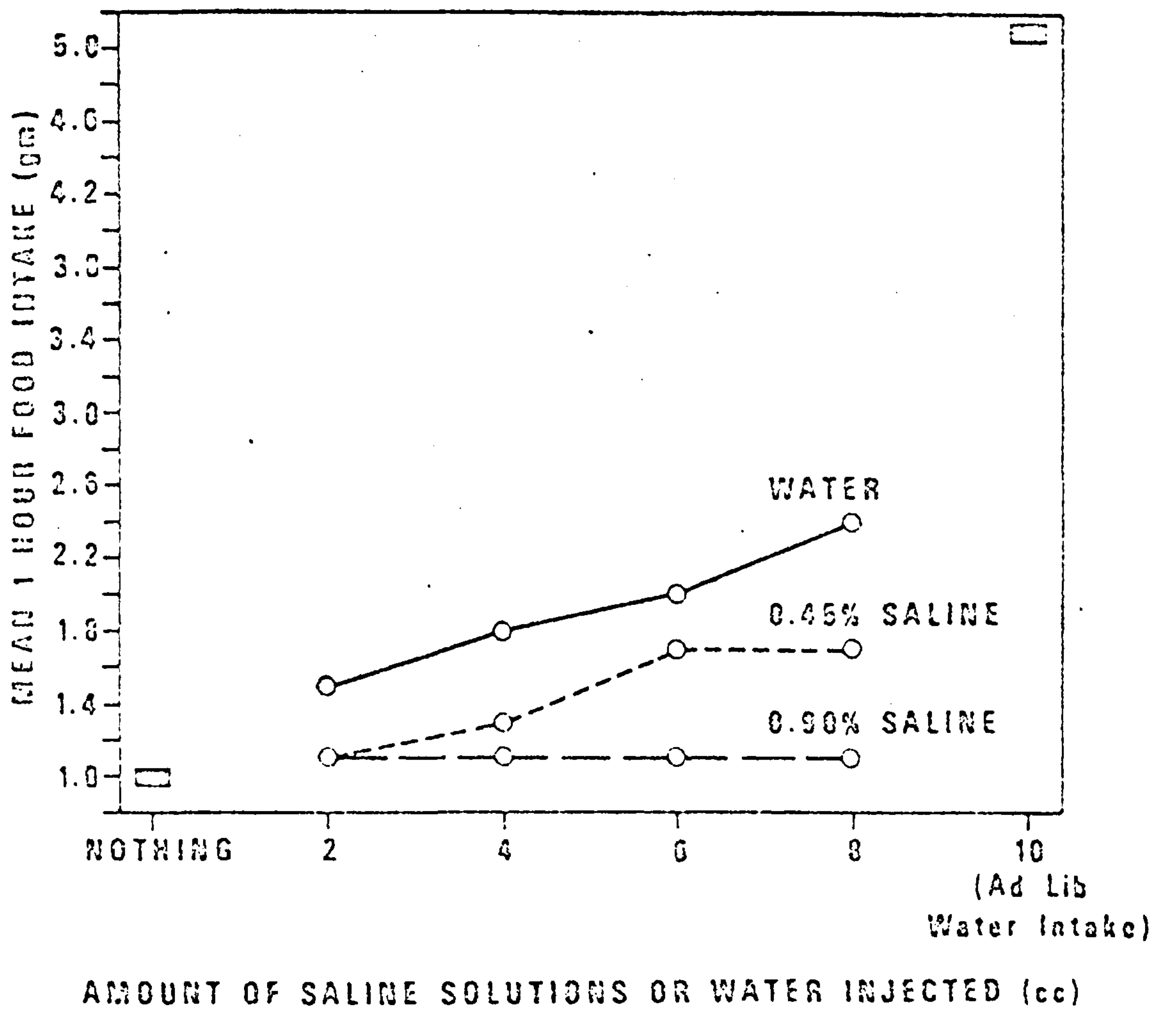

Fig. 2. Food Intake as a Function of Amount and Concentration of Saline Solutions Injected

"Nothing" indicates sham injection control. "Ad lib. water intake" indicates the amount of water drunk during a 30-minute period. The effect of this period of drinking on food intake is shown.

Table 2. Summary of Analysis of Variance:
Effects of Water and Saline Solution Injections on Food Intake

Source	df	MS	F	P
Solution Injected	2	21.20	22.08	<.01
Amount Injected	3	4.54	16.81	<.01
Solution x Amount	6	1.32	7.33	<.01
Subjects	27			
Subjects x Solution	54	.96		
Subjects x Amount	81	.27		
Subjects x Solution x Amount	160			
Total	333			

Since the subjects drank a mean of 10.7 cc of water after sham injection, the mean reduction in water intake was 1.5, 2.7, 4.1, and 5.2 cc respectively after 2, 4, 6, and 8 cc of water injected. Comparable figures for the increase in food intake were 0.5, 0.8, 1.0, and 1.4 gm. After sham injection food intake was 1.0 gm. For food intake the water/food ratio was 4, 5, 6, and 5.7 cc of injected water for eating one extra gm. of food and for water intake the ratio of injected water per cc of reduced water intake was 1.3, 1.5, 1.5, and 1.5 cc respectively for 2, 4, 6, and 8 cc of water injected. Injected water appeared to diminish in effectiveness with increased amounts in the case of food intake only and not in the case of water intake.

Since the mean increase in food intake after water injection was 0.93 gm., it can be concluded that water injection increased food intake, overall, by 0.93 gm. and, similarly, reduced water intake by 3.4 cc compared with the sham injection control. The mean amount of reduced water intake was 68 per cent of the amount of water injected. Injection of 0.45 per cent saline solution increased food intake by a mean of 0.45 gm. and reduced water intake by a mean of 1.6 cc. The mean amount of reduced water intake was 32 per cent of the amount of 0.45 per cent saline injected. Thus, the 0.45 per cent injection was almost exactly half as effective as water injection on both food and water intake.

If reduced water intake was taken as the extent of hydration, it can be calculated that, overall, 3.5 cc (ratio of 0.93 gm. to 3.4 cc) of water injection-induced "hydration" was effective for eating one gm.

of food. The comparable figure after *ad lib.* water intake was 2.3 cc (ratio of 4.1 gm. to 9.6 cc).

CHAPTER 4

DISCUSSION

Complex thirst after water deprivation has been regarded to be both volemic and osmotic (Corbit, 1969; Wolf, 1950). Since ingestion of dry food mobilizes body water into the stomach as shown by Lepkovsky et al. (1957), water deprivation may produce volemic as well as osmotic thirst depending upon the osmolarity of the liquid drawn into the stomach.

The results indicate that reduction of thirst after water deprivation induces appetite and that isotonic volemic restoration of body fluid is not enough to reduce complex thirst. Isotonic saline is effective in restoring extracellular but not intracellular fluid volume; water is more effective in restoring intracellular fluid volume. Restoration of extracellular fluid volume alone does not reduce thirst and increase appetite. Apparently cellular dehydration is the main cause of thirst and inhibition of eating. Water or hypotonic treatment is necessary to restore intracellular fluid volume which reduces thirst and, consequently, induces eating. Hsiao and Perline (unpublished data)* showed that thirst induced by total deprivation was also reduced by water but not by isotonic saline. Corbit (1967) has shown that hypervolemia does not alter drinking in rats (Corbit and Tuchapsky, 1968).

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Osmotic thirst induced by hypertonic saline solution and volemic thirst induced by bleeding or a hyperoncotic colloidal solution (osmotic pressure due to a colloidal solution) add to increase thirst (Corbit, 1968; Oatley, 1964). It is not known whether this experimentally induced complex thirst can be reduced partially by water or isotonic saline, but the present results indicate that complex thirst induced by water deprivation can be reduced only by water or hypotonic solution. Stricker and Wolf (1967) have shown that volemic thirst is reduced by isotonic saline and osmotic thirst by water, but reduction of complex thirst seems to require restoration of intracellular volume.

The results also indicate that the extent of restoration of the intracellular volume, resulting from varying amounts of water or hypotonic saline injection, is proportionally related to reduction of thirst. Reduction of thirst, in turn, is related to the return of appetite. The effectiveness of 0.45 per cent saline is almost exactly half that of water injection. Thus, within a short period, before the kidney effect of renal conservation of fluid can occur, the animal seems to function as a perfect osmometer, as proposed by Corbit (1969).

Hsiao and Bosse (1969) reported that rats prefer isotonic saline to water in a double-bottle test after total deprivation. However, when food was introduced, the rats switched their preference to water. Since rats can eat more food when given with water than with isotonic saline as the sole liquid source (Hsiao, 1967), water may have been chosen to maximize food intake. This result supports the present finding that food intake may induce hyperosmolarity and hypovolemia, but water is needed to reduce thirst and enable the rats to eat food; reduction of hypovolemia alone is not sufficient. It should be noted that Stricker and Wolf (1967) have shown that rats still prefer isotonic saline to water after hypertonic treatment. They explained this result in terms of taste and speed of absorption. Thirst alone may not be enough to produce a change of preference. Some influence from food ingestion seems to be required for the change.

It might be noted that thirst is increased in rats to a much greater extent after water deprivation than after total deprivation of food and water. Hsiao (1967) reported only 3.6 cc of water intake in one hour after 24-hour total deprivation, whereas 10.7 cc of water was drunk in one hour in this study after 22-hour deprivation (sham injection control).

Efficiency of hydration via water injection on food intake was 3.5 cc/gm. of food eaten. This figure is comparable to the efficiency measure of water drunk for eating food during a 24-hour period which was also 3.5 cc/gm. of food eaten (Hsiao and Lloyd, 1969). After ad lib. water intake rats ate more food per cc of water drunk (2.3 cc/gm.). This suggests that, within a short period, hydration accompanied by consummatory responses is more effective than hydration without those responses. This effect has been shown clearly by O'Kelly (1954) and O'Kelly and Beck (1960).

Overall, the following conclusions can be made:

1. Thirst induced by water deprivation seems to result from intracellular dehydration rather than extracellular dehydration. Water and hypotonic saline injections alter the extracellular fluid osmolarity which, in turn, hydrates the

intracellular compartment. On the other hand, isotonic saline injections expand the extracellular fluid volume without hydrating the intracellular compartment. Water and hypotonic saline injections are effective in reducing subsequent water intake and increasing subsequent food intake although isotonic saline injections do not produce any effect on later food and water intake.

2. The increase in food intake is proportional to the amount of water and hypotonic saline solution injected, but not proportional to the amount of isotonic saline injected.
3. Similarly, the decrease in water intake is proportional to the amount of water and hypotonic saline solution injected, but not proportional to the amount of isotonic saline injected.

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