Sodium intake in the Mongolian gerbil (*Meriones unguiculatus*) consequent to subcutaneous formalin injections

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Gerbils, like rats, respond to subcutaneous formalin injections with a dramatic but transitory increase in NaCl drinking. Unlike with rats, this polydipsic response does not reach maximum for at least 72 h and is not dose-related; nor does it occur with repeated testing. Since the adrenalectomized gerbil has already been shown to respond differently to NaCl when compared to the adrenalectomized rat, the results of these experiments are not particularly surprising. However, the fact that the control Ss injected subcutaneously with isotonic saline in all three experiments also increased NaCl ingestion is perplexing. The sodium reservoir hypothesis and electrolyte metabolism stability in desert mammals may explain some of these findings.

Recently the gerbil has been described as a highly adrenal-dependent animal based upon its inability to regulate chronic sodium deficiency following adrenalectomy. Whether sodium is made available as isotonic saline or in a dry diet, the gerbil shows no salt appetite and dies within a week unless given cortical hormone therapy (Cullen & Scarborough, 1970). This is in marked contrast to the adrenalectomized rat which can and does survive indefinitely by ingesting large quantities of sodium chloride (Fregly, 1958; Richter, 1936).

As another point of comparison, the gerbil has been found to have an adrenal-to-body-weight ratio three times that of the rat, along with adrenocortical morphological differences (Cullen, Pare, & Mooney, 1971). It seems reasonable to say, then, that the gerbil will respond differently from the rat vis à vis other manipulations of electrolyte balance. With this hypothesis in mind, the following experiments tested gerbils' sodium response to subcutaneous formalin injections. It has already been pointed out that the rat shows a marked and immediate (24-h) sodium appetite when given formalin subcutaneously (sc) (Braun-Menendez & Brandt, 1952) and that the appetite is dose related (Handal, 1965).

METHOD

The Ss were 60 male gerbils (*Meriones unguiculatus*) obtained from Tumblebrook Farms. Their weight range at the start of the experiments was 58-88 g, with a mean body weight of 71.4 g (SD = 6.2 g).

The drinking solutions consisted of distilled water and/or isotonic saline mixed on a weight per volume basis with distilled water as the solvent. The dry diets were either Purina Lab Chow or a sodium-potassium deficient test diet supplied by General Biochemicals. The latter diet was assayed by the Wisconsin Alumni Research Foundation and found to contain 0.0079% sodium and 0.0043% potassium. An additional 0.5% of KCl (wt/wt) was added to this diet to make it potassium replete. All food and drinking solutions were available ad lib and were weighed daily to the nearest gram. The animals were all housed in individual standard-sized Hoeltge rat cages in a temperature-controlled room (76 ± 2° F) with a 12-h light-dark cycle (light on 0800-2000) and a relative humidity of 37% (±2%).

The same basic design was employed in all three experiments. An initial 8-day baseline was obtained (B-1), followed by sc injections under the skin of the back of isotonic saline or 1.5% formalin (0.6% formaldehyde in isotonic saline adjusted to pH 7.4 with NaOH) and 5 days of additional solution consumption (FML-1). The whole procedure was then repeated, i.e., B-2, injection, FML-2.

The experimental design is depicted in Table 1 for all three experiments.

EXPERIMENT 1

This experiment comprised four groups of six Ss each. All Ss were fed the sodium-deficient diet. After baseline drinking of saline and water, each group was differentiated by the injection treatment. Group 1 got .025 formalin; Group 2, .25 ml formalin; Group 3, the control group, 2.5 ml of saline; and Group 4, 2.5 ml formalin. The 5 days comprising FML-1 then ensued, followed by a sodium-repletion period of 10 days, wherein .75% NaCl was added to the deficient diet along with access to water only. Starting on Day 23, all groups under identical conditions underwent the same treatments as above for a second time (i.e., B-2, injections, and FML-2).

Results

All quantitative comparisons carried out below employed analyses of variance (one-way or two-way for repeated measures). Differences were reported only if they equaled or exceeded the .01 probability level. Posttest analyses employed Winer's individual F-test comparisons for unweighted means solution (Winer, 1962).

For statistical simplification, the

Table 1
General Experimental Design

Group	N	B-1	Injection 1	FML-1	B-2	Injection 2	FML-2
1	6	E1: NaCl vs HOH Na-deficient Diet (8 Days) E2: NaCl—purina (8 Days) E3: NaCl vs HOH Purina (8 Days)	.025 ml Formalin	E1: NaCl vs HOH Na-deficient Diet (5 Days) E2: NaCl—purina (5 Days) E3: NaCl vs HOH Purina (5 Days)	Same as B-1	Same as Injection 1	Same as FML-1
				Experiment 1 Only			
2	6	Same	.25 ml Formalin	Same	Diet Repletion for 10 Days (.75% NaCl was Added to Na-deficient Diet)	Same as Injection 1	Same
3	6	Same	E1: 2.5 ml Saline E2: .25 ml Saline E3: .25 ml Saline	Same		Same as Injection 1	Same
4	6	Same	2.5 ml Formalin	Same	Same	Same as Injection 1	Same

only baseline days used for comparison were the last three.

Figures 1A and 1B depict the salt and water consumption of the Ss in this experiment. No differences were obtained between or within groups during B-1. On the third day (Day 11) after injection, however, both salt and water consumption had increased significantly for Groups 1 and 2, and on Day 12 all groups were consuming the test liquids in quantities significantly greater than base consumptions. With the one exception of Group 2, salt and water consumption across groups had returned to base levels by Day 13.

In spite of the disruption in baseline drinking evident during B-2 within groups, no differences were noted between groups for water or salt. After the second injection treatment, no significant consumption increases obtained for either solution. But all animals that had received 2.5 ml of formalin were dead before the end of FML-2.

EXPERIMENT 2

It was impossible to determine whether the polydipsia witnessed across groups in Experiment 1 was due to a sodium appetite or merely a nondiscriminative thirst from some unknown effects of the formalin. Consequently, in this experiment only isotonic saline was made available as a drinking fluid. If a nonspecific thirst had caused the polydipsia, then presumably much more saline would be ingested here than above, since it was the only drinking fluid available.

In addition, Purina Lab Chow was substituted as the solid diet. This methodological change was based on the absence of any apparent potentiation (when compared to pilot observations) occurring in Experiment 1 due to the diet sodium restriction. Other methodological modifications included (1) the omission of the 2.5-ml formalin-injected group, since these Ss had all died in the first experiment; (2) reduction of the control group injection from 2.5 ml saline to .25 ml; and (3) B-2 was begun immediately upon termination of FML-1.

Results

No consumption differences obtained between groups during B-1 and FML-1 regardless of injection treatment. And, as is evident from inspection of Fig. 2, there was again a substantial delay before increased salt consumption ensued (about 72 h), and the quantities imbibed on Day 11, FML-1, were significantly greater than base. Drinking, then, quickly returned to base levels. It is also clear that during B-2 there was considerable variability between groups and, with the exception of the saline controls,

salt consumption during this period was significantly elevated over B-1. On the other hand, no differences between or within groups were noted for FML-2, and B-2 was identical to B-1.

Although there was considerably more salt consumption during FML-1 of Experiment 2 as compared with that in the first experiment, total liquid consumption was lower (35.8 vs 52.0 g/100 g BW, respectively).

EXPERIMENT 3

The same design was used here as in Experiment 2 with one exception. Both water and isotonic saline were available as drinking fluids, as in Experiment 1.

Results

The results here largely support what had already been found. Baseline drinking of both salt and water was stable, followed by a delayed but dramatic salt and water polydipsia on Day 11 and a gradual return to baseline intakes. Again this was true for all groups, including the saline-injected controls (cf. Figures 3A and 3B). Mean total liquid consumption was greater than 89 ml/100 g BW/animal, with 10 of the 18 Ss drinking more than 100 ml/100 g BW on Day 11. Indeed, two Ss drank more than 150 ml. These amounts certainly constitute an astonishing amount of liquid for an animal weighing about 75 g.

As was true in Experiment 2, drinking during B-2 was quite variable. This was particularly true for water intake within groups. Between-group variability was minimal, with the one exception of the saline control group on Day 20. It was significantly higher than both other groups. As in both other experiments, no increased intakes obtained subsequent to the second injection treatment.

Food and weight data did not reveal any reliable changes in any of the experiments.

DISCUSSION

It is clear that the gerbil responds to formalin injection with a sharp increase in NaCl consumption. Nevertheless, unlike the rat, whose response is immediate and circumscribed to a 24-h period (Jalowiec & Stricker, 1970; Wolf & Steinbaum, 1965), the gerbil's response has a latency of 48-96 h. Peak consumption occurred on the fourth day after injection in Experiment 1 and on the third day in both Experiments 2 and 3.

The gerbil's NaCl consumption is at least comparable to the rat's if measured in grams of NaCl consumed (Handal, 1965; Wolf & Steinbaum, 1965). If total liquid consumption is compared, however, the gerbil drinks

considerably more. This is particularly noteworthy in Experiment 3.

The three experiments reported here differ procedurally on the basis of diet and consumable fluids. While a sodium-deficient diet was used in Experiment 1, with saline and water available for drinking, Purina and saline only were present in the second experiment. Nevertheless, the salt intakes between experiments were statistically comparable. Diet sodium deprivation, then, had no potentiating effect on NaCl consumption. Concurrent water availability does not explain this, since in Experiment 3, where both water and salt were also used but Purina was the diet, the salt intakes were again the same as those of the groups in Experiments 1 and 2.

Although a delayed response, the gerbil's NaCl consumption after formalin is reminiscent of what has been found in the rat. Wolf & Steinbaum (1965) have concluded that formalin induces a sodium deficiency in the rat by removing both sodium and water from the general circulation, thereby producing a sodium appetite. This could also be true for this desert mammal, although several of the present findings preclude an outright endorsement of this hypothesis. First of all, increased NaCl consumption did not occur after the second series of injections. If formalin were an adequate stimulus to produce a sodium appetite, why did it not continue to do so? Second, why was there not a graded dose-response relationship? Handal (1965) clearly showed that the amount of saline ingested by the rat after formalin was directly related to the quantity of formalin injected. Third, the increased salt consumption of the saline control Ss in all three experiments suggests that salt itself is as much an adequate stimulus as is formalin. But it is rather difficult to conceive of how isotonic saline injections could induce sodium deficiency. Certainly the phenomenon of increased salt drinking following sc saline injections is difficult to understand. Nevertheless, the reliability of the response was very impressive, and since observations made by others (Mays, 1969) establish the mean serum sodium of this mammal at 150.90 (±7.04 SD) mEq/l, it does not seem likely that the increased salt ingestion was in the interests of reestablishing an altered osmotic equilibrium.

It is not likely that the polydipsia exhibited in the three experiments is the result of a formalin-induced nonspecific thirst. Wolf & Steinbaum (1965) briefly entertained this hypothesis for the formalin-injected rat but discounted it when they found that rats drank much less water than isotonic saline when these fluids were
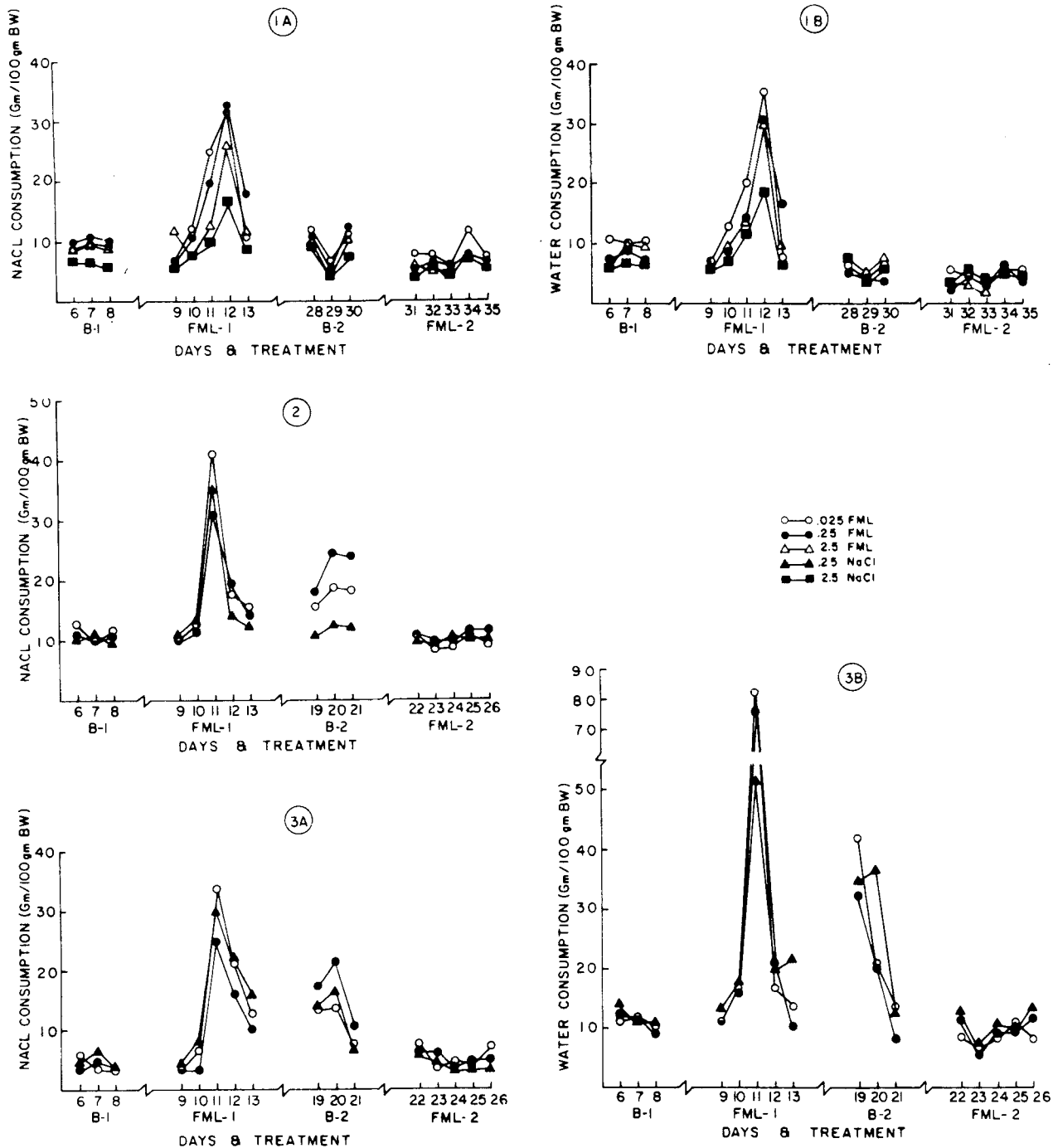
Fig. 1A-3B. NaCl and water consumption (g/100 g BW) for all three experiments. Experiment 1: 1A and 1B; Experiment 2: 2; Experiment 3: 3A and 3B.

simultaneously available. This was in contrast to what they found when formalin-injected rats could choose between hypertonic saline and water. In that case, water intake virtually doubled salt intake. In the work reported here, salt was available only in isotonic concentrations. While water intake either equaled salt intake (Experiment 1) or was dramatically greater (Experiment 3), in Experiment 2, where no water was available, total fluid consumption was significantly less than in the other experiments. If a nonspecific thirst were the cause of the increased NaCl consumptions, a much greater quantity of saline should have been consumed in Experiment 2, particularly since it was isotonic.

It is difficult at this point to explain these findings merely on the basis of

electrolyte homeostasis, again due to the behavior of the saline-injected controls. The possibility exists that formalin and saline injections were both highly stressful, thereby influencing the adrenal-pituitary axis and the subsequent polydipsia. The fact that those Ss receiving 2.5 ml in Experiment 1 died before the end of FML-2 attests to this. But if this were so, why was the polydipsia delayed so

long and why did it not recur upon repeated injections?

According to the sodium-reservoir model proffered by Wolf & Stricker (1967), sodium appetite is not directly related to the hyponatremia, hypovolemia, and increased aldosterone output that quickly ensues in the rat after formalin treatment. Rather, sodium appetite is elicited when sodium is depleted from a body sodium reservoir which is only gradually responsive to these mediating stimuli. As Jalowiec & Stricker (1970) have found, even though all three of these stimuli are present soon after formalin injection, sodium appetite is delayed for several hours. Since the gerbil is a desert mammal where fluid balance is always in jeopardy, it is not unlikely that it is more resistant to electrolyte imbalance. Such a disposition could explain the consumption delays witnessed here. Then the rebound effect indicated by the B-2 data might reflect the potentiating effect of a delayed and cumulative circulating aldosterone response. The outcome of this overcompensation could have been in the interests of reservoir repair and thereby explain the absence of a repeat performance during FML-2.

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