

Sodium Appetite: Some Conceptual and Methodologic Aspects of a Model Drive System¹

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The study of many fundamental problems of motivation may be facilitated by the utilization of sodium appetite as a model drive system. This paper summarizes the current state of knowledge of the behavioral, physiological, and, particularly, neurological mechanisms of sodium appetite and discusses a number of unique methodological and theoretical features which render sodium appetite especially amenable to laboratory investigation. A simple and reliable screening procedure for assessing deficits in sodium appetite after neurological damage is presented, and three techniques for acute elicitation of sodium appetite are compared.

Sodium appetite is a drive system which is especially well-suited for experimental analysis, and the interest of physiological psychologists in this system has been steadily increasing. Several reviews of various aspects of sodium appetite have appeared during the past two decades (Denton, 1965, 1966, 1967; Falk, 1961; Nachman and Cole, 1971; Richter, 1956; Stricker and Wolf, 1969; Wolf, 1969), and a comprehensive 300-item bibliography of sodium appetite and related topics is available.²

The purpose of this paper is to elucidate certain unique features of sodium appetite which render it especially amenable to laboratory investigation and to propose its utilization as a model drive system. The relevant conceptual features are presented in the context of brief reviews of the major findings from behavioral and physiological research and a somewhat more extensive review of the

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²Bibliography #75, "Sodium Appetite and Related Topics", may be obtained from: Publications Distribution, Brain Information Service, UCLA Center for the Health Sciences, Los Angeles, CA. 90024.

neurological research (which is not available elsewhere). We have omitted several findings and ideas which are otherwise interesting and important but not directly relevant to the purpose of this paper. Thus, the analysis of the literature is not intended to be comprehensive and reference to the cited papers is suggested for further evaluation of the field. The methodological features are presented with an emphasis on applications for neurological studies, although sodium appetite is equally valuable as a model system for behavioral and physiological investigations, and many of the methodological features apply to research in these areas as well. Finally, a simple and reliable method of screening for abnormalities in sodium appetite after neurological interventions is described, and three techniques for rapid elicitation of sodium appetite are compared.

BEHAVIORAL FEATURES

It appears that two factors motivate the ingestion of sodium by rats. The first is a gustatory factor. Rats ingest excessive amounts of sodium when it is in a palatable form (e.g., a weak sodium chloride solution) even though they are receiving abundant sodium elsewhere in their diet, and their body fluids are in normal equilibrium (Fregly *et al.* 1965; Young, 1949). The second factor is a regulatory one. The rat's sodium intake increases when there is a deficit of body sodium or when the hormonal conditions (increased mineralocorticoid levels) normally accompanying a deficit of body sodium are present (Richter, 1956). Such imbalances have surprisingly strong drive properties. Sodium appetite can motivate rats to learn and perform instrumental responses to obtain sodium (Wagman, 1963; McCutcheon and Levy, 1972), to ingest sodium-containing substances even though they are adulterated with aversive-tasting quinine (Lewis, 1960; Quartermain and Wolf, 1967), and to overcome a previously learned sodium aversion based on an association that salt ingestion is followed by illness (Cullen, 1969; Balagura and Smith, 1970; Stricker and Wilson, 1970; Frumkin, 1971).

Certain basic components of the sodium appetite system are innately organized. Rats do not have to learn to identify and ingest sodium when they need it (Falk and Herman, 1961; Nachman, 1962; Wolf, 1969). That is, within seconds after initial taste sampling, a rat which has been subjected to body sodium deficiency for the first time will avidly ingest any of a wide variety of sodium salts but totally reject nonsodium salts (except lithium, which presumably has a similar taste). The sense of taste seems critically important in the identification of sodium and the maintenance of appropriate intake levels (Borer, 1968; Mook, 1969; Smith, 1972). Studies investigating various aspects and implications of this innate behavioral organization have appeared recently and the relations between these phenomena and those recently uncovered in toxiphobia and diet selection research pose important problems for current learning theories (Garcia and Ervin, 1968; Kriekhaus, 1970).

PHYSIOLOGICAL STIMULI

The physiological stimuli for sodium appetite have been under investigation in several laboratories and a considerable amount is now known. Behavioral and internal sodium regulatory functions appear closely integrated and probably share common receptor, endocrine, and neural mechanisms (Stricker and Wolf, 1969). Thus, many of the findings and concepts of renal and cardiovascular physiologists have direct relevance for the appetitive system and not only supply immediate explanations of appetitive phenomena but also suggest fruitful directions for future research. Largely as a result of this fortunate source of data and ideas, several physiological stimuli for sodium appetite have been identified.

It seems quite certain that a decrease in blood volume is a sufficient condition for the elicitation of sodium appetite and that a decrease in blood sodium concentration is at least a contributory factor (Stricker and Wolf, 1969). Increased mineralocorticoid levels can also stimulate sodium appetite independently of body sodium homeostasis (Wolf, 1965; Fregly and Waters 1966). (Mineralocorticoids, secreted in states of sodium need, probably help stimulate the appetitive system in addition to stimulating other sodium regulatory functions such as the reabsorption of sodium from the renal tubules.)

The above findings indicate close physiological relationships between sodium appetite and thirst. Not only is the thirst mechanism responsive to blood volume and blood sodium concentration but it is responsive to a substance critically involved in control of mineralocorticoid secretion—angiotensin (Fitzsimmons and Simons, 1969). However, unlike thirst which is rapidly responsive to body fluid changes, there is a long latency between the induction of the physiological stimulating conditions and the induction of sodium appetite behavior (Stricker and Wolf, 1969). Furthermore, thirst can be immediately dissipated by parenteral restoration of blood volume and composition, but sodium appetite cannot be quickly satiated in this way (Nachman and Valentino, 1966).

In order to parsimoniously account for the variety of physiological changes which stimulate sodium appetite as well as for the long latency for initiation and parenteral satiation, we have proposed the so-called reservoir hypothesis (Stricker and Wolf, 1969). According to this hypothesis, sodium appetite, unlike thirst, is not directly responsive to changes in the extracellular fluids, but is regulated according to sodium levels in a body sodium reservoir (such as bone) which is directly, but slowly, responsive to changes in extracellular sodium levels and to the sodium transport actions of mineralocorticoids.

NEURAL MECHANISMS

Although the past decade has seen rapid progress in the elucidation of neural mechanisms mediating sodium intake in the rat, the problems encountered here seem enormous compared to those encountered in behavioral and physiological investigations. While we are already able to present general schemas of the behavioral and physiological aspects of sodium appetite, we are unable, as yet, to do so for the neurological aspects. However, we can identify certain major current issues, ideas, and findings, and present some preliminary speculations.

Certain structures of the limbic system possibly involved in motivation and certain structures of the mesencephalic tegmentum and thalamus possibly involved in gustation appear critical for normal regulation of sodium intake. The data on the limbic system are more extensive and will be discussed first.

Total or partial impairment of the normal voluntary increase in sodium intake in response to body sodium depletion or elevated mineralocorticoid levels has been observed after lesions in the ventromedial and lateral feeding areas of the hypothalamus and in the basolateral amygdala (Antunes-Rodrigues *et al.* 1970a, 1970b; Novakova and Cort, 1966; Quartermain *et al.* 1969, Wolf and Quartermain, 1967; Wolf, 1968a). Although the bases of the impairments have not yet been intensively analyzed, the best hypothesis to date is that, at least in the case of the lateral hypothalamus, the decrement in sodium appetite represents a specific instance of a general loss of appetitive motivation (Rogers *et al.* 1965). Rats with lateral hypothalamic lesions do not make appropriate behavioral responses to either a deficit or an excess of body sodium (Wolf and Quartermain, 1967).

The roles of pathways which interconnect the hypothalamus, amygdala, and associated limbic structures are also being elucidated. Chiaraviglio (1971) has found that section of the ventral amygdaloid pathway or the stria terminalis, which connect the amygdala with the hypothalamus, thalamus, and basal forebrain, produced decrements in sodium appetite. On the other hand, we have lesioned each of the major known hypothalamic pathways and found no observable decrement in sodium appetite, (Wolf, 1967; Jalowiec *et al.* 1970; Wolf, 1971). Some of these lesions disjoined the portion of the ventral amygdaloid pathway which turns caudally into the medial forebrain bundle to connect the lateral hypothalamic feeding area and the anterior amygdala, so that Chiaraviglio's positive results may be related to other segments of the ventral pathway. Our inability to find an effective pathway for transmission of critical hypothalamic sodium regulatory functions to extrahypothalamic sites presents an interesting neuroanatomical problem for future research.

Electrical and chemical stimulation studies confirm and extend the conclusions from lesion work that amygdaloid and hypothalamic structures are critical components of the central mechanism for sodium appetite. It has

recently been reported that electrical stimulation of basolateral amygdala and lateral hypothalamus enhance salt intake while electrical stimulation of corticomedial amygdala diminishes salt intake (Gentil *et al.* 1971). [It is of interest that lesions of the corticomedial amygdala may cause increased sodium intake (Gentil *et al.* 1968).] There is evidence that cholinergic stimulation of the amygdala causes increased saline intake (Chiaraviglio, 1971) while in the region of the third ventricle or posterior hypothalamus cholinergic stimulation causes decreased saline intake (Chiaraviglio and Taleisnic, 1969; Hendler and Blake, 1969). The above studies also investigated the effects of other neurochemicals but the results are not sufficiently clear to warrant further discussion here. However, it is worth noting that Stricker (personal communication) has found that treatment with 6-hydroxy-dopamine which eliminates more than 90% of brain catecholamines does not impair sodium appetite and that Buggy (1972) has reported enhanced sodium intake after injection of angiotensin into the septal-preoptic region.

Several structures of the central gustatory system may play an important role in sodium appetite. Lesions of the gustatory region of the thalamus and the reticular formation immediately caudal to this region produce impairments in sodium appetite but the effect is less consistent than that seen after hypothalamic lesions, and some animals completely escape the deficit (Wolf, 1968). Chiaraviglio (1972) found that lesions placed further caudally in the mesencephalic reticular formation at the level of the decussation of the brachium conjunctivum and ventrolateral to the central grey disrupt sodium appetite. She also found that stimulation of this region with FeCl_3 enhanced sodium intake. It is very interesting that the effective mesencephalic sites correspond with the trajectory of a recently described ascending gustatory pathway from the pontine taste area (Norgren and Leonard, 1971).

There are a number of brain stem and forebrain structures whose destruction does not clearly impair the ability of the rat to increase its sodium intake in response to body sodium depletion or elevated mineralocorticoid levels. These include (1) limbic or rhinencephalic structures such as rostral, dorsomedial, and caudal hypothalamus, preoptic area, olfactory bulbs, septum, and hippocampus (Chiaraviglio, 1969; Kim, 1960; Murphy and Brown, 1970; Wolf, 1964, Wolf, 1967); (2) dorsal thalamic and subthalamic sites surrounding the gustatory relay (Wolf, 1968; Wolf, 1971); (3) several mesencephalic regions (Chiaraviglio, 1972); and (4) the entire neocortex (Wolf *et al.* 1970).

There have been a number of studies showing changes in saline taste thresholds and preference-aversion functions or in spontaneous intake of a given saline solution following brain lesions (e.g., Chiaraviglio, 1969; Covian and Antunes-Rodrigues, 1963; Cort, 1963; Donovan *et al.* 1969; Kawamura *et al.* 1970; Kissileff and Epstein, 1962; Oakley and Pfaffmann, 1962; Schmidt *et al.* 1968; Vilar *et al.* 1967; Grace, 1968). The relations between the findings of these studies and of those dealing with regulatory sodium intake

are in many cases unclear. For example, brain lesions may completely disrupt regulatory sodium intake without affecting the normal taste preference for palatable saline solutions (Wolf and Quartermain, 1967). On the other hand changes in spontaneous saline intake may be secondary to disruptions of diverse physiological functions such as renal, endocrine, or cardiovascular processes which regulate salt and water homeostasis and thus may be manifested in the absence of any alteration of the ability to regulate sodium intake. In other cases the change in saline intake may be secondary to more general alterations in gustatory or reinforcement mechanisms. Of course all these functions and the brain sites mediating them are relevant to the overall sodium appetite system, and the above studies must be taken into account in any attempt to gain a general picture of the neural mechanisms for sodium appetite. Covian and co-workers, who have done the most work on effects of brain lesions on spontaneous saline intake, have, in some studies, utilized measures of body sodium balance and renal function as well as observations on appetitive responses to induced sodium deficiency in order to elucidate some of the underlying variables. In a recent study (Saad *et al.* 1972), these workers investigated the interactive effects of multiple lesions of limbic structures on spontaneous saline intake. They concluded "that the hypothalamus is the main structure controlling sodium intake and that the amygdala and septal area have modulating influences on the hypothalamus. In turn, septal area overcomes the action of the amygdala."

Finally, several investigators have been searching for receptor sites for sodium appetite. Denton has hypothesized, on the basis of several selective infusion studies, that the induction of sodium appetite is directly mediated by "intracellular sodium depletion in the specific cells of the central nervous system subserving the appetite" (Denton *et al.* 1970). As noted before, we have suggested that the receptors are associated with a body sodium reservoir. In accordance with Denton's hypothesis of a cerebral site, it may be that glial cells act as sodium reservoirs for neurons, and modify relevant neuronal activity when their sodium content is diminished. On the other hand it is quite likely that there is more than one receptor system. Lin and Blake (1970) found that perfusion of the liver with NaCl solutions via the portal vein resulted in decreased sodium intake in rats and proposed a hepatic site for the receptors. Chernigovsky (1962) obtained evidence that intragastric receptors associated with the vagus nerve mediate sodium intake in dogs.

METHODOLOGICAL ADVANTAGES

Because of the many essential functions of body sodium and because of its involvement in human cardiovascular disfunction, it is of interest to study sodium appetite in its own right. However, the potential importance of

sodium appetite to the solution of central problems of physiological psychology lies in its utilization as a model drive system. In addition to the inherent characteristics of sodium appetite such as its innate organization and its close relation to internal sodium regulatory processes and associated primary drives such as thirst, it affords a number of important methodological advantages. Thus, its special amenability to laboratory investigation can quite plausibly be expected to facilitate the elucidation of more general mechanisms of homeostatic drives. Denton (1971) has recently made a very clear and cogent statement of the relevant theoretical considerations. "Salt hunger is a classic example of an innate neural organization generating a behavioural drive in the instance of nutritional deficiency . . . For study on how specific behaviours are activated, and how drives are satisfied it is a wonderful experimental field—particularly because it lends itself to precise measurement of deficit and resultant behaviour."

Standard rat chows contain an excess of sodium, so that one may assume that adult rats purchased from the supplier or raised in the laboratory under *ad lib* feeding conditions have had minimal experience with sodium deficiency. Furthermore, one can be quite certain that such rats have never had the opportunity to taste sodium independently of other strong concomittant taste stimuli. Thus, one can study a relatively virgin drive system in a mature animal without special manipulation of past experience.

Other important methodological advantages lie in the ease and accuracy with which sodium appetite can be assessed in the laboratory rat. The sodium salt can be present *ad lib* in solution along with the standard food and water diet. It takes negligible time and effort to record daily fluid intake when graduated liquid dispensers are used. The amount of salt solution ingested by rats under baseline conditions can be controlled by varying the concentration. With sodium chloride, a 1% solution is ingested in relatively large quantities—often more than 30 ml per day—while a 3% solution is almost totally avoided (Bare, 1949). The acceptability of the sodium salt can also be controlled by using different anions, e.g., sodium chloride is strongly preferred to equimolar sodium carbonate or sodium iodide solutions (Fregly, 1958; Morrison, 1972; Nachman, 1962). The palatable solutions are useful when experimental manipulations cause a decrease in sodium appetite because they provide a well elevated baseline. The aversive solutions are good for observing increases in sodium appetite because they give a highly stable, near zero baseline.

We have found sodium appetite most useful in the analysis of the neural control of motivation. Consider, for example, the problems encountered in studying central ablation effects upon appetitive drives such as hunger and thirst. When brain lesions result in aphagia adipsia, quite laborious and complex experiments are often necessary to determine the relative participations of motivational, sensory, and motor impairments in the feeding and drinking deficits (Williams and Teitelbaum, 1959; Rodgers *et al.* 1965;

Marshall *et al.* 1971). Generally, complete postoperative aphagia and adipsia are not permanent. Spontaneous feeding and drinking gradually reappear and more sophisticated behavioral analyses of remaining deficits become possible. However, certain basic problems concerning the role of practice or experience in the recovery process are difficult to approach experimentally (DiCara, 1970). Rats must be fed and watered daily, and while postoperative experiences of food and water ingestion can be controlled by intragastric loading, it is unlikely that one could prevent the animal from experiencing some hunger and thirst without continual infusions of food and water. Furthermore, the role of a lifetime of *preoperative* experience with the satisfaction of the urge to eat and drink in escape or recovery from lesion effects poses another knotty problem. For example, we have repeatedly observed that while rats with lateral hypothalamic lesions may recover relatively normal *ad lib* food and water intake within a few days after lesioning, they show little evidence of recovery of sodium appetite in spite of substantial postoperative experience (Wolf and Quartermain, 1967) or prolonged postoperative survival (up to six months after achieving stage 4 of recovery, unpublished observations). It is possible that the alimentary functions which recover after lateral hypothalamic damage are mediated by other parts of the brain which contain information on (or "programs" for) food and water ingestion as a result of repeated preoperative feeding and drinking experiences, while the failure of recovery of sodium appetite is due to the absence of such learned programs for sodium intake.

In summary, it is quite obvious that many problems commonly encountered in the neurologic analysis of motivation can be circumvented by studying sodium appetite. Of primary importance is the fact that experience with both sodium deficiency and sodium intake can be easily controlled both before and after brain surgery. As discussed earlier, the rat requires very little sodium to maintain homeostasis and superabundant amounts can be given in the standard diet. Sodium appetite can be tested for the first time in experimentally naive rats either immediately following induction of lesions (when there are no confounding impairments) or well after recovery from postoperative aphasia, adipsia, or other incapacitating deficits. The ability of the lesioned animal to ingest food and water necessarily eliminates several potentially confounding variables which might otherwise impede the interpretation of the results. Finally, specific relevant sensory deficits such as loss of gustatory discrimination can be assessed simply by determining the rat's preference-aversion function for various salt solutions.

TESTING METHODS

One methodological problem with sodium appetite is that it cannot be readily induced by simple deprivation as can hunger and thirst. It takes at

least a week of total sodium deprivation to elicit a small increase in sodium appetite in a normal rat (Fregly *et al.* 1965). During the past few years, we have been investigating artificial methods of rapidly inducing a strong sodium appetite with the aim of developing a more reliable test for screening brain-lesioned rats living in standard individual cages under *ad libitum* feeding conditions which require only a few minutes per day per rat for maintenance and for obtaining the relevant measures. Following is a description of the testing conditions and of three quite different techniques for inducing a specific, intense, and consistent sodium appetite under these conditions.

Rats are housed in individual cages and given 0.50 M NaCl solution, 0.30 M KCl solution, and water in standard graduated dispensers with metal drinking spouts which protrude through the fronts of the cages about one inch apart. A 100-ml beaker containing condensed milk (the sole food) is attached to a front corner of the cage near the fluid dispensers. The fluids and food are available *ad lib* and the relative positions of the substances remain constant (rather than being alternated daily as in taste-preference experiments) to minimize the time to learn the discrimination and to obtain a stable baseline. A 0.50 M solution of NaCl is used because it is concentrated enough to be quite unpalatable so that intake normally drops to less than 1 ml per day within 3 or 4 days and yet dilute enough so that our sodium appetite induction procedures elicit a substantial (5-10 ml) increase in intake. The 0.30 M KCl solution is used to identify cases in which there is a loss of discriminative ability on the sodium appetite test. The 0.50 M NaCl solution and the 0.30 M KCl solution appear to be about equally unacceptable to normal nondeficient rats. While standard lab chow can be used instead of condensed milk, the milk offers several advantages. It is a thick and pasty substance which is not spilled or scattered so that it yields a very accurate measure of food intake simply by weighing the beaker. While it contains all the nutrients necessary to maintain dietary balance during the time course of the experiment, it contains only about a third as much sodium as standard chow (0.1% vs 0.3%) so that sodium appetite cannot as readily be satiated by ingesting the food rather than the NaCl solution. Finally, it remains fresh for a few days at room temperature. (Note that the above comments refer to Borden's Eagle or Magnolia brand condensed milk—we have no information on other brands.) Usually fluid intakes are sufficiently stable for sodium appetite testing within about 5 days. Only one additional day of measurement is necessary to complete the screening test.

We shall present data on the effects of 3 different sodium appetite-inducing treatments upon fluid and food intake under the above testing conditions. The first two treatments, subcutaneous injection of formalin or administration of a diuretic drug have been used to elicit sodium appetite by many investigators under conditions similar to those used here. Both of these treatments have anorexic and probably stressful side effects which render

them less than ideal for use in screening tests. For this reason we present a third treatment (fludrocortisol) which appears free of these side effects and thus seems preferable for this purpose.

The three treatments induce sodium appetite in different ways. Subcutaneous formalin causes a sequestration of circulating sodium in local damaged cells as well as the accumulation of sodium-containing edema fluid at the injection site (Stricker and Wolf, 1969). Thus, the rat is effectively depleted of body sodium without external loss. Furosemide is a diuretic drug which acts on the kidneys to cause large amounts of sodium to be excreted in the urine and, thus, induces sodium appetite by external loss (Jalowiec, 1971). Fludrocortisol is a synthetic corticoid which combines extremely potent mineralocorticoid and glucocorticoid activity (Fried, 1955). Since we found that a simultaneously administered glucocorticoid potentiates the appetitive effect of mineralocorticoids (Wolf, 1965), we expected this compound to have a very powerful sodium appetite-inducing effect. Fregly (1967) has shown that doses of fludrocortisol above the optimal dose for sodium retention can enhance the sodium intake of adrenalectomized rats. As noted earlier, the mechanism of action of mineralocorticoids upon sodium appetite has not yet been elucidated, but it is certain that no diminution of circulating sodium is involved (Wolf, 1964), and it is likely that a putative body sodium reservoir which elicits receptor activity is depleted.

Figure 1 shows results obtained under the above testing conditions from groups of 6 experimentally naive adult male rats which had never been

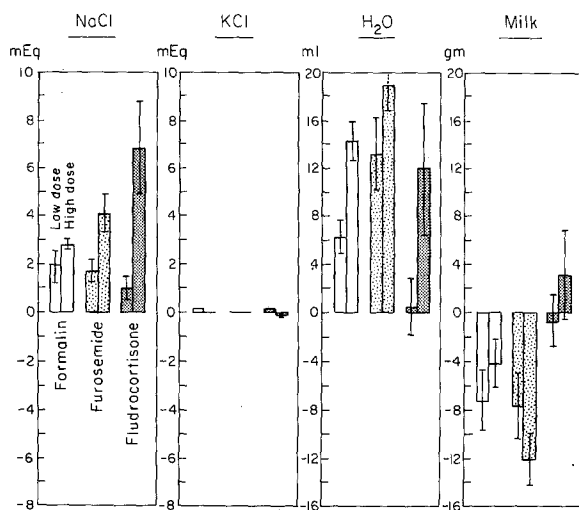


Fig. 1. Mean changes in intake of various substances from day preceding to day following low and high dosage treatments with each of three natrorexic agents. Variance measure is standard error of mean.

subjected to sodium deprivation prior to the administration of the experimental treatments. All three treatments were administered by subcutaneous injection under the thick skin of the back. It is good general practice to gently knead the substance into the tissue following injection. The treatments were given following the measurement period of the final baseline day, and the changes in intake from this measurement to the next day's measurement provide the relevant data.

Each of the three treatments was administered at two dose levels. The low (threshold) dose was estimated on the basis of preliminary dose-response studies to produce a marginally significant increase in sodium intake in a group of 6 rats under the present conditions. For each treatment, the high dose was twice the threshold dose. The low formalin dose group was injected with 1.25 ml and the high dose group with 2.5 ml of 1.5% formalin (15 parts of 10% formalin phosphate in 85 parts distilled water). The low furosemide dose group was injected with 10 mg of furosemide (Lasix-Hoechst) in 1 ml of aqueous solution. The high furosemide dose group was given 2 separate 10 mg injections—the second administered 4 hr after the first. The dosage was divided in this way because the diuretic effect of a single dose is asymptotic at 10 mg but renal function returns to normal within 4 hr so that a second diuresis can then be initiated. The low fludrocortisol dose group was injected with 2.5 mg and the high dose group with 5.0 mg of fludrocortisol (Florinef-Squibb) dissolved in 1 ml of peanut oil.

Figure 1 shows changes in sodium, potassium, water, and milk intake from the day before treatment to the day after treatment. Mean intakes of these substances during the day preceding treatment were approximately as follows: sodium, 0.1 mEq, potassium, 0 mEq, water, 20 ml, and milk 30 g. Statistical analyses of change scores were by correlated T tests, analyses of variance, and Duncan tests for individual comparisons.

All three treatments produced a substantial increase in NaCl intake without affecting KCl intake. With each treatment, the high dose produced a large and highly reliable increase in NaCl intake ($p < .01$), while the low dose produced a smaller and only marginally reliable increase (p between .05 and .10). Both low and high doses of formalin and furosemide caused significant increases in water intake ($p < .05$), while neither dose of fludrocortisol caused a significant increase (although there is a tendency for increased water intake in the high dose group). Similarly, both formalin and furosemide caused diminutions of milk intake ($p < .02$), while fludrocortisol had no observable effect at either dosage. It is interesting to note that, at the present dose levels, doubling the dose of fludrocortisol has a proportionally larger effect on sodium intake than does doubling the dose of the other drugs ($p < .01$).

The anorectic side effects of formalin and furosemide may be secondary to general stress or debilitation. This seems especially likely with formalin. There is evidence that subcutaneous formalin acts as an aversive

stimulus (Woods *et al.* 1971) and can cause local skin ulceration (Porter and Relinger, 1972), in addition to its well-known general stress effects (Selye, 1937). On the other hand, E. M. Stricker has informed us that formalin may not cause a diminution of food intake if the accompanying sodium depletion is circumvented. In order to obtain further evidence on possible stressful side effects of formalin, we performed close observations of the spontaneous behavior of groups of 4 rats during an 8-hr period following injection of each of the three substances. While there were no observable effects of either furosemide (10 mg) or fludrocortisol (5 mg) on spontaneous behavior, there were obvious effects of even the small (1.25 ml) dose of formalin. The rats assumed abnormal prone postures with eyes partially closed and dragged their rear legs when locomoting. They seemed sensitive to touch at the injection site and squealed frequently when handled. Possibly a long acting local anesthetic might counteract these effects, but until this is demonstrated we recommend the discontinuance of the use of formalin for sodium appetite experiments.

Furosemide remains a potentially useful technique. The LD_{50} is 680 mg/kg given intravenously—about 10 times the high dose used here (Jalowiec, 1971). It should be possible to elicit a sufficiently reliable increase in sodium appetite with much smaller doses of furosemide if it is given in conjunction with a few days of sodium deprivation and a sensitive brief access test is used (Handal, 1965; Jalowiec, 1971; Porter and Relinger, 1972; Wolf and Quartermain, 1966).

The above findings and considerations suggest that fludrocortisol is preferable to formalin or furosemide for use in screening for deficits in sodium appetite under the present testing conditions. The 5.0 mg dose elicits a large (10-20 ml) increase in intake of 0.50 *M* saline from a near zero base line within a 24-hr period. At this dosage, fludrocortisol has no effect on KCl or milk intake, and the tendency for increased water intake is unquestionably secondary to the elevated intake of the strongly hypertonic saline solution.³ We have injected fludrocortisol into rats deprived of NaCl solution and found no change in water intake, and Richter (1956) has reported similar findings for desoxycorticosterone.

The testing conditions and sodium appetite-inducing treatments suggested here were developed with the goals of maximizing methodologic simplicity, efficiency, and reliability. The procedure is suggested for obtaining supplementary information at the completion of experiments primarily di-

³In studies conducted after the submission of this paper, we discovered an anorectic effect of fludrocortisol which appears on the second day after injection. Our initial observations suggest that this is due to the glucocorticoid properties of the drug and is not secondary to aversive side effects. Because of the long latency of onset of this effect, it does not confound the results of the present sodium appetite test which is completed prior to its onset. We are currently investigating the basis of the anorectic effect of fludrocortisol, for it may well offer the advantage of adding a separate regulatory variable which can be tested within the present experimental paradigm.

rected toward other aspects of alimentary behavior as well as for use in experiments specifically concerned with identifying neural structures mediating sodium appetite. However, the procedure should not be taken as perfect. Obviously the conditions bear little resemblance to natural ones—behavioral or physiological. Fortunately, many highly sophisticated and well-validated techniques for the behavioral analysis of sodium appetite are available (see references under *Behavioral Features*) to follow up initial findings gained by use of the simple screening technique.

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