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PRODUCTION OF CONDITIONED DIURESIS IN MAN *

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Although recent evidence suggests that the neurohypophysis may participate in volume regulation under special circumstances (1, 2), it is primarily sensitive to alterations in osmotic equilibrium. Its output of antidiuretic hormone (ADH) is thought to be regulated by the effective osmolality of the plasma. However, since Verney's original demonstration of the role of the central nervous system in ADH release in dogs (3), clinical evidence has accumulated indicating that inappropriate ADH secretion may have important effects upon the course of patients with diffuse structural (4), metabolic (5), and paroxysmal (6) disorders of the brain. Antidiuresis, characteristic of that produced by the action of ADH, has also been observed after stimulation of subcortical sites distant from the hypothalamus both in primates (7) and in man (8). Furthermore, several reports have described an increase in water diuresis in response to the suggestion of water-drinking under hypnosis (9, 10). The experiments described here were designed to ascertain whether pathways from the higher centers of the nervous system can be activated selectively and predictably in normal human subjects to control the secretion of ADH, and also to test whether this neurohormonal regulatory mechanism is susceptible to the acquisition of new patterns of reactivity under the influence of controlled experience, that is, whether it can participate in a "learning" process.

Although there are no assay procedures for estimating small changes in ADH concentration within the range found in the blood of hydrated human subjects, fluctuations in the level of this hormone may be estimated from measurements

of changing urine osmolality and minute volume, provided that the excretory rates for electrolytes are constant and no gross changes in glomerular filtration rate occur (11, 12). The rapid administration of a water load of moderate size provides a simple, repeatable stimulus that causes suppression of ADH levels without affecting other renal regulatory mechanisms. The dilution of the plasma produced by the ingested water stimulates sensitive receptor sites in the anterior hypothalamus and inhibits the secretion of vasopressin. When the concentration of this hormone in the blood decreases, the reabsorption of water by the renal tubules decreases also, and a state of physiologic diabetes insipidus ensues, with a copious flow of dilute urine.

The signal to which the neurohypophysis responds in this case is the fall in plasma osmolality. Attempts have been made to "condition" this neurohypophyseal response by using dogs that were exposed to auditory or visual signals which repeatedly preceded the administration of large water loads. Investigators have reported that such conditioning is very difficult to accomplish in this manner (13). Those who have been apparently successful have published data on urinary composition from which no firm conclusions have been reached as to the regulatory mechanisms involved (14-16). In the present study, an attempt has been made to ascertain whether a single swallow of cold tap water, administered to normal human subjects in fixed environmental surroundings, can, by repeatedly preceding the ingestion of large water loads in a stereotyped manner on a number of occasions, acquire the property of producing a diuresis characteristic of decreased circulating ADH.

METHODS

Four healthy young male subjects (medical students) were maintained on measured schedules of water intake

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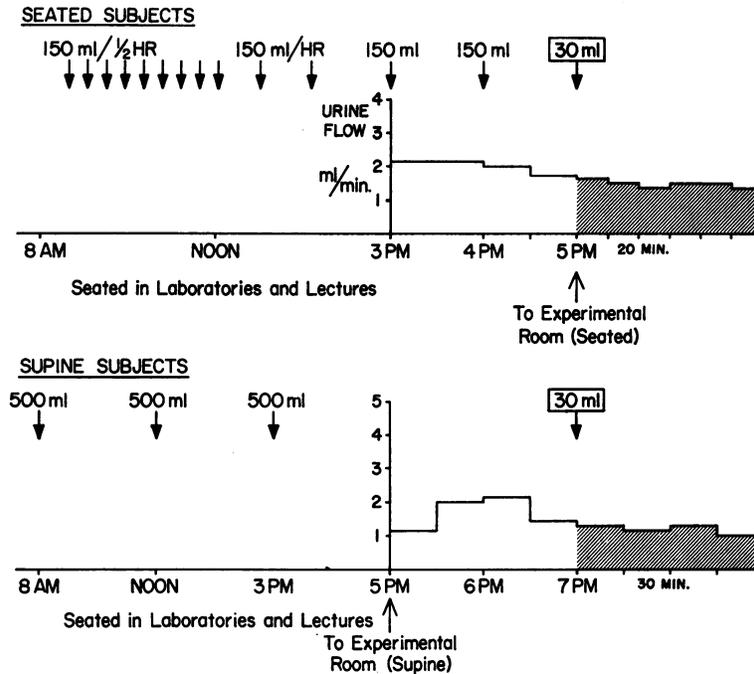


FIG. 1. HYDRATION SCHEDULE AND EXAMPLES OF CONTROL EXPERIMENTS.

(1,200 and 2,000 ml per day) and a diet selected to avoid gross fluctuations in salt intake. They engaged in a stereotyped routine of sedentary activity and refrained from drinking alcohol within 24 hours of the experiment. None of the subjects smoked. All experiments for each subject were carried out at the same time of day, in the same room, in a laboratory in which the ambient temperature was controlled to within $\pm 2^\circ$ C. Urine specimens were voided in the standing position at 20- to 30-minute intervals, directly into graduated cylinders. Before the experiments were undertaken, it was ascertained that the subjects could void regularly at these specified time intervals. Collections were timed from the end of each voiding and measured to the nearest 1 ml. Samples were then frozen and subsequently analyzed for osmolality by the Fiske osmometer, for sodium and potassium by the Baird-Atomic flame photometer, for chloride by the titration method of Schales and Schales (17), and for creatinine by the modified method of Taussky (18).

To establish an adequate and reproducible state of hydration, each subject drank an excess of water on the morning of the experimental day, until his urine flow exceeded 2 ml per minute. The water intake was then reduced during the afternoon, and except for the 30-ml swallow, stopped entirely 1 or 2 hours before the experiments, which were then carried out over the subsequent periods of declining flow rate. In experimental sessions, any significant increase in minute volume arising from this base line of decreasing flow rates could then be ascribed with some confidence to variables introduced by the experimental procedure.

A typical control experiment for a seated subject is shown in the top half of Figure 1. This subject drank 150 ml of water every half-hour during the morning until a substantial urine flow rate was achieved. Then, during the afternoon, he drank 150 ml hourly, measuring, but discarding, timed urine specimens. The last half-hour's specimen was collected when the subject came to the laboratory. This served as a control sample for that experimental day. At about 5:00 p.m., at least 4 hours after his last meal, he entered the laboratory and received immediately a single 30-ml swallow of cold tap water. Thereafter, timed urine specimens were collected every 20 minutes for 2 hours.

To investigate the modifying effects of the recumbent position upon renal function (19) during the conditioning procedure, two subjects were studied in the supine position. The protocol of a typical experiment is shown in the lower half of Figure 1. This subject drank 500 ml of water at breakfast, at noon, and at 3:00 p.m. Two hours later, when urine flow was on the decreasing limb of diuresis, he entered the laboratory and lay down, collecting urine specimens without further hydration for another 2 hours. This interval was found necessary to allow the diuresis of recumbency to subside. A single swallow of tap water was then given, and urine was collected as it had been in the seated experiments.

Four or five days of control experiments were carried out for each subject. Thereafter, the subjects were conditioned in the following manner. The procedure up to the time of their arrival at the laboratory was identical in every way. They were not informed that the routine would be changed. Upon their arrival at the

laboratory, however, they received, instead of a single swallow of water, 750 ml of tap water at room temperature, which they ingested within 5 minutes. After 5 to 15 daily repetitions of this procedure in the same setting and under the same circumstances, the subjects were tested for evidence of "conditioning." Coming to the laboratory without knowledge that the routine would be changed, they were given a single 30-ml swallow of water, exactly as had been done during the control sessions before conditioning. Urine samples were collected as in control experiments. After 1 or more of these experimental days had been recorded, the subjects were "reconditioned" by 1 to 6 more days on which they were again given the full water load. Finally, the single swallow was again tested for acquisition of the capacity to produce diuresis. In this way, three to seven experiments were carried out with the single swallow after conditioning for each of the four subjects.

RESULTS

The results of these experiments are summarized in Tables I and II and Figures 2 to 5.

Altered response to 30-ml swallow. After the conditioning by repeated water ingestion, swallowing a 30-ml sample of water in the customary laboratory environment was consistently followed by an increase in urine flow above the flow rates for the 30-minute control collection period before

the swallow. Before the conditioning, but while subjects were on identical schedules of hydration, the swallow was not followed by any consistent change in urine flow rates. The mean change for 18 experiments on four subjects before conditioning was - 0.13 ml per minute, with random variability ($p > .5$), whereas the mean change for 17 experiments on the same subjects after conditioning was + 1.38 ml per minute ($p < .001$). Details of these data form Table I. The mean urine flow rate during the control period of $\frac{1}{2}$ hour before the swallow was almost identical before and after conditioning, indicating comparable states of pre-experimental hydration in the two series.

By calculation of the mean values for urine flow in all collection periods, a line graph was constructed showing the mean excretory patterns for the two series (Figure 2). The mean flow rates for the 2 hours before the swallow were not significantly different. In the experiments before conditioning, a pattern of steadily declining urine flow was recorded after the swallow. After conditioning, however, urine output for all subjects rose significantly after the single swallow of water, exceeding the levels recorded by

TABLE I

Effect of conditioning on urine flow following 30 ml H₂O orally

Subject, age, BSA	Before conditioning			After conditioning				
	Date	Control	Maximum	Δ	Date	Control	Peak	Δ
		ml/min	ml/min	ml/min		ml/min	ml/min	ml/min
S.R.	5/11/61	0.71	1.00	+0.29	5/29/61	0.50	0.75	+0.25
(seated)	5/14/61	1.60	0.90	-0.70	6/ 5/61	1.80	2.65	+0.85
22 yrs.	5/15/61	1.06	1.15	+0.09	10/25/61*	2.06	3.60	+1.54
2.00 m ²	5/16/61	0.85	1.10	+0.25				
	5/17/61	1.48	1.70	+0.22				
K.K.	3/25/61	3.87	1.40	-2.47	4/ 6/61	0.93	2.60	+1.67
(seated)	3/26/61	5.47	2.40	-3.07	4/20/61	2.84	4.53	+1.69
23 yrs.	3/29/61	1.45	2.48	+1.03	4/24/61	1.00	3.45	+2.45
1.97 m ²	3/30/61	1.63	2.80	+1.17	5/ 1/61	0.77	0.90	+0.13
					5/ 2/61	1.00	3.55	+2.55
B.B.	6/13/61	1.23	1.20	-0.03	5/ 4/61	2.80	4.20	+1.40
(supine)	6/14/61	1.00	1.13	+0.13	5/11/61	1.46	3.60	+2.14
22 yrs.	6/15/61	0.73	0.80	+0.07				
1.85 m ²	6/16/61	0.70	1.03	+0.33	7/12/61	2.40	3.25	+0.85
	6/18/61	1.53	1.33	-0.20	7/13/61	2.16	3.30	+1.14
					7/14/61	1.90	3.50	+1.60
S.B.	6/13/61	0.87	0.83	-0.04	7/20/61	1.57	3.50	+1.93
(supine)	6/14/61	1.33	2.19	+0.86				
23 yrs.	6/15/61	1.95	1.60	-0.35	6/23/61	1.60	1.90	+0.30
1.74 m ²	6/17/61	1.90	2.06	+0.16	7/ 4/61	1.13	3.10	+1.97
					7/ 5/61	2.00	2.96	+0.96
Mean		1.63	1.50	-0.13		1.64	3.01	+1.38
				($p > .5$)			($p < .001$)	

* S.R. was reconditioned by being given full water loads on six occasions before this experiment.

MEAN DATA - 4 SUBJECTS

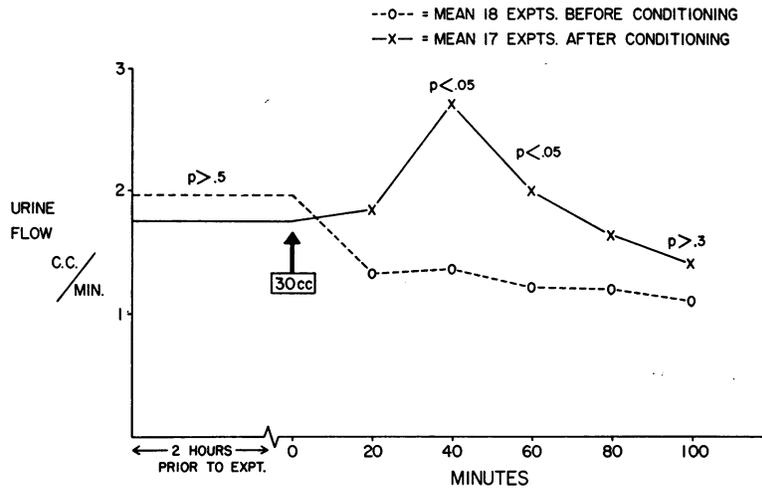


FIG. 2. MEAN URINE FLOW RATES BEFORE AND AFTER CONDITIONING.

these subjects during similarly timed collection periods in the experiments before conditioning by differences significant beyond the 5% level.

Composition of acquired diuretic response. The composition of a typical diuresis occurring after conditioning is graphically represented in Figure 3, the salient characteristics being a phasic rise in urinary flow and an accompanying fall in osmolality, with relatively stable excretory rates for other urinary constituents.

To investigate the consistency of this pattern

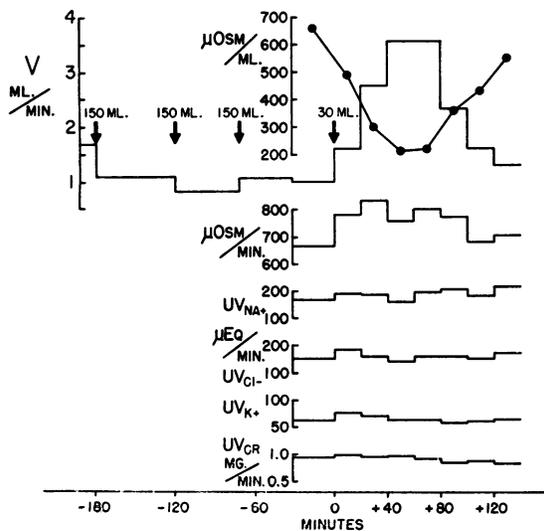


FIG. 3. COMPOSITION OF TYPICAL RESPONSE TO 30 ML OF WATER AFTER CONDITIONING.

among subjects and to allow a detailed comparison between results before conditioning and diuretic responses after conditioning for all subjects, we analyzed the urine samples collected during 12 of the 35 experiments for osmolality, sodium, potassium, chloride, and creatinine content. For the two subjects most extensively studied (K.K. seated and B.B. supine), two experiments before conditioning and two after conditioning were analyzed; the remaining two subjects were represented by data from one experiment before and one after conditioning. For each subject, experiments to be analyzed were selected, insofar as possible, so that flow rates during control periods before and after conditioning would be of comparable magnitude. Thus, the data from the two occasions to be compared reflect similar initial states of water balance, leaving the conditioning experience as the relevant variable presumably responsible for differences in urinary volume and composition following the swallow of water on the two occasions.

Table II compares these experiments before and after conditioning in terms of mean values and ranges for urine flow (V), urine osmolality (U_{osm}), rate of sodium excretion ($U_{Na}V$), rate of chloride excretion ($U_{Cl}V$), rate of potassium excretion ($U_{K}V$), and rate of creatinine excretion ($U_{Cr}V$) for the control collection periods and for periods exhibiting peak diuresis after conditioning (peak period). Mean values and ranges

TABLE II
Effect of single swallow on urinary composition

		Before conditioning*			After conditioning†			Mean difference‡ p	
		Control period	Peak period	Change§	Control period	Peak period	Change§		
		Mean range	Mean	Mean range	Mean range	Mean	Mean range		
V	ml/min	1.39 1.00 to 1.95	1.58	+0.19 -0.65 to +1.03	1.59 1.00 to 2.06	3.43	+1.84 +0.96 to +2.55	+1.65	<.001
	μOsm/ml	631 397 to 958	630	-1 -144 to +157	503 289 to 666	249	-254 -62 to -453	-253	<.005
U _{osm}	μOsm/min	811 647 to 993	853	+42 -86 to +103	749 585 to 982	849	+100 -177 to +241	+58	>.4
	U _{Na} V μEq/min	209 148 to 268	234	+25 -43 to +90	181 64 to 364	222	+41 -12 to +168	+16	>.5
U _{Cl} V	μEq/min	154 102 to 194	172	+18 -6 to +32	149 61 to 210	155	+6 -54 to +48	+12	>.5
U _K V	μEq/min	65 42 to 94	68	+3 -18 to +19	70 56 to 80	72	+2 -17 to +10	-1	>.5
U _C V	mg/min	1.08 .86 to 1.28	1.09	+0.01 -.10 to +.27	1.15 0.78 to 1.36	1.23	+0.08 -.19 to +.26	+0.07	>.5

* Data from: S.R., 5/15/61; K.K., 3/29/61, 3/30/61; B.B., 6/13/61, 6/14/61; S.B., 6/15/61—see Table I.
 † Data from: S.R., 10/25/61; K.K., 4/24/61, 5/2/61; B.B., 7/14/61, 7/20/61; S.B., 7/5/61—see Table I.
 ‡ Calculated as mean change for experiments after conditioning minus mean change for experiments before conditioning.
 § Peak period mean minus control period mean.
 || Abbreviations: V = urine flow, U_{osm} = urine osmolality, U_{Na}V = rate of sodium excretion, U_{Cl}V = rate of chloride excretion, U_KV = rate of potassium excretion, and U_CV = rate of creatinine excretion.

for urine flow and osmolality in control periods before conditioning were generally similar to control periods after conditioning. There was no statistical significance for the mean differences in flow (0.20 ml per minute, $p > .5$) and osmolality (128 μOsm per ml, $.4 > p > .3$) between these two series of experiments. There is no evidence, then, that the subjects presented themselves in consistently different states of hydration on the two occasions compared. During experiments before conditioning, there was no significant rise in flow or fall in osmolality. In all experiments after conditioning, however, there was a rise in flow that was paralleled by a fall in osmolality, on one occasion reaching -453 μOsm per ml, indicating the addition of considerable quantities of free water to the urine during the conditioned diuresis. The mean osmolar output rose somewhat in both sets of experiments, but the trend was not entirely consistent. Although mean U_{osm}V increased more in the experiments after conditioning than in those before, this difference

between the two sets of experiments (Table II, far right) is very unlikely to be of significance ($p > .4$). The output of electrolytes generally followed the pattern of total osmolar output. The mean excretion rate for sodium increased slightly on control days, somewhat more so after conditioning, but the mean change after conditioning was not significantly different from that observed on control days. Chloride and potassium excretion also tended to rise, but again mean differences were not significant. The creatinine output showed no consistent change in either series of experiments. The mean coefficient of variation of excretory rates for creatinine for all experiments was 7.7%, indicating that bladder emptying was satisfactory.

A comparison between the composition of the urine obtained during conditioned diuresis and that obtained during diuresis in response to water loads is made in graphic form for two subjects in Figure 4. The conditioned diureses (left, top and bottom) illustrate the two varieties

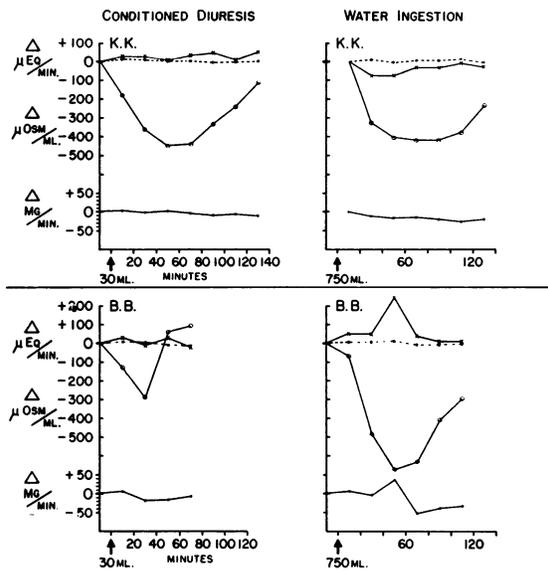


FIG. 4. COMPOSITION OF DIURESIS AFTER WATER LOADING COMPARED TO CONDITIONED DIURESIS. Na \times — \times ; K \bullet — \bullet ; Osm \ominus — \ominus ; Cr \bullet — \bullet .

of timing observed in the characteristic fall of osmolality. K.K. showed a rather prolonged suppression of osmolality, although this returned toward base line somewhat sooner than in his full diuretic response to 750 ml. In the pattern

exhibited by B.B., the conditioned response clearly reproduced only the initial phases of the response to 750 ml, with a compensatory rise in osmolality following the initial dip. In general, excretion rates for electrolytes and creatinine were similar in all diureses. B.B., however, in response to 750 ml, showed an isolated apparent rise in sodium and creatinine output in one collection period during which a precipitous increase in flow rate of 3.7 ml per minute to 17.9 ml per minute occurred.

Relationship of diuretic response to base-line flow rates. If the data from Table I for the experiments after conditioning are plotted graphically (Figure 5), it becomes clear that the magnitude of increase in minute volume after conditioning was not directly related to the magnitude of base-line flow rates, but rather remained fairly constant over a wide range of control values. There was, however, considerable variability in magnitude of response for each subject from day to day (Table I). Several factors which may have been responsible for such variability have been defined in subsequent experiments (20).

Effects of the number of days of conditioning with large water loads. The amount of conditioning necessary to alter the response to a single

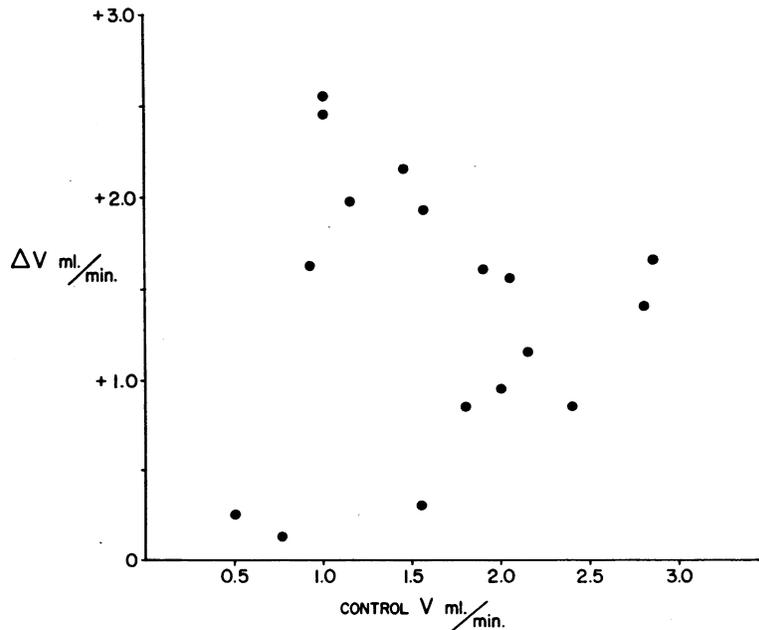


FIG. 5. MAGNITUDE OF CONDITIONED DIURESIS AS A FUNCTION OF CONTROL FLOW RATES.

swallow of water varied considerably among subjects. All four showed statistically augmented responses after 15 conditioning periods. One subject showed a pronounced conditioned response to a single swallow after only eleven repetitions of the conditioning stimulus. Two others, tested after 5 and 9 days, gave minimal responses only. On the other hand, subjects apparently could be "overconditioned" to the point that the swallow of water was no longer effective: one subject gave two clear-cut diuretic responses after 15 conditioning days, but showed no responses after 6 additional days of stimulation with large water loads.

DISCUSSION

The elicitation of physiologic responses by previously neutral stimuli was described by Pavlov as the "conditioned reflex" (21). These words fail to suggest, however, the association process by which the new response was acquired, a process basic to "learning" and "memory" as they are usually considered in purely central neural processes. Further knowledge of the properties of this mechanism and its interaction with central neuronal systems for emotion is basic to a better understanding of physiological disturbances arising out of patients' life situations. Although the magnitude of the diuresis produced within these short periods of regular conditioning was modest, further experimentation has shown that flow rates as high as 7.9 ml per minute in seated subjects and 12.3 ml per minute in the supine can be produced under altered schedules of conditioning (20). Such rates of water loss could be physiologically damaging if maintained for any length of time.

The "triggering" action of the 30-ml swallow of water apparently made it possible to demonstrate this effect after only 15 repetitions of the conditioning sequence, and seems to have overcome the obstacles of "overconditioning" that may have been responsible for the difficulty encountered in demonstrating this phenomenon in dogs (22). In producing diuresis, an "appropriate" conditional stimulus, such as a swallow of water, seems to be superior to more casually associated environmental stimuli such as bells or lights. The environment of the conditioning room, however, appeared to have an important permissive effect

upon the acquired diuretic response to the swallowing of water; 150 ml taken in preparation for the experiments either in the subject's dormitory or classrooms, but under similar conditions of position, activity, and temperature, failed to produce diuresis of comparable magnitude to that produced by 30 ml taken in the conditioning room in which the subject had previously been given large water loads.

Although the single swallow might acquire the property of initiating diuresis, there was no consistent shortening of the latency for peak diuresis following large water loads within these relatively short periods of conditioning. On occasional days after the tenth conditioning session, however, minute volume rose significantly *before* water ingestion. Further studies have shown that with longer periods of intermittent conditioning, anticipatory responses can be reliably maintained (20).

In one seated and one supine subject, the 30-ml swallow was repeated on several consecutive days without further reinforcement by large water loads. A definite diuretic response was obtained on each of the first 3 or 4 days, but upon further repetition of the swallow on subsequent days, it ceased to produce flow rate changes greater than those observed on days before conditioning ("extinction"). However, when these subjects were returned to the routine of large water loads for as little as one day, another diuretic response usually could be obtained before extinction again supervened. The acquisition of the diuretic response to the single swallow thus appeared to be temporary, the effect ceasing to be demonstrable after the stimulation involved in the swallow had been repeated several times without the physiological sequelae of significant water absorption.

Several features of this conditioned diuresis suggest that it was the result of a decrease in the concentration of circulating ADH. There was the characteristic delay of 20 to 40 minutes after the swallow before peak rates of urine flow occurred. This period is thought to be necessary for the metabolic destruction of pre-existing, circulating hormone (23). Analysis of the composition of the urine showed that the single swallow after conditioning promoted an increased flow of urine with a decreased osmolality; excretion rates for other constituents remained unchanged.

This pattern was similar to that found after ingestion of large water loads. Regularly, free water clearance (estimated from a plasma osmolality of 280 μOsm per ml) rose from negative to positive. The described changes in urinary composition are highly characteristic of decreased levels of circulating ADH. A rise in free water clearance, however, may be produced in certain circumstances by osmotic loading (24), pronounced alterations in glomerular filtration (25), or infusions of aldosterone (26). Under these conditions, the alterations in free water excretion are always accompanied by pronounced changes in solute excretion, and in none of these instances has urine osmolality fallen from clearly hypertonic to very hypotonic levels. The apparent increase in solute excretion rates observed in one of our subjects after a large water load may best be explained by washout of solute previously accumulated in higher concentrations within the renal dead space (27). The fact that consistent changes in creatinine and electrolyte excretion did not occur in our experiments supports the inference that decreased ADH, and not any of these other mechanisms, was responsible for the conditioned diuresis.

A diuresis of water and salt has previously been reported from this laboratory (28) and by Miles and de Wardener (29) as occurring during threatening situations. Other workers have reported augmented water diuresis in normal subjects given large doses of cortisone (30, 31). The mechanism of these phenomena is uncertain, but a decrease in circulating ADH has been thought to play an important role in them. In the present study, the familiarity of the subjects with the laboratory and the tranquillity and predictability of the experimental situation made it unlikely that any emotional upheaval would take place, or that subjects would feel threatened by the procedure. The neural mechanisms involved therefore could not have been entirely the same as those involved in the "stress diuresis" previously described, which was usually associated with an overt display of apprehension and anxiety, and often with a catabolic response, leading to an increase in the excretion of nitrogen and electrolytes. An increase in free water clearance was its most prominent and its only consistent characteristic.

The data from these experiments help to exclude other possible diuretic mechanisms and provide strong indirect evidence that a decrease in secretion of ADH from the posterior pituitary forms the efferent mechanism for this conditioned diuresis. The afferent portion is apparently complex, involving stimulation of visceral structures innervated by the V, VII, IX, and X cranial nerves (the swallow), as well as exteroceptive stimuli of sight, sound, and smell. The vagus nerve has previously been implicated in water diuresis following atrial stretch (32), indicating the existence of a neural mechanism for modification of neurohypophyseal function by peripheral stimuli. It seemed unreasonable to suspect that the 30-ml swallow had acquired some means of depressing plasma osmolality and was influencing posterior pituitary function by this means. But to test this possibility, plasma osmolality was measured at $\frac{1}{2}$ - to 1-hour intervals during two conditioned diureses and was found actually to rise somewhat after the 30-ml swallow, whereas the ingestion of 750 ml of water was observed to produce a fall of 10 μOsm per ml.

It is not surprising that effects similar to those described in this paper have been reported previously in the literature as having occurred by accident during the course of repeated experimentation on the same subject. White (33) reported, as an incidental finding, that one of his subjects who was studied before and after large water loads showed a considerable increase in urine flow and fall in urine osmolality after water ingestion, but before any fall in plasma osmolality had occurred, instead of showing the usual lag in urinary response to plasma dilution. In his monograph, Smith suggested that "an anticipatory conditioned response may have occurred" (34).

The observations of Hollander (35) suggest that regulatory mechanisms governing salt excretion may also be liable to modification during repeated experimentation on the same subject. More significant than the possibility of producing artifacts in many types of experiments, conditioning procedures may prove to be a valuable experimental technique for the study of neuroregulatory systems in the intact human.

Thus, it would appear that afferent stimuli, ordinarily incapable of affecting neurohypophyseal ADH output, acquired the ability transiently to

suppress circulating levels of this hormone simply by preceding plasma dilution in a stereotyped manner on a number of occasions. The "conditioned" responses demonstrated in these experiments must be an integral part of the homeostatic system, serving to prepare the organism specifically for a particular form of physiological challenge. By this means, self-regulation may occur in anticipation as well as in compensation, the organism responding in terms of past experience in preference to current homeostatic demands.

SUMMARY

Normal human subjects, maintained on standard schedules of hydration and activity, were conditioned by receiving 750-ml water loads in a stereotyped manner on five to fifteen occasions. Subsequently, a single 30-ml swallow produced a phasic diuresis lasting 40 to 80 minutes in all subjects. Identical 30-ml swallows taken in control experiments before conditioning had no such effect, being followed by a pattern of decreasing urine flow rates.

The conditioned diuresis was similar to standard water diuresis in composition. Analysis of urinary composition showed an addition of free water to the urine without significant alteration in electrolyte and creatinine excretion. The evidence implicating neurogenic suppression of antidiuretic hormone release from the neurohypophysis as the efferent mechanism for this diuresis is critically discussed.

The evidence suggests that new kinds of hormonal response can be established in the human through repeated associative experience.

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