nalectomy, bilateral adrenalectomy or unilateral adrenalectomy and contralateral adrenal enucleation and given 1% sodium chloride solution to drink. Their response to the daily administration of 100 μg of d-aldosterone acetate was compared. Hormone-treated or adrenal enucleated rats both developed hypertension at about the same rate and with a comparable incidence and severity, and in this respect the two influences were not additive. In contrast to adrenal enucleation, aldosterone treatment caused impairment of growth and thymus involution. Thymus glands of aldosterone-treated bilaterally adrenalectomized rats were nonetheless larger than those of untreated enucleated rats. Although adrenal enucleation induced saline polydipsia and caused kidney enlargement, both responses were significantly greater in hormone-treated rats. The implication of these findings with respect to the role which endogenous aldosterone hypersecretion has been suspected of playing in the genesis of adrenal-regeneration hypertension is considered.

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Effect of Hydrochlorothiazide on Preference Threshold of Rats for NaCl Solutions.* (32281)

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During an earlier study in which the diuretic agent, hydrochlorothiazide (HCZ), was administered chronically to rats with post-desoxycorticosterone acetate (DCA)-induced hypertension, the impression was gained that HCZ increased the NaCl intake of these rats above that of controls given DCA alone. The experiments reported here were carried out to verify this observation and to study it in more detail. Both the time course for development of the NaCl appetite after HCZ administration and the effect of this diuretic agent on preference (taste) threshold for NaCl solution were studied and are described below.

Methods. Experiment 1. Effect of chronic administration of HCZ on spontaneous NaCl intake by rats. Eighteen male rats of the Carworth CFN strain weighing 420 to 460 g were kept in individual cages in a thermoregulated

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(26 ± 1°C) room which was illuminated from 8 A.M. to 6 P.M. The rats were divided into 3 equal groups. All rats were given finely powdered Rockland Rat Diet in spillproof feeders(1). Group 1 served as control while Groups 2 and 3 received the same food containing 0.3 and 0.6 g HCZ/kg respectively. All rats were given tap water to drink except during weeks 2 and 6, when choice was offered between tap water and 0.15 M NaCl solution. Intakes of water, NaCl solution and food were measured daily during these periods. The fluid containers were infant nursing bottles with cast aluminum drinking fountains as described by Lazarow(2).

A second, different group of 18 male rats of the Carworth CFN strain weighing 400 to 470 g were maintained under conditions identical to those described above (Exp. 1B). The rats were also divided into 3 equal groups. Again, Group 1 served as control while Groups 2 and 3 received 0.3 and 0.6 g HCZ/kg food respectively. All rats were given tap water to drink until the 8th week of drug treatment at which time each rat was given choice between tap water and 0.15 M NaCl solution to drink. Intakes of water, NaCl solution and food were measured daily.

The intakes of the groups in both experiments were compared by an analysis of variance using orthogonal comparisons between groups(3).

Experiment 2. Effect of acute administration of HCZ on spontaneous NaCl intake by rats. Twenty male rats of the Carworth CFN strain weighing 350 to 425 g were used. The animals were maintained in individual cages under conditions identical to those described in Experiment 1. The rats were allowed 3 days to adjust to experimental conditions after which daily measurement of water, 0.15 M NaCl solution and food intakes began for 4 days. The rats were divided randomly into 4 equal groups and at the end of the 4th day Group 1 served as control; Group 2 received 0.1 g HCZ/kg food; Groups 3 and 4 received 0.3 and 0.6 g HCZ/kg food respectively. Measurements of intakes continued daily for an additional 4 days.

Intakes of the groups were compared before and after drug treatment by means of an analysis of covariance using the Biomed Series program BMDO4V and the IBM 709 computer.

Experiment 3. Effect of HCZ on preference threshold of rats for NaCl solution. Ten male rats of the Carworth CFE strain weighing 360 to 425 g were used. They were maintained under conditions identical to those described in Experiment 1. Five of the rats received HCZ in food at 0.6 g/kg for 4 months prior to beginning the experiment. The remaining 5 animals received the same diet without HCZ. Tap water and food were available *ad libitum* during the 4-month period. At the beginning of the experiment the rats were caged individually and given choice between two bottles each containing distilled water. The fluid and food containers were the same as those described in Experiment 1. The food was finely powdered Rockland Rat Diet. One week was allowed for adjustment to the cages and bottles. During this time intakes were measured daily to ascertain that each rat drank roughly equal amounts from each bottle. Positions of the 2 bottles on each cage were interchanged daily to avoid habit formation in selection of drinking fluid. At the end of this period, 2-day test periods were begun. During the first test period, each rat was offered choice between 2 bottles of distilled water. During the second test period, choice was offered between distilled water (bottle A) and 0.0003 M NaCl solution (bottle B). All NaCl solutions used were made from a concentrated stock NaCl solution by serial dilution. Each dilution was checked for accuracy by determination of chloride concentration(4). During subsequent 2-day periods, each rat was given choice between distilled water and the following molar NaCl solutions in chronological sequence: 0.0006, 0.001, 0.003, 0.006, 0.009, 0.012, 0.015, 0.018, 0.025, 0.050, 0.075, 0.100, and 0.150. Daily intakes of water and NaCl solutions were measured and expressed as ml/100 g body weight/day to correct for differences in body weight.

The criterion of preference threshold used was similar to that of Richter(5): namely, the concentration of NaCl solution at and above which simultaneous mean volume taken

TABLE I. Effect of Dietary Administration of Hydrochlorothiazide on Spontaneous Intakes of 0.15 M NaCl Solution, Water and Food by Rats.

	No. of rats	Mean body wt (g)	Intakes (ml or g/100 g body wt/day) of: .15 M NaCl soln.	Water	Total fluid	Food	Ratio of NaCl/total fluid ingested
Experiment 1A							
2 wk of drug treatment							
Control	6	470	6.0 ± .6*	3.8 ± .3	9.8 ± .7	5.6 ± .2	.61 ± .02
Hydrochlorothiazide (.3 g/kg diet)	6	452	11.0 ± 1.9†	4.0 ± 1.3	15.0 ± 1.7†	5.5 ± .1	.67 ± .13
Hydrochlorothiazide (.6 g/kg diet)	6	457	11.9 ± 1.7†	3.1 ± 1.3	15.0 ± 1.7†	5.7 ± .1	.75 ± .12
6 wk of drug treatment							
Control	6	490	5.7 ± .8	3.8 ± .5	9.5 ± .7	4.9 ± .2	.60 ± .06
Hydrochlorothiazide (.3 g/kg diet)	6	485	11.4 ± 1.8‡	3.2 ± 1.0	14.6 ± 1.6‡	5.4 ± .1	.73 ± .11
Hydrochlorothiazide (.6 g/kg diet)	6	478	9.8 ± 1.3†	2.8 ± 1.3	12.7 ± 1.3†	5.4 ± .1	.73 ± .13
Experiment 1B§							
Control	6	457	4.8 ± 1.6	3.5 ± .5	8.3 ± 1.4	4.4 ± .2	.56 ± .07
Hydrochlorothiazide (.3 g/kg diet)	6	432	11.7 ± 1.9‡	1.4 ± .3†	13.1 ± 1.7†	4.5 ± .2	.87 ± .04‡
Hydrochlorothiazide (.6 g/kg diet)	6	427	11.9 ± 2.1‡	2.5 ± .6	14.4 ± 2.8	4.7 ± .1	.79 ± .06†

* Standard error of mean.
† Significantly different from control ($P < .05$).
‡ Significantly different from control ($P < .01$).
§ Eight weeks of drug treatment.

from the test bottle exceeded that taken from the reference (water) bottle. To determine the preference threshold concentration, simultaneous intakes of distilled water and NaCl solution were graphed for each individual rat. The concentration at which NaCl intake increased and remained elevated, while water intake decreased and remained low (cross-over concentration) was then determined. Individual data for rats in each group were averaged and the means were compared statistically using a t test(3).

Results. Experiment 1. Administration of HCZ for 2 to 8 weeks increased significantly spontaneous salt intake when choice was offered between water and 0.15 M NaCl solution to drink (Table I). Neither water nor food intake was consistently affected by treatment. However, total fluid intake (water + 0.15 M NaCl solution) increased. The ratio of NaCl ingested to total fluid ingested increased on the average with drug treatment but was significantly greater than the control ratio only after 8 weeks of treatment (Table I).

Experiment 2. Acute administration of HCZ also increased significantly spontaneous intake of NaCl solution but failed to affect either water or food intake (Table II). The increase in intake of NaCl solution occurred with all doses of HCZ used and did not appear to be dose-dependent within this range of doses. It may be noted that intake of NaCl solution by the control group increased during the treatment period. No reason can be advanced for this although its occurrence made it important to compare all results by an analysis of covariance. The ratio of intake of NaCl solution to total fluid was increased significantly by drug treatment. The results of this experiment are in accord with those of Experiment 1 obtained after chronic administration of HCZ.

Experiment 3. Using the mean cross-over concentration as the criterion, HCZ-treated rats detected the difference between water and NaCl solution when the latter was 0.012 M while controls detected the difference between the two when the NaCl concentration was 0.050 M (Fig. 1). Thus, the preference threshold of HCZ-treated rats lies between 0.009 and 0.012 M while that of control rats lies between 0.025 and 0.050 M. If the cross-

TABLE II. Effect of Hydrochlorothiazide on Spontaneous Intakes of 0.15 M NaCl Solution, Water and Food by Rats.

	Intakes during control period (ml or g/100 g BW/day)				Intakes during treatment period (ml or g/100 g BW/day)			
	Water	.15 M NaCl soln.	NaCl /total	Food	Water	.15 M NaCl soln.	NaCl /total	Food
Control	5.5	3.9	.36	4.9	5.0	6.1	.53	4.5
.1 g HCZ/kg food	5.0	3.8	.36	4.6	2.1	11.7	.84	3.9
.3 g HCZ/kg "	4.2	3.9	.46	4.8	1.8	11.9	.87	4.7
.6 g HCZ/kg "	6.0	3.0	.31	5.0	2.8	9.4	.76	4.5

Analysis of covariance

		Water			.15 M NaCl solution			NaCl/total			Food		
Source	df	MSS	F	P	MSS	F	P	MSS	F	P	MSS	F	P
Treatment	3	6.07	2.47	>.05	31.55	4.04	<.05	.099	3.54	<.05	.47	.59	>.05
Error	16	2.46			7.81			.028			.81		
Total	19												

over concentrations are determined individually and the means of each group compared, the HCZ-treated rats differentiated between water and the NaCl solution at a mean NaCl concentration of 0.010 M while controls differentiated at a mean NaCl concentration of 0.029 M (Table III). Thus, HCZ-treated rats could differentiate between NaCl solution and water at approximately one-third the concentration required by control rats. In spite of the greater sensitivity of treated rats for detecting NaCl, their maximal NaCl intake did not differ from that of controls. Body weights of both groups of animals increased during the course of the experiment but failed to differ significantly from each other. Food intakes of the two groups did not differ from each other at any time during the experiment.

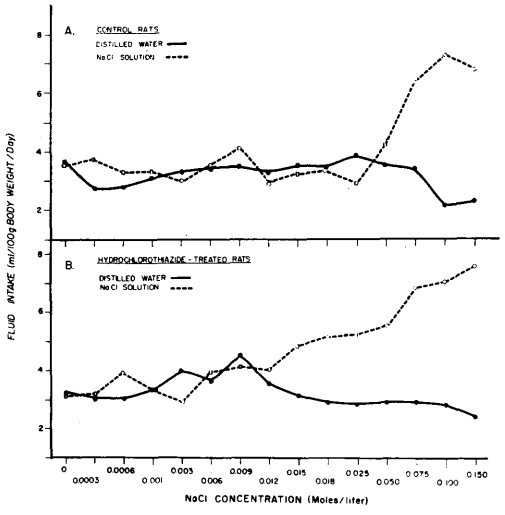

FIG. Spontaneous intakes of NaCl solution (○) and distilled water (●) are shown for control rats (part A) and hydrochlorothiazide-treated rats (part B).

Discussion. Administration of HCZ to rats increased their spontaneous salt intake after either days or weeks of treatment. The ability to differentiate between distilled water and NaCl solution was also increased by HCZ, the preference threshold of treated rats being one-third that of control rats. The preference

TABLE III. Effect of Treatment with Hydrochlorothiazide on Preference Threshold of Rats for NaCl Solution.

Treatment	No. of rats	NaCl preference threshold (moles/l)	P*
Control	5	.029 ± .007†	<.05
Hydrochlorothiazide treated (.6 g/kg food)	5	.010 ± .004	

* Probability value.
† ± 1 standard error of mean.

threshold of the control rats observed here was higher than that reported by Weiner and Stellar (.009 M) (7) and Richter (0.0094) (8) but was within the range of variability observed previously in this laboratory for derivatives of the same strain (6). The preference threshold of HCZ-treated rats (Table III) was higher than that observed previously for rats treated chronically with the antithyroid drug, propylthiouracil (0.004 M) (6) although treatment with either

drug was accompanied by similar NaCl appetites and reduced preference thresholds for NaCl solution. Although propylthiouracil is known chiefly as an antithyroid drug, it has weak diuretic activity(9). HCZ is known chiefly as a diuretic agent but has been shown recently to possess antithyroid-like activity (10,11). Thus chronic administration of HCZ at a concentration of 0.6 g/kg food increased thyroid weight, altered thyroid histology and increased rate of release of radioactive iodide from the thyroid gland(10,11). It is likely that the similar effects of the two compounds on NaCl appetite and preference threshold for NaCl are related to their effect on thyroid gland. Other experimental procedures that reduce thyroid activity and increase NaCl intake are surgical thyroidectomy(12) and administration of either amphenone-B(13) or piperidyl-sulfonic acid(14). It is of interest that all experimental procedures thus far tested inducing a chronic appetite for NaCl *viz.*, adrenalectomy, hypothyroidism and administration of large doses of desoxycorticosterone acetate, are also accompanied by a reduced preference threshold for NaCl solution (6,8,15). To this list may now be added treatment with HCZ.

The apparent relationship between reduced preference threshold for NaCl solution and NaCl appetite has not been explained satisfactorily although a number of possibilities exist and have been presented recently(16). For example, it has been suggested that these two phenomena may be related directly to the Na/K ratio of the saliva bathing taste receptors and indirectly to blood level of aldosterone which appears to regulate salivary Na/K ratio(16). Venning *et al*(17) have shown that urinary aldosterone excretion, and presumably adrenal aldosterone secretion, increased when patients with essential hypertension were treated chronically with chlorothiazide, an analog of hydrochlorothiazide. In addition, chlorothiazide has been shown to reduce significantly both the mean salivary Na concentration and the mean salivary Na/K ratio in 8 human hypertensive patients (18). It is also possible that the salt appetite is induced by central mechanisms responding to either altered aldosterone or electrolyte concentration of blood or to altered extracellular fluid volume. Changes in body sodium content resulting from increased renal sodium loss induced by the drug may also play a role (16). It is difficult to state which, if any, of the suggestions mentioned above can explain the spontaneous salt appetite of treated rats. Further studies are required to separate these possibilities or to provide more compelling explanations.

Summary. When given choice between water and 0.15 M NaCl solution to drink, rats treated with the diuretic agent, hydrochlorothiazide (0.3 and 0.6 g/kg food), for days or weeks increased their salt intake 70 to 150% above the level of controls while simultaneous water and food intakes were unaffected by treatment. Chronic administration of hydrochlorothiazide (0.6 g/kg food, 4 months) lowered the preference threshold of rats for NaCl solution from 0.029 to 0.010 mole/liter. This lowering of NaCl preference threshold by hydrochlorothiazide may be an important factor influencing the increased spontaneous NaCl intake of treated rats.

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Effect of Thyrocalcitonin on Duodenal Calcium Transport.* (32282)

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The gastrointestinal tract is unnecessary for mediation of the hypocalcemic effect of thyrocalcitonin(1). It is possible, however, that this hormone does exert an effect upon intestinal calcium absorption, but reports in the literature regarding this possibility are conflicting(2-4). This investigation was undertaken to determine the effect of thyrocalcitonin on calcium fluxes in the rat duodenum in acute studies using an *in vivo* perfusion technique.

Methods. Male Sprague-Dawley rats weighing 230-300 g which were fed normal laboratory chow (calcium content 1.2%) served as experimental and control animals. Food was withheld for 24 hours before study but the rats were permitted tap water *ad libitum*.

Anesthesia was produced with intraperitoneal *Dial*® with urethane solution (Ciba) (0.7-1.0 ml/kg). Through a midline abdominal incision the distal end of the bile duct was ligated and transected. The proximal 8-13 cm of small intestine were cannulated with metal adapters attached to polyvinyl tubing. The inflow cannula was inserted through an incision in the pylorus and the tubing tied in place with suture. Similarly, the outflow cannula was positioned and tied 1-2 cm distal to the ligament of Treitz. Before perfusion the isolated segment of gut was washed with 0.9% NaCl and then emptied with air. The abdomen was closed over the cannulas and body temperature was maintained with a heating pad.

Fluid was perfused by recirculation at a rate of 2.0 ml/min for one hour from a reservoir containing 12 ml of solution by use of a proportioning pump (Technicon). The perfusing solution of pH 6.5 contained 0.85 mM $CaCl_2$, 137 mM NaCl, 20 mM THAM, 0.2% polyethylene glycol and 0.02 μc/ml $^{45}CaCl_2$ (Nuclear Chicago, specific activity $>$ 10 curies/g).

Just before the start of perfusion 1.0 ml of blood was withdrawn from the inferior vena cava. At the beginning of the perfusion, thyrocalcitonin† (approximately 250 MRC milliunits/kg body weight in a total volume of 2.5 ml/kg of body weight) was injected rapidly through a femoral vein. The control animals received the vehicle (0.9% NaCl of pH of 3.0, 2.5 ml/kg body weight) only. At the end of the perfusion period, blood was withdrawn from the inferior vena cava. The gut was removed and the contents and any excess fluid expressed by gently pressing the tissue flat on absorbent paper. The perfused segment of gut was weighed on a torsion balance (Bethlehem Instrument Co.).

Polyethylene glycol was analyzed turbidometrically with a spectrophotometer (Research Specialties Model 4000 Spectromatic). Calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 303) and radioactivity was estimated in a

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