eel gastric mucosa.

Summary. By the everted intestinal sac technique, a crude homogenate of eel gastric mucosa was shown to enhance selectively the vitamin B_{12} uptake of the distal portion of the eel intestine. The active principal in ESH was nondialyzable and destroyed by boiling for 10 min. Weak binding of vitamin B_{12} to ESH was demonstrated. These findings suggest that a rudimentary intrinsic factor mechanism for vitamin B_{12} asborption is present in the eel.

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The Salt Appetite of Adrenalectomized Rats as Influenced by Deoxycorticosterone and Hydrochlorothiazide* (33979)

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Administration of the diuretic agent, hydrochlorothiazide (HCZ), to rats was accompanied both by a spontaneous NaCl appetite and a reduction in the preference (taste) threshold for NaCl solution (1). These changes have also been reported to accompany bilateral adrenalectomy in rats (2, 3). The similarities of the responses to these two diverse experimental procedures are striking and suggest that a common mechanism may exist for both. Since administration of the proper dose of deoxycorticosterone acetate (DOCA) to adrenalectomized rats reduced their exaggerated NaCl intake (4, 5), it seemed worthwhile to determine whether a similar response occurred when DOCA and HCZ were administered simultaneously. The present experiments were designed to study the interrelationship between these two drugs as well as to test the effect of HCZ on the spontaneous NaCl intake of adrenalectomized rats.

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Methods. Two separate experiments were performed. Both used male rats of the Blue Spruce Farms Strain which were bilaterally adrenalectomized 5–7 days prior to the experiment. During this period and throughout the experiment the rats were maintained on finely powdered Purina laboratory chow and given choice between distilled water and 0.15 *M* NaCl solution to drink. Food containers were spill-proof and have been described in detail (6). Fluid containers consisted of infant nursing bottles with cast aluminum fountains (7). The NaCl intake of each rat was measured during 1 week prior to beginning the experiment to assure that all rats were completely adrenalectomized. Only those rats ingesting 65% or more of their total fluid intake as NaCl solution were used.

Expt. 1. The purpose of this experiment was to study the interrelationship between simultaneous administration of DOCA and HCZ with respect to the spontaneous NaCl intake of adrenalectomized rats. Thirty rats were placed into three equal groups. Group 1

TABLE I. Effect of Hydrochlorothiazide and Deoxycorticosterone Acetate Singly and Together on Spontaneous Intakes of 0.15 *M* NaCl Solution, Water, and Food by Adrenalectomized Rats.

Treatment	Measurement made	Intakes (ml or g/100 g body wt) during: Control period[a]	Treatment with hydrochlorothiazide[b]
Adrex. + oil	0.15 *M* NaCl soln	11.1	14.8
	Water	4.0	2.0
	NaCl/total fluid	0.74	0.88
	Food	6.2	6.1
Adrex. + DOCA (50 μg/100 g of body wt)	0.15 *M* NaCl soln	5.9	11.5
	Water	6.9	2.3
	NaCl/total fluid	0.46	0.83
	Food	7.4	6.2
(100 μg/100 g of body wt)	0.15 *M* NaCl soln	6.1	14.0
	Water	7.4	3.3
	NaCl/total fluid	0.44	0.80
	Food	7.3	6.9

[a] Each period represents 4 days.
[b] Dose: 0.6 g of hydrochlorothiazide/kg of food.

received a subcutaneous injection of 0.20 ml of peanut oil once daily during a 4-day period. Groups 2 and 3 received, respectively, 50 and 100 μg of DOCA/100 g of body weight once daily during the same period. The rats were caged individually and given choice between distilled water and 0.15 *M* NaCl solution to drink. The bottles and fountains were washed thoroughly every second day. The positions of the bottles on the cages were interchanged daily to avoid habit formation in selection of drinking fluid. Daily measurements were made of food and fluid intakes and body weight.

At the end of the 4-day period, all rats were given powdered Purine laboratory chow into which was mixed 0.6 g of HCZ/kg of food. The drug was first triturated with a small amount of food and then mixed in a tumbling mixer at 6 rpm for 45 min. Those rats receiving DOCA continued to receive this steroid during the 4 days HCZ was administered in food. Daily measurements were made of food and fluid intakes and body weight during this period also.

The experiment was designed so that a 3 × 2 factorial analysis could be used to analyze the results. The statistical analysis was performed using the IBM 360 computer and Biomed Series program BMD02V.

Expt. 2. The purpose of this experiment was to test the effect of HCZ on the spontaneous NaCl intake of adrenalectomized rats. Ten rats were placed randomly into two equal groups. Body weight and intakes of water, 0.15 *M* NaCl solution and food were measured for 5 days, at the end of which five of the rats were given the same food into which was mixed 0.6 g of HCZ/kg. Measurement of intakes and body weight continued for an additional 5-day period. All data were compared by means of a *t* test for the 95% confidence limit (8). Since the experimental results were expressed as a percentage change from the control period, an arc sin transformation was used to normalize the data (9).

Results. Tables I and II show the effects of DOCA and HCZ, singly and in combination, on the spontaneous intakes of 0.15 *M* NaCl solution, water and food. In addition, the ratio of the volume of NaCl ingested compared to the total fluid volume (NaCl solution + water) ingested is given. Administration of HCZ significantly ($p < 0.01$) reduced water intake. Administration of DOCA

TABLE II. Results of an Analysis of Variance for Factorially Designed Expt. 1.

Source of variation	*df*	Sums of squares	Mean squares	*F*	*p*
a. Water intake					
Hydrochlorothiazide	1	95.5154	95.5154	22.33	<0.01
DOCA	2	29.1562	14.5781	3.41	0.05
Interaction	2	9.5442	4.7721	1.12	>0.05
Within replicates	24	102.6536	4.2772		
Total	29	236.8694			
b. 0.15 *M* NaCl soln intake					
Hydrochlorothiazide	1	248.3714	248.3714	30.37	<0.01
DOCA	2	93.6982	46.8491	5.73	<0.05
Interaction	2	23.0453	11.5226	1.41	>0.05
Within replicates	24	196.2779	8.1783		
Total	29	561.3928			
c. Ratio NaCl/total fluid intake					
Hydrochlorothiazide	1	0.6366	0.6366	33.86	<0.01
DOCA	2	0.2038	0.1019	5.42	<0.05
Interaction	2	0.0859	0.0430	2.29	>0.05
Within replicates	24	0.4518	0.0188		
Total	29	1.3781			

significantly ($p = 0.05$) increased water intake. Both treatments given simultaneously failed to interact with respect to water intake ($p>0.05$). Administration of HCZ significantly ($p< 0.01$) increased both intake of NaCl solution and the ratio of NaCl/total fluid ingested. Administration of DOCA significantly ($p < 0.05$) reduced both intake of NaCl solution and the ratio of NaCl/total fluid ingested. There was no significant interaction of these two drugs on NaCl intake when administered simultaneously. Food intake was not affected significantly by either treatment or by the combination of the two.

The results of the second experiment reveal that HCZ significantly increased ($p< 0.01$) the percentage change in NaCl intake while decreasing significantly the percentage change in water intake (Table III). Neither food intake nor body weight was altered significantly by treatment with HCZ.

Discussion. Earlier studies showed that a U- or V-shaped dose–response relationship existed between intake of NaCl solution and dose of mineralocorticoid hormone administered to adrenalectomized rats (5). In the case of DOCA, the greatest reduction in intake of NaCl solution occurred when approximately 100 μg/100 g of body weight/day was administered to adrenalectomized rats (5). Doses greater than this threshold dose returned NaCl intake progressively toward that of untreated, adrenalectomized controls. The doses used in this study, 50 and 100 μg/100 g

TABLE III. Effect of Hydrochlorothiazide on Percentage Change from Control Period in Food, Water, NaCl Intakes and Body Weights of Adrenalectomized Rats.

	Change[a] (%) from control period:			
	Food	Water	NaCl solution	Body wt
Control	+27.4 ± 7.1[b]	+20.8 ± 16.9	−16.8 ± 6.1	+19.7 ± 2.5
Hydrochlorothiazide treated	+13.8 ± 5.4	−46.1 ± 2.4[c]	+48.1 ± 6.1[c]	+18.2 ± 1.4

[a] Arc sin transform.

[b] One standard error of mean.

[c] Significantly different from control ($p <0.01$).

of body weight/day, were chosen because they lay on the descending limb of the dose–response curve of this earlier study and would be expected to reduce the spontaneous NaCl intake of adrenalectomized rats. That these doses reduced NaCl intake is shown in Table I. However, it appears that the 50-μg dose reduced NaCl intake to the same level as the 100-μg dose. Since the dose–response relationship is U-shaped, perhaps an intermediate dose, such as 75 μg, would have reduced NaCl intake below that of the 50 μg dose. If so, it would suggest that the 100-μg dose was actually on the ascending limb of the dose–response curve for these rats rather than at the apex of the curve as had been observed for the rats in the previous study (5). Unfortunately, the fact that only two doses of DOCA were used prevents further speculation. In any event, the doses chosen reduced NaCl intake significantly and again suggest the possibility that blood level of mineralocorticoids is at least one factor influencing spontaneous NaCl intake.

The reduction in the spontaneous salt intake of adrenalectomized rats by DOCA was prevented by administration of HCZ. These results are made interesting by their parallelism with the results of studies reported by Beyer (10), Kagawa and Drill (11) and Renzi *et al.* (12). These investigators showed that thiazides and other diuretics counteract the sodium-retaining properties of administered mineralocorticoid hormones. Thus, Renzi *et al.* (12) showed that acute administration of HCZ (0.32 mg/kg) reversed the effect of DOCA (2.5 mg/rat) on urinary excretion of sodium and potassium in normal rats loaded by stomach with 0.2% saline solution. They also reported that administration of HCZ to adrenalectomized rats increased their urinary excretion of sodium and potassium under these same conditions. In the present study, administration of HCZ to adrenalectomized rats increased their spontaneous NaCl intake significantly (Table III), reflecting further parallelism between the present studies and those of Renzi *et al.* (12). The fact that HCZ increased both urinary excretion of sodium (12) and spontaneous NaCl intake of adrenalectomized rats is especially significant since it eliminates any possibility of participation of adrenal steroids in these responses. The results suggest that the increased intake of NaCl solution accompanying HCZ administration to DOCA-treated, adrenalectomized rats may be related to an increased sodium loss induced by the diuretic agent. The possibility that increased sodium excretion mediates increased sodium intake requires elucidation. Some mechanisms were suggested recently by which the spontaneous salt intake of intact rats may be controlled (5). Those applicable to the present study include a salt appetite induced by changes in salivary sodium and potassium concentrations and by changes in central nervous mechanisms responding either to altered electrolyte concentration of blood or to altered extracellular fluid volume (5). Changes in both body sodium content and distribution induced by HCZ may also play a role (13, 14). It cannot be stated at present which suggestion best explains the mechanisms by which HCZ increased NaCl intake of adrenalectomized rats or, indeed, how this change was translated into specific behavioral activity.

Summary. Subcutaneous administration of desoxycorticosterone acetate (DOCA) to adrenalectomized rats at 50 and 100 μg/100 g of body weight/day reduced their spontaneous NaCl intake significantly. When hydrochlorothiazide (HCZ, 0.6 g/kg of food) was administered concurrently, the effect of DOCA was prevented and NaCl intake increased. The HCZ also increased the NaCl intake of untreated adrenalectomized rats. Hence, the presence of adrenal steroids is not required for this response. The results suggest that increased intake of NaCl solution by HCZ-treated, adrenalectomized rats may be related to increased urinary excretion of sodium. However, the mechanism by which increased sodium loss may mediate increased sodium intake requires elucidation.

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Studies on the Measurement of Insulin Antibodies in Controlled and Resistant Human Diabetic Patients by Polyacrylamide Gel Electrophoresis* (33980)

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During the past several years a number of methods have been developed for the assay of insulin and insulin antibody levels in biological materials (1–6). The nonspecific absorption of insulin to protein, and ^{131}I insulin isotope exchange between insulin and other proteins is a major drawback in many of the conventional immunological methods of assaying insulin and insulin antibody titers using ^{131}I insulin in biological materials.

To overcome this, we have now developed a new method by which the antibody or insulin levels in any given sample can be accurately and reproducibly determined without interferences as found in other techniques. For this we use the polyacrylamide analytical gel electrophoresis method of Ornstein (7) and Davis (8). The procedure is as follows: An insulin solution containing 800 mU/ml (80 mU of insulin-^{131}I, 720 mU of cold insulin) was prepared then diluted serially. To a series of tubes, each containing 0.3 ml of large-pore sample gel pH 6.6–6.8 and 1.5 mg of the unknown antiinsulin serum (AIS), 5–40 mU insulin contained in 0.05 ml of each dilution was then added. Glass columns 5/16 × 6 in. containing 2.5 ml of polymerized 7% acrylamide gel (pH 8.6) and 0.2 ml of polymerized large-pore stacking gel were then prepared. A 0.2-ml sample of gel was then added to each column. The gels were electrophoreized for 1.5 hr using Tris–glycine buffer (pH 8.3) and 4 mA current/column. A standard curve was then drawn by using the serial dilutions and plotting cpm vs. mU. The exact amount of insulin added to each gel column was then calculated by subtracting the cpm of the remaining 0.1 ml from the cpm of starting 0.3 ml of gel and converting this to mU from the standard curve.

After electrophoresis, the gels were removed from the columns and cut in 0.5-mm slices. The sample gel, stacking gel, and 5 slices of 7% separating gel were then counted. The total cpm/gel were then converted to mU from the standard graph. A plot of mU taken vs. mU bound is then made. From this graph the potency of the AIS in mU/mg of serum may be calculated. With known potency, AIS, the procedure

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