

EFFECT OF ADRENALECTOMY ON BLOOD PRESSURE  
IN SALT-FED, HYPERTENSION-PRONE RATS\*

FAILURE OF HYPERTENSION TO DEVELOP IN ABSENCE OF EVIDENCE  
OF ADRENAL CORTICAL TISSUE

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▣ The role of the adrenal cortex in experimental hypertension has been clouded by the fact that in studies with the most commonly used animals, the dog and rat, reports have often been at variance. The early work of Goldblatt (1) and of Page (2) probably still represents the general consensus relative to renal hypertension in the dog: without substitution therapy (NaCl or adrenal steroids), adrenalectomy prevents the development of chronic hypertension. Similarly, supportive therapy is thought necessary for the maintenance of present hypertension, after adrenalectomy. Rogoff, Nixon, and Stewart (3) however, reported that severe, acute hypertension developed in three dogs after adrenalectomy and bilateral renal artery constriction in the absence of specific therapy, including salt; and, in adrenalectomized-nephrectomized dogs, Turner and Grollman (4) also reported that the animals developed hypertension when peritoneal lavage was used to correct electrolyte abnormalities. In the rat, Fregly (5) and Gross (6) have reported that renal hypertension developed after adrenalectomy, provided that either salt, alone, or salt-retaining steroids were also administered. If salt was provided, Crane and Ingle (7) and Fregly (8) reported that the adrenals were unnecessary for salt-hypertension to be induced in rats; and Brandt, Dubin, and Sapirstein (9) found that salt hypertension in rats could be maintained in the absence of the adrenals when dietary salt was allowed. Skelton (10-11) has suggested that both the adrenal and salt play an essential role in the pathogenesis of adrenal regeneration hypertension. Knowlton et al. (12) found that the administration of cortisone to adrenalectomized rats resulted in a lesser degree of hypertension if the animals received extra sodium rather than a restricted sodium intake. Masson et al. produced hypertension in uninephrectomized rats by prolonged administration of renin and NaCl. This effect was prevented by adrenalectomy, but restored fully by cortisol, and only partially by deoxycorticosterone acetate (DOCA) (13).

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Three rather incompatible interpretations may be proposed from the foregoing: (a) The adrenals are not needed for development of hypertension. (b) The adrenals are causally related to experimental hypertension. (c) The adrenals will permit the expression of hypertension but do not themselves produce hypertensinogenic substances.

Use of the rat as the experimental animal is complicated by the indubitable fact that it has the capacity to regenerate functioning adrenal cortical tissue from adrenal accessories and rests (14, 15) and, possibly, even from cells in the coelomic wall (16). Colonies differ in this capacity (14, 15). Accessories have often been found in the renal vein and tissues immediately surrounding it, as well as in the epididymis (14). Such sites would commonly be overlooked in a routine autopsy survey. Among the five colonies of rats studied by Gaunt (14), accessory cortical tissue was generally found in those animals that lived more than 1 month after adrenalectomy, and he concluded that accessory tissue was probably present in all such survivors. In such instances, the function of salt (or salt-retaining steroids) is probably to allow the animal to survive long enough for these presumably dormant cells to proliferate and develop into functioning cortical cells. We were left with the same impression by some studies preliminary to those reported here: among rats with complete surgical adrenalectomy, there were always a few that developed frank hypertension when chronically maintained on a high salt intake. In such hypertensive animals, more than half proved at autopsy to have histologically identifiable adrenal cortical tissue, and it seemed possible that the remainder had obscurely placed, newly regenerated, adrenal tissue which we had failed to locate. In the present study, therefore, we have used biochemical, physiological, and anatomical criteria to assess the presence or absence of adrenal cortical tissue. By these criteria we found that, in rats with a strong genetic predisposition to developing hypertension from chronic NaCl ingestion, as well as from other techniques (17-21), salt hypertension failed to develop in the absence of evidence that the rats possessed functioning adrenal cortical tissue.

#### *Material and Methods*

The rats employed in this experiment were derived originally from a Sprague-Dawley strain by selective inbreeding. Based on their capacity to resist or yield to the hypertensive effect of a high NaCl diet, they were called the resistant (R) and sensitive (S) strains (17, 18). In the experiments reported in this paper, only S strain rats of both sexes were used. All animals were housed in an air-conditioned, constant temperature room, lit from 8:00 a.m. to 5:00 p.m. daily. The animals were weaned at 21-23 days and then were maintained on tap water (0.5-0.7 mEq of sodium/liter, on repeated analyses), and a special low salt chow (0.38% NaCl), until they weighed about 100-110 g. At that time (*circa* 30-40 days of age), they were divided into four groups (Table I), and subjected to the procedures and diets described below.

TABLE I  
Classification of Groups

Group	No. of rats and sex	Operation	Postoperative diet		
Ia	6 male 6 female	Bilateral adrenalectomy	Sodium-free diet, distilled water (until death)		
			Low salt (0.38% NaCl), tap water(1st-6th wk)	High salt (8% NaCl), tap water (7th-10th wk)	Sodium-free diet, distilled water (11th-13th wk)
Ib	6 male 6 female	Bilateral adrenalectomy	Sodium-free diet, distilled water (21 days)		
			Low salt (0.38% NaCl), tap water(1st-6th wk)	High salt (8% NaCl), tap water (7th-10th wk)	Sodium-free diet, distilled water (11th-13th wk)
IIa	6 male 6 female	Intact	Sodium-free diet, distilled water (21 days)		
IIb	6 male 6 female	Intact	Sodium-free diet, distilled water (21 days)		
			Low salt (0.38% NaCl), tap water(1st-6th wk)	High salt (8% NaCl), tap water (7th-10th wk)	Sodium-free diet, distilled water (11th-13th wk)
III	19 male 11 female	Bilateral adrenalectomy	Low salt chow (0.38% NaCl) Solution (0.43% saline + 2.5% glucose (1st wk)	4% NaCl chow Solution (0.43% saline + 2.5% glucose) (2nd wk)	High salt chow (8% NaCl) Solution (0.11% saline + 1.25% glucose) (3rd wk)
			High salt chow (8% NaCl) Tap water (4th-10th wk)	Sodium-free diet, distilled water (11th-13th wk)	
IV	4 male 6 female	Sham adrenalectomy	Sodium-free diet, distilled water (11th-13th wk)		

1. *Adrenalectomized Rats Fed a "Sodium-Free" Diet (0.008-0.013% Na).—*

*Groups Ia and Ib:* This experiment was undertaken to determine whether rats from the S strain could survive on a virtually sodium-free diet after adrenalectomy, and before adrenal regeneration reasonably could have occurred.

*Group Ia:* Using 12 S rats (6 males, 6 females), bilateral adrenalectomy was performed in one stage at the time corresponding to that of the secondary adrenalectomy for group III animals (see below). Bilateral adrenalectomy was carried out in one stage, rather than two, as was done in the test animals, in order to minimize the probability that functioning adrenal cortical tissue was present. It was considered possible that unilateral adrenalectomy might be a sufficient stimulus to initiate regeneration of adrenal rests in some animals. Low salt diet (0.38% NaCl) and tap water had been continued until adrenalectomy was done. Immediately after adrenalectomy, all rats were placed on distilled water and an artificial "sodium-free" diet, containing a concentration of 0.008-0.013% Na by analysis. This regimen was continued until they died.

*Group Ib:* Because it had been reported that older rats were able to survive adrenalectomy longer than younger ones (22), older animals were also adrenalectomized at the age corresponding to that at which test animals were placed on the sodium-free diet. Six male and six female S rats were maintained on low salt chow (0.38% NaCl) and tap water for 6 wk after weaning; from wk 7-10 they were maintained on high salt chow (8% NaCl) and tap water. During the 11th wk, bilateral adrenalectomy was performed as in group Ia, after which, they were placed on the sodium-free chow and distilled water until death.

2. *Intact Rats Fed a Sodium-Free Diet—Groups IIa and IIb.*—This experiment was done to determine whether S rats with adrenals intact could survive significantly longer than similar, adrenalectomized rats on the sodium-free regimen.

12 S rats (6 males, 6 females) were used as the controls for group Ia. They were not operated on, but were placed on the same dietary regimen at the same age, and maintained on this regimen for 21 days. Since younger animals are more sensitive to sodium restriction than older ones, due to their growth requirements, survival of those younger rats would make it almost unnecessary to do the same control study for group Ib. Nonetheless, a similar study was made on 12 animals of the same age used in group Ib.

3. *Adrenalectomized Rats on a High Salt Diet—Group III.*—This experiment was made to determine whether rats without evidence of adrenal cortical function would develop hypertension from a high NaCl intake.

30 S strain rats (19 males, 11 females) were adrenalectomized in two steps at 2 wk intervals. Unilateral adrenalectomy was performed under ether anesthesia through a single skin incision over the lumbar spine. The low salt chow and tap water diet were continued until the second adrenalectomy, 2 wk later. After this second operation, carried out in similar fashion to the first, the rats were fed special diets and fluids as follows:

*1st wk:* Low salt chow (0.38% NaCl) and a solution of 0.43% saline plus 2.5% glucose as drinking fluid.

*2nd wk:* 4% NaCl chow and the solution of 0.43% saline plus 2.5% glucose.

*3rd wk:* High salt chow (8% NaCl) and a solution of 0.11% saline and 1.25% glucose.

*4th wk and thereafter:* High salt chow and tap water.

This feeding regimen was arrived at empirically, during preliminary studies when a prohibitively high postoperative mortality resulted from more rapid exposure to the high salt chow.

Blood pressure and weight were measured prior to the second adrenalectomy, and once a week, thereafter, for 10 wk. Blood pressure was measured by the microphonic method under ether anesthesia, induced by a flowing oxygen-ether mixture (21).

Corticosteroid level in the plasma of individual rats was measured at the time of, and

every 2 wk after, the second adrenalectomy. From animals fasted overnight, 0.5 ml blood was obtained by nicking the tail under light ether anesthesia in the morning between 10 and 11:00 a.m., using heparin as an anticoagulant. Plasma was obtained by centrifugation, immediately after the bleeding. Corticosteroid level in plasma was determined by the fluorimetric procedure of Mattingly (23). A reagent blank and cortisol standard (2.0  $\mu\text{g}$ ) were run through the procedure with each batch of six samples. Two check samples, one from pooled rat plasma and one from a previous day's run were included each day. Since a cortisol standard was used, the free plasma 11-hydroxycorticoid concentration in  $\mu\text{g}/100$  ml of plasma was expressed as cortisol. However, the principal glucocorticoid in rat plasma is reported to be corticosterone, not cortisol (24-26). The two steroids have the same emission maximum, but differ in time development and amplitude. Our analysis of reference steroids indicated that values for cortisol could be converted to corticosterone by dividing by 3.2; we have not done this, since it would represent merely a recalculation of primary data, rather

TABLE II  
Results of Steroid Determination in Plasma from Salt-Fed, S Rats. Comparison of Sampling Procedures from Same Animals on Different Days

Rats	Female		Male	
	Tail blood, anesthesia	Decapitation, no anesthesia	Tail blood, anesthesia	Decapitation, no anesthesia
No. in group	7		18	
Average concentration of steroid $\mu\text{g}$ cortisol/100 ml	244	225	113	64
SE	13	17	9	14
Difference	19		49	
<i>P</i>	NS		0.005 < <i>P</i> < 0.01	

than the actual measurements. We adhered rigorously to a detailed procedure, which was reflected in a standard deviation of less than 1.5%, as determined by 25 analyses on the same check sample on different days. The basal plasma corticosteroid levels in 89 intact R and S rats, 16 wk old, on high salt chow for the previous 10 wk, were as follows:

*Corticosteroid (cortisol)*

18 R male 166.2  $\pm$  9.5 ( $\pm$ SE)  $\mu\text{g}/100$  ml (Range 100-261  $\mu\text{g}$ )  
 13 R female 228.2  $\pm$  10.6 ( " ) " ( " 155-274 " )  
 34 S male 148.7  $\pm$  4.3 ( " ) " ( " 105-193 " )  
 24 S female 214.7  $\pm$  10.9 ( " ) " ( " 102-285 " )

R male < R female } *P* < 0.001  
 S male < S female }  
 R male = S male } *P* > 0.05  
 R female = S female }

A comparison of our sampling method and sampling by decapitation without anesthesia

showed slightly higher values with our technique (Table II). 11 wk after adrenalectomy, all rats in group III were put on the sodium-free regimen (see above), on the assumption that animals without adrenal cortical tissue would succumb on this low NaCl intake. Animals still alive 21 days later were sacrificed. At autopsy, a careful survey was made through-

TABLE III  
*Blood Pressure and Plasma Corticosteroid Concentration in Adrenalectomized Male S Rats on High Salt Diet, Group III*

Rat No.	Time after operation, wk												Survival on sodium-free diet*	Adrenal tissue at autopsy	
	0		2		4		6		8		10			Gross	Micro
	Blood pressure	Ste-roid	Blood pressure	Ste-roid	Blood pressure	Ste-roid	Blood pressure	Ste-roid	Blood pressure	Ste-roid	Blood pressure	Ste-roid			
mm Hg	µg/100 ml														
1	126	114	86	18	156	28	156	47	226	73	†	—	11	+	+
2	126	78	128	5	150	42	136	59	210	70	†	—	21§	—	
3	98	133	130	29	140	39	152	49	166	31	202	76	19	+	+
4	112	93	86	6	124	18	120	21	184	28	220	28	21§	+	+
5	132	153	116	2	128	8	120	26	142	28	166	37	15	+	+
6	130	122	120	19	168	30	140	58	194	24	192	66	19	—	
7	130	88	118	6	148	13	136	30	194	28	224	51	20	—	
8	110	112	142	23	150	18	146	67	220	83	†	—	21§	+	—
9	128	90	102	3	112	15	110	10	140	15	156	12	14	—	
10	142	136	100	10	118	6	138	8	136	7	148	9	5	—	
11	118	121	104	6	126	6	118	7	130	7	142	4	7	+	—
12	118	116	120	5	126	10	124	4	¶				**	—	
13	120	78	106	8	146	21	¶						**	—	
14	142	96	112	6	¶								**	—	
15	130	126	112	11	¶								**	—	
16	136	153	108	2	¶								**	—	
17	126	95	110	8	¶								**	—	
18	120	131	114	3	¶								**	—	
19	124	150	¶										**	—	
n...	19		18		13		12		11		11				

\* Survival of less than 12 days is presumptive evidence of lack of adrenal cortical function.

† Placed on sodium-free diet during 9th wk.

§ Sacrificed.

|| No microscopic examination made because no tissue found on gross inspection.

¶ Died.

\*\* Died during high salt phase of study.

n, total number of rats at that time.

out the abdominal and thoracic cavities for possible accessory adrenal tissue. The renal veins and testes (14) were not included in the survey, however. Suspicious tissue was fixed in formalin for microscopic examination of adrenal cortical tissue. No attempt was made to assess the quantity of such tissue present.<sup>1</sup>

4. *Intact Rats on a High Salt Diet—Group IV.*—This study was carried out to confirm

<sup>1</sup> We are indebted to Dr. Horton Johnson for all microscopic evaluations.

the capacity of the high salt regimen used in Group III to cause hypertension in intact rats from the S strain.

10 S rats (4 males, 6 females) were the controls for group III. Sham adrenalectomy was performed in two stages at the corresponding times for the group III animals with the adrenals exposed but not removed. All animals were otherwise treated like those in Group III, except that autopsies were not performed.

TABLE IV  
Blood Pressure and Plasma Corticosteroid Concentration in Adrenalectomized Female S Rats on High Salt Diet, Group III

Rat No.	Time after operation, wk												Survival on sodium-free diet*	Adrenal tissue at autopsy	
	0		2		4		6		8		10			Gross	Micro
	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid			
mm Hg	µg/100 ml														
20	122	120	120	14	132	27	138	23	142	37	166	39	16	+	+
21	140	185	118	8	136	20	130	42	174	53	170	63	18	+	+
22	132	147	130	41	142	50	140	100	244	105	†	—	‡	+	—
23	132	150	104	12	140	12	128	14	136	18	148	25	21	+	+
24	124	110	108	39	162	35	194	108	234	65	†	—	‡	+	+
25	102	192	128	7	132	24	128	19	130	23	156	23	6	+	—
26	118	66	106	3	134	10	130	9	134	9	134	18	6	+	—
27	138	194	134	14	142	13	130	17	130	10	148	16	4	—	¶
28	130	151	106	6	116	7	110	14	134	16	136	7	9	—	¶
29	144	123	138	8	116	14	130	15	138	15	170**	20	5	—	¶
30	124	77	116	11	130	4	132	9	136	12	142	18	9	—	¶
n...	11		11		11		11		11		9				

\* Survival of less than 12 days is presumptive evidence of lack of adrenal cortical function.

† Died.

‡ Died during high salt phase of study.

§ Sacrificed.

¶ No microscopic examination made because no tissue found on gross inspection.

\*\* At 11 wk this animal had blood pressure of 144 mm Hg, steroid 19 µg/100 ml.

OBSERVATIONS

*Adrenalectomized Rats on Sodium-Free Diet—Groups Ia and Ib.*—All rats in group Ia died between 3 and 10 days after adrenalectomy, with a mean of  $5.8 \pm 2$  (sd) days. All rats in group Ib died between 5 and 10 days after adrenalectomy, with a mean of  $7.5 \pm 1.4$  (sd) days. There was no difference apparent between the sexes. Hence, a survival of less than 12 days (mean + 3 sd) on the sodium-free regimen was used as presumptive evidence for lack of functioning adrenal cortical tissue. (In a group of 12 S rats, 11 months old, not included in this study, survival after precisely the same treatment ranged from 8–19 days, with a mean of  $14.7 (\pm 3.3)$  days. This confirms the observation that older rats

survive adrenalectomy longer than younger ones (22). It also emphasizes the need for having control and test animals of similar ages.)

*Intact Rats on Sodium-Free Diet—Groups IIa and IIb.*—All rats survived

TABLE V

*Adrenalectomized Rats with Hypertension (2 Consecutive Blood Pressures  $\geq 140$  mm Hg or 1 Blood Pressure  $\geq 160$  mm Hg)*

Rat No.	Sex	Last blood pressure	Adrenal cortical function	Criteria for adrenal cortical function		
				Plasma steroid concentration $\geq 42$ $\mu\text{g}/100$ ml*	Survived on sodium-free diet†	Histologically confirmed adrenal cortical tissue—autopsy
		<i>mm Hg</i>				
1	Male	226	Yes	+	—	+
2	"	210	"	+	+	—
3	"	202	"	+	+	+
4	"	220	"	—	+	+
5	"	166	"	+§	+	+
6	"	192	"	+	+	—
7	"	224	"	+	+	—
8	"	220	"	+	+	—
9	"	156	"	—	+	—
20	Female	166	"	—	+	+
21	"	170	"	+	+	+
22	"	244	"	+		—
23	"	148	"	—	+	+
24	"	234	"	+		+
29	"	144	No	—	—	—
n. . . . .	15					
Mean blood pressure						
<i>mm Hg</i> . . . . .		194.8				
SE . . . . .		8.70				

\*  $42$   $\mu\text{g}/100$  ml exceeds by 3 s.d. the mean plasma concentration of corticosteroids among rats 2 wk after surgical adrenalectomy. See Results, group III.

† Less than 12 days presumptive evidence of lack of adrenal cortical tissue.

§  $46$   $\mu\text{g}/100$  ml during sodium-free regimen.

|| Died during high salt phase.

without apparent distress until they were sacrificed after 21 days. It was concluded that if surgically adrenalectomized rats survived this regimen for 21 days, normal adrenal cortical function must be presumed to be present by virtue of accessory glands or regeneration of rests.

The combined results of experiments on adrenalectomized (groups Ia and Ib) and intact (groups IIa and IIb) rats on this sodium-free regimen led to the



following operational postulates: (a) Death in <12 days = no functioning adrenal cortical tissue. (b) Death from >12 and <21 days = some functioning adrenal cortical tissue. (c) Survival for >21 days = normally functioning adrenal cortical tissue.

*Adrenalectomized Rats on High Salt Diet—Group III (Tables III–VI).*—One rat (No. 19, Table III) died postoperatively during the first 2 wk and has not

TABLE VI  
*Adrenalectomized Rats without Hypertension*

Rat No.	Sex	Last blood pressure	Adrenal cortical function	Criteria for adrenal cortical function		
				Plasma steroid concentration $\geq 42 \mu\text{g}/100 \text{ ml}^*$	Survived on sodium-free diet	Histologically confirmed adrenal cortical tissue
		<i>mm Hg</i>				
10	Male	148	No	—	—	—
11	"	142	"	—	—	—
12	"	124	"	—	†	—
13	"	146	"	—	†	—
14	"	112	"	—	†	—
15	"	112	"	—	†	—
16	"	108	"	—	†	—
17	"	110	"	—	†	—
18	"	114	"	—	†	—
25	Female	156	"	—	—	—
26	"	134	"	—	—	—
27	"	148	"	—	—	—
28	"	136	"	—	—	—
30	"	142	"	—	—	—
n.....	14					
Mean blood pressure, <i>mm Hg</i> ....		130.9				
SE.....		4.53				

\* See legend for Table V.

† Died during high salt phase.

been included in this evaluation. In previous studies, we have defined hypertension as persistent systolic blood pressures  $\geq 140 \text{ mm Hg}$  (21), a definition arrived at during observations ordinarily extending over periods of up to 12 months. In the current study, blood pressures after adrenalectomy were labile and the period of observation shorter. We have, therefore, for this study somewhat arbitrarily defined hypertension as two consecutive systolic pressures  $\geq 140 \text{ mm Hg}$  or a single observation  $\geq 160 \text{ mm Hg}$ . Our extensive experience

with these rats indicated that such end points might overestimate the presence of *chronic* hypertension, but would not underestimate it.

The primary data on blood pressure, plasma steroid concentration, survival on the sodium-free regimen, and anatomical evidence of adrenal cortical tissue

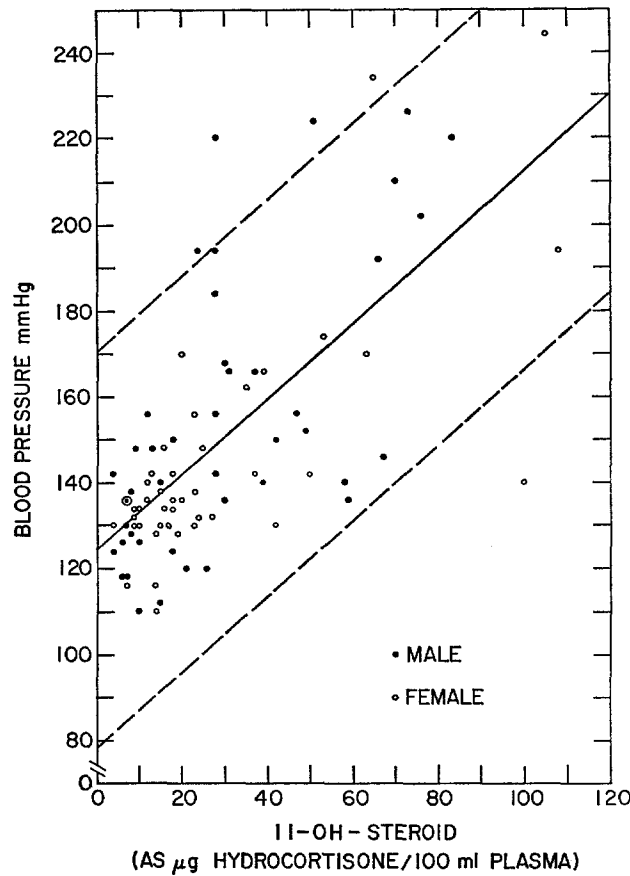


FIG. 1. Correlation between blood pressure and plasma steroid concentration in adrenalectomized S rats on high salt diet.

are summarized, by sex, in Tables III and IV. In Tables V and VI, the animals have been regrouped on the basis of the presence or absence of hypertension (as defined above), with an assessment of whether or not adrenal cortical function was present. The average corticosteroid level in the plasma of adrenalectomized rats (group III), 2 wk after second adrenalectomy was  $11.5 \pm 10.1$  (SD)  $\mu\text{g}/100$  ml.  $42 \mu\text{g}/100$  ml. (mean + 3 SD) was therefore considered presumptive evidence for significant adrenal function. Except for one animal (No. 29, Tables

IV and V), all rats with evidence of hypertension showed evidence of functioning adrenal tissue. The converse was also true. Rat 29 had no adrenal cortical function by the three criteria used in this study, but its pressure was marginally elevated (144 mg Hg) prior to adrenalectomy. After adrenalectomy the blood pressure was low, but later returned to preoperative levels or possibly higher (10 wk, 170 mm Hg; 11 wk, 144 mm Hg). As the single exception it is difficult

TABLE VII  
*Blood Pressure and Plasma Corticosteroid Concentration in Intact Rats on High Salt Diet, Group IV*

Sex	Time after operation, wk											
	0		2		4		6		8		10	
	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid	Blood pressure	Steroid
	mm Hg	µg/100 ml										
Male	128	157	188	123	180	99	196	103	176	185	220	159
"	134	122	162	126	186	176	184	197	160	103	204	77
"	132	115	158	110	178	117	194	109	174	140	224	190
"	140	188	192	123	184	144	192	152	184	168	208	119
n.....	4		4		4		4		4		4	
Mean.	133.5	145.5	175.0	120.5	182.0	134.0	191.5	140.3	173.5	149.0	214.0	136.3
SE....	2.5	16.9	8.7	3.6	1.8	16.8	2.6	21.8	5.0	17.9	4.8	24.5
Fe- male	124	206	170	206	178	195	198	174	180	211	208	120
"	130	174	152	176	170	176	190	161	168	169	196	108
"	144	157	182	153	196	103	180	152	166	178	204	126
"	124	199	146	148	160	200	178	210	174	188	190	158
"	124	178	198	164	190	115	190	133	184	198	178	174
"	140	193	166	133	170	109	Died	Died				
n.....	6		6		6		5		5		5	
Mean.	131.0	184.5	169.0	163.3	177.3	149.7	187.2	166.0	174.4	188.8	195.2	137.2
SE....	3.6	7.4	7.8	10.4	5.5	18.5	3.7	12.9	3.4	7.4	5.3	12.4

to estimate the importance of this finding. The difference between the mean blood pressures of the groups in Tables V and VI was highly significant ( $P < 0.001$ ). The death of rat 1 (Tables III and V) after 11 days on the sodium-free regimen was probably due to the severity of the hypertension, rather than to lack of adrenal cortical function, since the plasma steroid concentration was approaching normal (80 µg/100 ml) after 1 wk on the sodium-free regimen (4 days before death). Fig. 1 displays the observed blood pressures and blood steroid levels from wk 4-10 (85 observations on 23 animals). There is a significant positive correlation between the two parameters. The regression equa-

tion is  $y = 0.85x + 124.5$ . The residual standard deviation of  $y$  on  $x$  is 22.4 mm Hg. The standard deviation of the regression coefficient is ( $\pm 0.10$ ).

*Intact Rats on High Salt Diet—Group IV (Table VII).*—Hypertension, as expected, developed rapidly in all of these intact animals on a high salt intake. By the end of the 4th wk, the average blood pressure was approximately 180 mm Hg, and after the 10th wk about 200 mm Hg. Plasma corticosteroid concentrations remained within the normal range for males and females of this S strain throughout the 10 wk on salt. 9 of the 10 rats in this group survived the 10 wk on salt, as well as the subsequent 3 wk on the sodium-free regimen. The one death occurred in a female, after anesthesia for blood pressure measurement, at the end of the 4th wk on salt.

#### DISCUSSION

In adrenalectomized hypertension-prone rats, we found a high degree of correlation between evidence of functioning adrenal tissue and the development of salt hypertension. But for one exception, high blood pressure occurred in the rats, only in combination with strong evidence of adrenocorticoid function, and vice versa. Further, there was a direct and significant correlation between the values of systolic blood pressure and of plasma steroid concentration, as measured by a fluorescence method. When salt hypertension developed in our operated rats, it did so much more slowly than in the sham-operated, intact controls, suggesting that adrenal cortical function was not fully replaced by the high salt intake alone. It seems probable that the animals were able to survive adrenalectomy because of the concurrent salt intake, but developed hypertension only after some adrenal cortical function returned.

The validity of our criteria for the presence of adrenal tissue and function is critical. We used three tests, one of which was biochemical, the second, physiological, and the third, anatomical. The biochemical parameter, presence of fluorescent material in serum, is probably most reliable and has the advantage of being quantitative. The fluorescence appeared to be caused almost exclusively by circulating glucocorticoids. It had the same emission maximum as standards of corticosterone and cortisol. This fluorescence virtually disappeared immediately after adrenalectomy, and reappeared slowly. It seems reasonable to use the plasma concentration as an *index* of adrenal corticoid function, although theoretically low values might occur with a high production rate. The physiological test, survival on a sodium-free diet, is also considered reliable (14). In our own study, no recently adrenalectomized rat survived when placed immediately after operation on the sodium-free regimen, whereas all sham-operated controls did. We feel, therefore, that survival on such a regimen is strong presumptive evidence for the presence of functioning cortical tissue. The anatomical evidence, identification of adrenal tissue, is strong evidence if positive, but inconclusive if negative, for the reasons cited in the introduction. It may not

be necessary that a fully functioning adrenal cortex be present for hypertension to evolve. It is well established, for instance, that adrenal regeneration hypertension (10) develops during a time when the uninephrectomized, salt-fed rats have a demonstrably deficient corticosterone and aldosterone secretion (27). The adrenal function was deficient in our operated rats that developed hypertension, as evidenced by their plasma corticosterone concentrations, which were significantly lower than in the intact controls, although higher than in those that failed to develop hypertension.

Relative to salt-induced experimental hypertension, Crane and Ingle (7) reported that in uninephrectomized rats, adrenalectomy generally ameliorated its development. In a few rats, however, hypertension was marked, and severe kidney lesions were found. Adrenal remnants, or accessories, apparently were searched for, but not found. Tests for residual adrenal cortical function were not made. They concluded that adrenal cortical hormones played a supporting role, whereas the toxicity of the NaCl and the associated electrolyte imbalances were the primary cause of such hypertension.

Fregly (8) also concluded that, in rats, the adrenals were not necessary for hypertension to be induced by administration of hypertonic NaCl solutions as the only drinking fluid. In all his experiments, the food was standard Purina Chow (in our experience this contains about 1% NaCl), and drinking fluid containing NaCl was always available. After adrenalectomy, a 1–3 wk control period preceded the administration of hypertonic NaCl solutions in various strengths for periods ranging from about 13 to 28 wk. Hypertension developed 3–8 wk after the animals began to drink 1.75% NaCl solution. Four criteria were used for completeness of the adrenalectomy: (a) slower growth rate (5); (b) increased NaCl appetite (28); (c) faster cooling rate after exposure to cold (29); (d) gross inspection for adrenal tissue. Fregly reported that no accessory adrenal tissue was found in any rat. The first three criteria are all indirect evidence of complete absence of adrenal cortical tissue, and their sensitivity as end points might be limited if, in fact, regenerating adrenal tissue were present in amounts below those required for normal function, but sufficient for the development of hypertension, as is true for adrenal regeneration hypertension. The fourth criterion is subject to the limitations described in our introduction. In sum, despite Fregly's conclusion that the salt-fed, adrenalectomized rats became hypertensive, it is at least possible that they had some functioning adrenal cortical tissue.

The correlation between plasma steroid concentration and hypertension demonstrated in Fig. 1 is not interpreted to mean that the steroid measured in plasma is responsible for the hypertension. This is hardly likely, since intact normotensive animals from the strain genetically resistant to hypertension, and intact hypertensive rats from the hypertension-prone strain, had similar plasma steroid concentrations. Hence, if there is a difference in adrenal function be-

tween the two strains responsible for the development of hypertension, this difference must be associated with a substance other than that measured here. We have no data to settle this issue now, but we suspect that, rather than having a primary role in hypertension, the adrenals play a permissive one, providing a necessary part of the environment for an extraadrenal hypertensinogenic factor to become manifest. If this is true, the correlation shown in Fig. 1, then, would be an expression of the necessity of some aspect of adrenal cortical function, without more specific implications. Furthermore, if hypertension cannot develop in the absence of adrenal cortical function, during the gradual return of such function by regeneration, a correlation between increasing blood pressure and increasing evidence of function might be expected. However, unless the adrenal were playing a primary role in the hypertension when adrenal cortical function approached normal, the correlation in Fig. 1 no longer would prevail. That seems to be the situation here.

On the basis of available evidence, we conclude that some adrenocortical function is necessary for salt hypertension to develop. Our evidence does not settle the question whether the action is causative, or whether corticosteroids play a supporting role for some extraadrenal hypertensinogenic principle.

#### SUMMARY AND CONCLUSIONS

In adrenalectomized, genetically hypertension-prone rats, a high degree of correlation was found between evidence of functioning adrenal tissue and the development of salt hypertension. There is considerable evidence that some rats have the capacity to regenerate functioning adrenal cortical tissue from accessory glands and microscopic rests, sometimes in remote locations. Therefore, the criteria for *continued* absence of adrenal function after surgical adrenalectomy are critical. In this study we used three tests to validate the presence, or absence, of adrenal function: (a) a biochemical test, the quantitative, serial measurement of plasma glucocorticoids in individual rats; (b) a physiological test, the ability to survive a virtually sodium-free diet; and (c) the anatomical search for histological evidence of adrenal cortical tissue. Among those animals that developed hypertension after adrenalectomy, the correlation between plasma steroid concentration and blood pressure was statistically significant. We suspect that this correlation exists only during the period when cortical tissue is regenerating; it does not exist among intact animals with and without hypertension induced by salt.

It was concluded that some adrenocortical function is *necessary* for salt hypertension to develop. The evidence was insufficient to settle the question whether the action of corticosteroids is causative, or whether they play a supporting, although necessary, role for an extraadrenal hypertensinogenic factor to become manifest.

## BIBLIOGRAPHY

1. Goldblatt, H. 1937. Studies on experimental hypertension. V. The pathogenesis of experimental hypertension due to renal ischemia. *Ann. Intern. Med.* **11**:69.
2. Page, I. H. 1938. The effect of bilateral adrenalectomy on arterial blood pressure of dogs with experimental hypertension. *Amer. J. Physiol.* **122**:352.
3. Rogoff, J. M., E. N. Nixon, and G. N. Stewart. 1939. The adrenals in experimental hypertension. *Proc. Soc. Exp. Biol. Med.* **41**:57.
4. Turner, L. B., and A. Grollman. 1951. Role of adrenal in pathogenesis of experimental renal hypertension as determined by a study of the bilaterally adrenalectomized nephrectomized dog. *Amer. J. Physiol.* **167**:462.
5. Fregly, M. J. 1957. Adrenal glands in the development of renal hypertension in rats. *Amer. J. Physiol.* **191**:542.
6. Gross, F. 1960. Adrenocortical function and renal pressor mechanisms in experimental hypertension. *Essential Hypertension, Int. Symp., Bern.* 92.
7. Crane, W. A. J., and D. J. Ingle. 1959. Pathogenic effects of salt loading in the presence and absence of the adrenal glands. *Endocrinology.* **65**:693.
8. Fregly, M. J. 1960. Production of hypertension in adrenalectomized rats given hypertonic salt solution to drink. *Endocrinology.* **66**:240.
9. Brandt, W. L., W. M. Dubin, and L. A. Sapirstein. 1951. Studies on salt hypertension. Effects of adrenalectomy and nephrectomy. *Amer. J. Physiol.* **164**:73.
10. Skelton, F. R., 1956. Adrenal regeneration hypertension and factors influencing its development. *Arch. Intern. Med.* **98**:449.
11. Oelsner, T., and F. R. Skelton. 1961. Complementary role of adrenal cortex and salt in adrenal-regeneration hypertension. *Amer. J. Physiol.* **200**:759.
12. Knowlton, A. I., E. N. Loeb, H. C. Stoerk, J. P. White, and J. F. Heffernan. 1952. Induction of arterial hypertension in normal and adrenalectomized rats given cortisone acetate. *J. Exp. Med.* **96**:187.
13. Masson, G. M. C., K. Aoki, M. Matsunaga, and E. R. Fisher. 1966. Role of adrenals in renin-induced hypertension. *Lab. Invest.* **15**:449.
14. Gaunt, R. 1933. Adrenalectomy in the rat. *Amer. J. Physiol.* **103**:494.
15. Gaunt, R., J. H. Gaunt, and C. E. Tobin 1935. Colony differences in survival of adrenalectomized rats. *Proc. Soc. Exp. Biol. Med.* **32**:888.
16. MacFarland, W. E. 1945. The vital necessity of adrenal cortical tissue in a mammal and the effects of proliferation of cortical cells from dormant coelomic mesothelium. *Anat. Rec.* **93**:233.
17. Dahl, L. K., M. Heine, and L. Tassinari. 1962. Effects of chronic excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exp. Med.* **115**:1173.
18. Dahl, L. K., M. Heine, and L. Tassinari. 1962. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature (London).* **194**:480.
19. Dahl, L. K., M. Heine, and L. Tassinari. 1963. Effects of chronic excess salt ingestion: Role of genetic factors in both DOCA-salt and renal hypertension. *J. Exp. Med.* **118**:605.
20. Dahl, L. K., M. Heine, and L. Tassinari. 1965. Effects of chronic excess salt ingestion. Further demonstration that genetic factors influence the develop-

- ment of hypertension. Evidence from experimental hypertension due to cortisone and to adrenal regeneration. *J. Exp. Med.* **122**:533.
21. Dahl, L. K., K. D. Knudsen, M. A. Heine, and G. J. Leitl. 1968. Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. *Circ. Res.* **22**:11.
  22. Cowie, A. T. 1949. The influence of age and sex on the life span of adrenalectomized rats. *J. Endocrinol.* **6**:94.
  23. Mattingly, D. 1962. A simple fluorimetric method for the estimation of free 11-hydroxycorticoids in human plasma. *J. Clin. Pathol. (London)* **15**:374.
  24. Zenker, N., and D. E. Bernstein. 1958. The estimation of small amounts of corticosterone in rat plasma. *J. Biol. Chem.* **231**:695.
  25. Bush, I. E. 1953. Species differences in adrenocortical secretion. *J. Endocrinol.* **9**:95.
  26. Guillemin, R., G. W. Clayton, J. D. Smith, and H. S. Lipscomb. 1958. Measurement of free corticosteroids in rat plasma: Physiological validation of a method. *Endocrinology.* **63**:349.
  27. Brogi, M. P., and C. Pellegrino. 1959. The secretion of corticosterone and aldosterone by the rat adrenal cortex regenerating after enucleation. *J. Physiol. (London)* **146**:165.
  28. Richter, C. P. 1936. Increased salt appetite in adrenalectomized rats. *Amer. J. Physiol.* **115**:155.
  29. Iampietro, P. F., M. J. Fregly, and E. R. Buskirk. 1956. Maintenance of body temperature of restrained adrenalectomized rats exposed to cold: Effect of adrenal cortical hormones. *Can. J. Biochem. Physiol.* **34**:721.