Aldosterone Hypersecretion in "Non-Salt-Losing" Congenital Adrenal Hyperplasia

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ABSTRACT Patients with the "non-salt-losing" form of the adrenogenital syndrome were studied before and after suppression of adrenal cortical activity with carbohydrate-active steroids. The response of aldosterone secretion to sodium deprivation was measured; in some patients response to adrenocorticotropic hormone (ACTH) was measured as well.

The aldosterone secretion was normal and responded normally to sodium deprivation in all patients studied during suppression with carbohydrate-active steroids. This finding suggests that 21-hydroxylation of progesterone is normal in this syndrome.

The sole abnormality in the production of aldosterone in these patients was found to be excessive secretion of aldosterone while they were not receiving suppressive doses of carbohydrate-active steroids. This finding strongly supports the view that the biogenetic pathways through which aldosterone is produced from progesterone are intact in this syndrome.

No patient showed hypertension or hypokalemic alkalosis despite very high aldosterone secretion rates. This observation suggests that the hyperaldosteronism is secondary to a tendency to sodium loss in the patient whose ACTH production is not suppressed.

These studies provide additional evidence in support of the hypothesis that the salt-losing and "non-salt-losing" forms of adrenogenital syndrome are genetically and biochemically distinct.

INTRODUCTION

The abnormalities of steroidogenesis which characterize congenital adrenal hyperplasia include (*a*) a defect in the biosynthesis of cortisol, (*b*) decrease in normal regulatory inhibition of adrenocorticotropic hormone (ACTH) with increased secretion of ACTH and (*c*) overproduction of steroids "proximal" to the site of the block in the biogenetic pathway of cortisol. Since overproduction of pregnanetriol [1] and of pregnanetriolone (1) has been demonstrated in this disease, it has been assumed that the block in steroidogenesis involves the 21-hydroxylation of 17α-hydroxyprogesterone (2). A defect in 21-hydroxylation of 17α-hydroxyprogesterone has been shown in vitro for two patients with the "salt-losing" form of congenital adrenal hyperplasia (3).

A simple defect in the 21-hydroxylation of 17α-hydroxyprogesterone will not explain the difference between the "salt-losing" form of congenital adrenal hyperplasia, in which secretion of aldosterone is defective (4), and the "non-salt-losing"

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[1] The following abbreviations have been used for the steroids discussed: pregnanediol, pregnane-3α 20α-diol; pregnanetriol, pregnane-3α, 17α, 20α-triol; pregnanetriolone, 3α, 17α, 20α-trihydroxypregnane-11-one; progesterone, Δ^4-pregnene-3, 20-dione; 17α-hydroxyprogesterone, Δ^4-pregnene-17α-ol-3, 20-dione.

form, in which there is no defect in aldosterone secretion (see below). We have suggested (4) that patients with the "salt-losing" variety of the disorder may have a second enzymatic defect, namely, a relative inability to perform 21-hydroxylation of progesterone. As a corollary of this hypothesis, the production of aldosterone by patients with the "non-salt-losing" syndrome should be excessive. A biosynthetic block in 21-hydroxylation of 17α-hydroxyprogesterone should result in excessive quantities of 17α-hydroxyprogesterone, and this in turn would lead to excessive quantities of progesterone; in the absence of a biosynthetic block between progesterone and aldosterone, aldosterone production should be increased. In the present studies, we have explored this hypothesis. We have examined further the effect of sodium deprivation on aldosterone secretion in patients with "non-salt-losing" congenital adrenal hyperplasia (*a*) while they received no treatment with adrenal steroids, (*b*) during stimulation with exogenous ACTH and (*c*) during continuous treatment with exogenous carbohydrate-active steroids to inhibit endogenous ACTH production, and thereby inhibit any consequent change in production of ACTH-dependent "salt-losing" hormones.

METHODS

12 patients with "non-salt-losing" congenital adrenal hyperplasia were studied. Table I lists the essential clinical and laboratory data on each patient. Two experimental designs were employed. The first may be seen by inspection of Table II and III and Fig. 1. Treatment was discontinued for as long a period as possible before study. The period ranged up to 22 yr.[2] Patients were given a metabolic diet containing 60 mEq or more of sodium per m^2 of body surface each day; without exception, they had been on ad lib. diets before this study. All studies were carried out with standard metabolic regimen in an air-conditioned unit in which patients remained throughout the study. Urinary sodium and potassium was measured daily, serum potassium and carbon dioxide content and hematocrit at intervals, and blood pressure at least 4 times a day. Aldosterone secretion rate was measured. ACTH was then administered for a 4 day period at a dosage of 40 U/day intramuscularly. Aldosterone secretion rate was again measured on day 2 and, in all patients except two, on day 4 of treatment with ACTH. Aldosterone secretion rate was again measured in most cases 2 or 3 days after ACTH was stopped. Throughout this period, the initial sodium intake was maintained. Dietary sodium was then lowered to 9 mEq/day, and aldosterone secretion rate was again measured on days 3–5 of this regimen (Table III).

The second protocol may be seen by inspection of Table IV and of Fig. 2. On this protocol, patients were treated continuously with prednisone at dosages (5, 10, or 15 mg/day) sufficient to lower 17-ketosteroid excretion to normal. They were depleted of sodium as described above by eating a diet containing 9–14 mEq of sodium per day for periods of 4–8 days. Aldosterone secretion rate was measured in each. The patients then received increments of dietary sodium of 60 or more mEq per m^2 of body surface per day, and aldosterone secretion rate was

[2] Patient D.M. could not discontinue exogenous replacement steroid but was given exogenous ACTH before admission, and the steroid was discontinued on the day of admission. Thus she was off steroid for 6 days before the first secretion rate.

TABLE I
Patients and Previous Treatment

Patient	Age	Surface area	Chromosomal sex	Therapy before study	Duration	Duration discontinued	ACTH before admission
		m^2					
R. H.	11	1.47	Female	Δ_1F 10 mg/day	1 yr	3 wk	None
D. H.	14	1.44	Female	Δ_1F 7.5 mg/day	1 yr	3 wk	None
E. B.	22	1.57	Female	None	—	22 yr	None
D. M.	17	1.45	Female	E100 mg/day	2 yr	0	40 U q 3 d × 5*
B. M.	6	1.05	Male	E 50 mg/day	4 yr	3 wk	40 U q 2 d × 7
S. M.	10	1.55	Male	E 75 mg/day	4 yr	3 wk	40 U q 2 d × 7
B. H.	16	1.45	Male	Δ_1E 7.5 mg/day	8 yr	10 months	None
R. W.	6	1.05	Female	None	—	6 yr	None
R. Wo.	8	1.23	Male	Δ_1E 10 mg/day	1 yr	2 wk	None
B. W.	4	0.79	Female	Δ_1E 5 mg/day	5 months	0	None
J. G.	18	1.40	Female	Δ_1E 10 mg/day	13 yr	0	None
D. C.	5	0.94	Female	Δ_1E 10 mg/day	1 yr	0	None

ACTH, adrenocorticotropic hormone; E, cortisone; Δ_1E, prednisone; Δ_1F, prednisolone.
* Every 3rd day for five injections.

TABLE II

Aldosterone Secretion Rates, Blood Pressure, Serum Potassium, and Carbon Dioxide before Suppressive Treatment

Patient	Sodium intake	Days*	Potassium intake	Urinary 17-KS	Aldosterone secretion rate	Urinary sodium, day of secretion rate	Blood pressure	Serum potassium	Serum CO_2
	mEq/day		*mEq/day*	*mg/day*	*μg/day*	*mEq/day*	*mm Hg*	*mEq/liter*	*mEq/liter*
R. H.	109	8	50	8	554	97	114/70	4.4	28
D. H.	109	8	50	7.5	492	104	110/50	4.1	29
E. B.	109	7	65	22	1697	102	106/70	3.9	23
D. M.	109	6	80	6	460	69	108/60	4.3	26
B. M.	109	6	50	5.8	117	50	100/56	4.3	25
S. M.	109	6	60	9.9		87	78/42	4.2	26
B. H.	109	4	35	19.4	277	102	110/80	4.3	24
R. W.	133	4	83	30	112	110	94/50	3.9	24
R. Wo.	83	8	90	16	169	82	130/80	3.8	26
B. W.				13			110/70	4.4	26
J. G.				18			106/64	4.6	19
D. C.				14			90/60	3.7	22

KS, ketosteroid.

* Minimum number of days on metabolic diet.

measured on the 4th day of this regimen. Two of these patients were studied by sodium deprivation both "off" and "on" suppressive therapy (Fig. 3). A third child (a male pseudohermaphrodite with normal adrenal function) was studied in an identical manner as shown in Fig. 4.

Aldosterone secretion rate was measured as described previously (4). In this study, all collections were carried out for complete 24-hr periods, and the metabolite of aldosterone which was measured (the 18-glucuronide) was virtually all excreted within 24 hr. This excretion was demonstrated by the procedure previously described (4), carried out on the 24-hr urine collected immediately after the specimen used for the secretion rate.

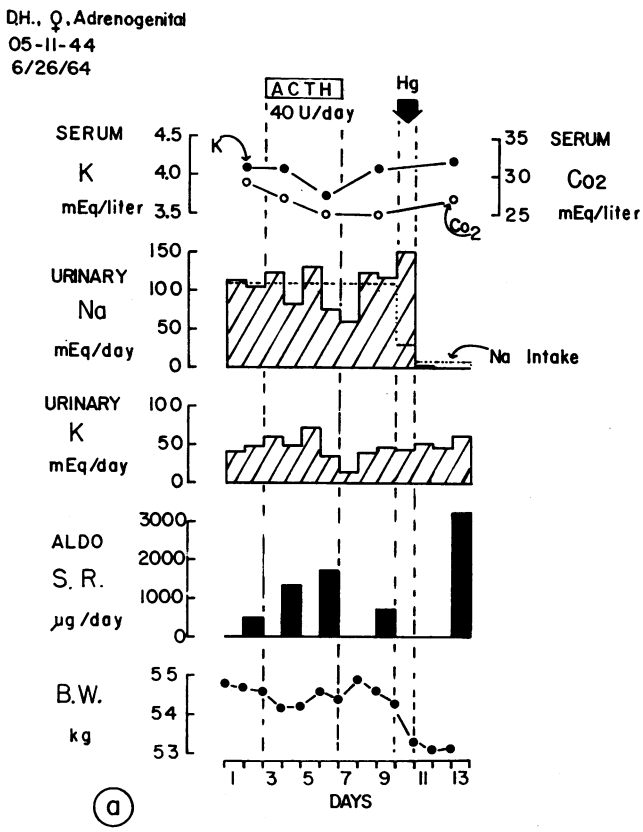
The radiochemical purity of the final eluate of aldosterone monoacetate used to measure secretion rates was verified on 15 specimens by measurement of the $^{14}C/^{3}H$ ratio and that of various derivatives, purified through additional chromatographic procedures; in particular some of the specimens allowed as many as three additional chromatographic separations (or a total of seven). The results are shown in Table V. They indicate that reasonable radiochemical purity is achieved by the methods employed.

TABLE III

Effect of Sodium Deprivation before Suppressive Treatment

Patient	Sodium intake	Days*	Mercurial diuretic on 1st day	Aldosterone secretion rate	Urinary sodium, day of secretion rate	Blood pressure	Serum potassium	Serum CO_2
	mEq/day			*μg/day*	*mEq/day*	*mm Hg*	*mEq/liter*	*mEq/liter*
R. H.	9	4	Yes	2513	2	110/70	4.8	26
D. H.	9	4	Yes	3244	2	106/74	4.2	27
E. B.	9	3	No	2411	32	120/80	3.8	25
	9	8		1756	14	112/86	3.8	26
D. M.	9	5	Yes	616	6	90/62	5.2	27
B. M.	9	5	Yes	266	7	92/30	5.0	25
S. M.	9	5	Yes	419	10	100/62	4.5	27
B. H.	9	3	Yes	632	9	110/90	4.2	27
R. W.	13	13	No	254	9	106/60	4.4	23
R. Wo.	8	4	No	275	14	112/64	3.6	24

* Minimum number of days with sodium deprivation.



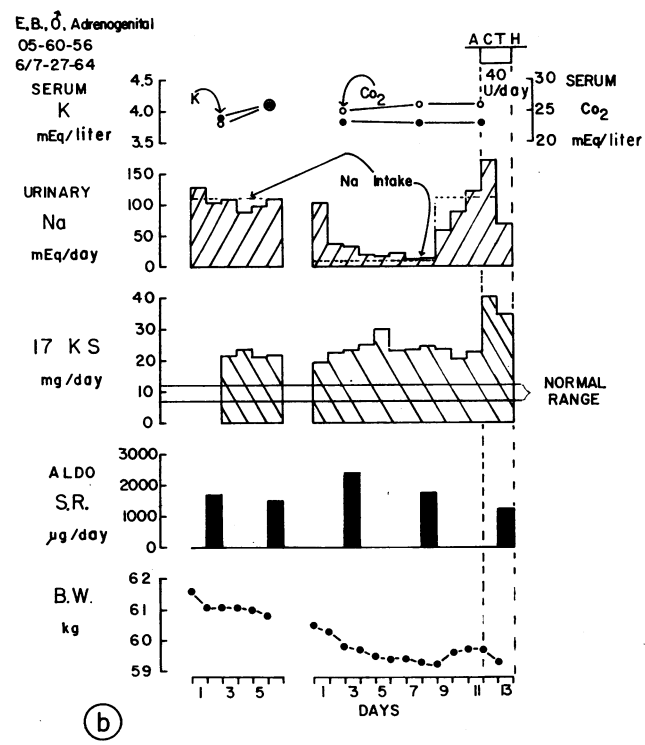


FIGURE 1 Design of experiment in patients studied without suppressive medication: (*a*) in D.H., who had previously been treated with prednisone; (*b*) in E.B., who had never been treated. *Aldo SR*, aldosterone secretion rate; *KS*, ketosteroid.

TABLE IV

Effect of Sodium Deprivation during Suppressive Treatment

Patient	Urinary 17-KS	Control sodium intake	Days*	Control aldosterone secretion rate	Sodium intake	Days‡	Mercurial diuretic on 1st day	Aldosterone secretion rate	Urinary sodium day of secretion rate
	mg/day	*mEq/day*		*μg/day*	*mEq/day*			*μg/day*	*mEq/day*
R. W.	2.6	134	4	49	9	8	No	116	27
R. Wo.	4.5	83	4	74	9	4	No	194	29
B. W.	1.4	61	4	121	13	6	No	361	8
J. G.	2.5	182	4	26	14	7	No	326	2
D. C.	2.8	124	4	92	12	7	No	330	15

KS, ketosteroid.

* Minimum number of days with "control" intake.

‡ Minimum number of days with sodium deprivation.

Hematocrit was determined on venous blood by the micro method. Serum and urinary sodium and potassium were measured by flame photometry. Blood urea nitrogen and serum carbon dioxide were measured by the Technicon AutoAnalyzer. Urinary 17-ketosteroids were measured by a modification of the Zimmerman reaction (5).

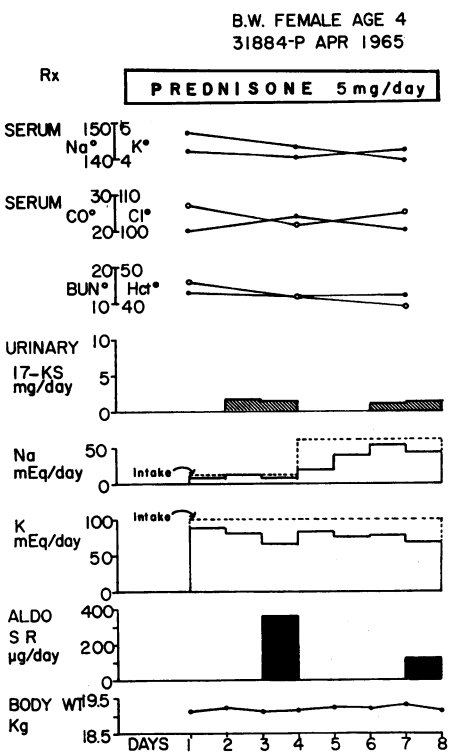


FIGURE 2 Design of experiment in patients studied only during steroid suppression by change of sodium intake. *BUN*, blood urea nitrogen; *Hct*, hematocrit.

RESULTS

Results are shown in Tables I–VI and Figs. 1–6. The basic diets contained each day 60 mEq/m² or more of sodium and potassium. During the period of time when the patients were receiving no carbohydrate-active steroid, the aldosterone secretion rates ranged from 112 μg/day in R.W. to 1697 μg/day in E.B. (Table II). Five of the eight secretion rates were definitely elevated above the normal range; two of the remaining three (R.W. and R.Wo.) were significantly higher before adrenal suppression than after prednisone therapy (112 vs. 49 μg/day and 169 vs. 74 μg/day. Results obtained in a group of normal subjects, and these patients are shown in Fig. 5.

With sodium restriction, aldosterone secretion increased in all subjects, to reach rates ranging from 254 μg/day in R.W. to 3244 μg/day in D.H. (Table III). The values with sodium deprivation in R.H., D.H., and E.B. exceed any that we have seen with sodium deprivation in normal subjects; and those in D.M. and B.H. (616 and 632 μg/day, respectively, achieved with 5 and 3 days of sodium deprivation respectively) are in the highest range of any values measured in these laboratories after mercurial diuretics.

In the patients receiving prednisone at dosages sufficient to decrease 17-ketosteroid excretion to normal (Table IV), aldosterone secretion rates ranged from 26 μg/day in J.G. to 121 μg/day in B.W. These values fall within the normal range for patients receiving, as these were, 60 mEq or more of sodium per m² each day. In each patient subjected to sodium restriction while suppression

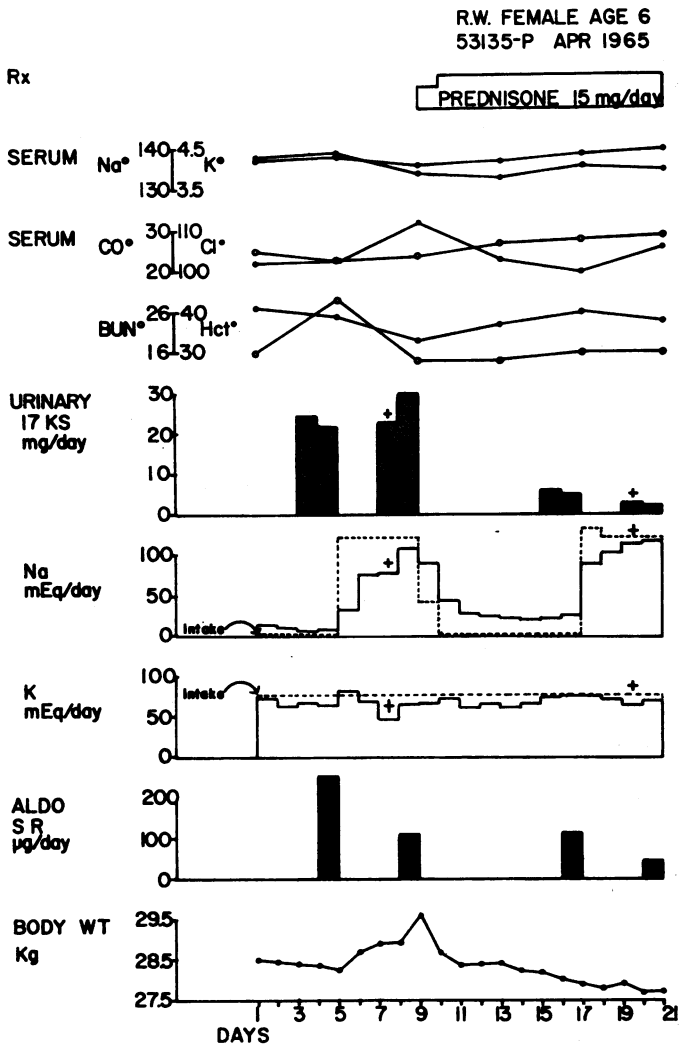


FIGURE 3 Design of experiment for patients studied both with and without steroid suppression on low and high sodium intake.

with carbohydrate-active steroids was continued, aldosterone secretion rate rose (Table IV); the increase was comparable to that found in normal subjects subjected to sodium deprivation without the use of mercurial diuretics.

In two patients the effect of sodium deprivation on aldosterone secretion rate was measured on identical metabolic regimens before and during suppression with prednisone, 15 mg/day, a dosage which reduced 17-ketosteroid excretion to normal. The results from these two patients can be seen by comparing Tables II, III, and IV for patients R.W. and R.Wo. The studies in patient R.W. are shown also in Fig. 3. The aldosterone secretion rate was two to three times higher with sodium deprivation than it was with sodium loading. All values, however, were approximately twice as high without suppression by prednisone as they were during suppression. In contrast, the control patient showed no significant change between the secretion rates with sodium loading before and during prednisone therapy but demonstrated a significant increase in the secretion rate during sodium depreviation and prednisone therapy (Fig. 4). Thus, the uncontrolled biogenetic defect approximately doubled aldosterone secretion in these patients.

Seven patients not receiving suppressive therapy with carbohydrate-active steroids, received ACTH, 40 U/day intramuscularly. The effect on aldosterone secretion rates is shown in Table VI. The results ranged from a steady increase in aldosterone secretion rate over a 4 day period in R.H. and D.H. to a slight (D.M.) or a marked (E.B.)

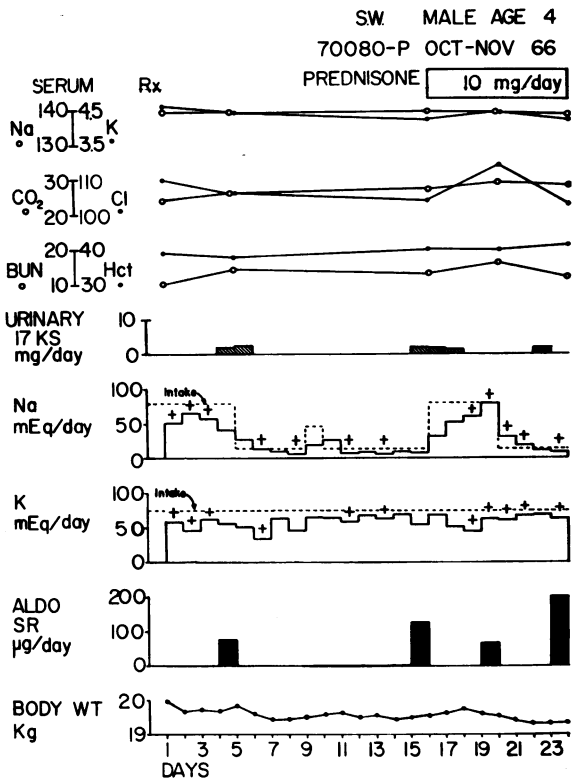


FIGURE 4 Design of experiment and results of study of control subject both with and without steroid suppression on low and high sodium intake.

decrease. In two patients (B.M. and S.M.) the aldosterone secretion rate decreased between day 2 and day 4. This last is the usual pattern of response in normal subjects (6) and has been observed also in the adrenogenital syndrome (7).

Despite the high values for aldosterone secretion rate found in some of these subjects while they were not receiving suppressive doses of carbohydrate-active steroids, all patients demonstrated normal blood pressure, and no patient developed hypokalemia or alkalosis (Tables II and III).

DISCUSSION

It is clear from the evidence presented and from previous studies (8, 9) that the biosynthetic pathways for aldosterone production are not defective in the "non-salt-losing" form of the adrenogenital syndrome. Thus, patients produced up to 1697 µg of aldosterone per day without the stimulus of sodium deprivation. With the stimulus of sodium deprivation aldosterone secretion increased in all patients. When the patients were receiving suppressive doses of carbohydrate-active steroids such as to reduce their excretion of 17-ketosteroids to normal, aldosterone secretion rates and their response to sodium deprivation were normal in every respect. These findings confirm and extend the cases studied with similar methods by Kowarski, Finkelstein, Spaulding, Holman, and Migeon (4 cases) (8) and New, Miller, and Peterson (2 cases) (9). If there were in these patients a block in the 21-hydroxylation of progesterone, the inhibition of ACTH production with carbohydrate-active steroids, superimposed on such a block, should lead to the inhibition of aldosterone production so that subnormal quantities would be produced. The normal values found with suppression and the normal response to sodium deprivation despite such suppression argue strongly for normal biogenetic pathways from progesterone to aldosterone in this syndrome.

The results (Table II) reveal a surprising discrepancy between the aldosterone secretion rates on the one hand, and the clinical and serum chemical findings on the other. Whereas aldosterone se-

TABLE V

Carbon-Tritium Ratios of Aldosterone Monoacetate after Further Purification by Chromatography

Number	Aldosterone secretion rate	Chromatography			
		4th	5th	6th	7th
250	379	0.21	0.25	0.24	0.22
253	396	0.70		1.50	1.57
254	276	0.15	0.22	0.22	0.17
255	303	0.15	0.22	0.23	0.21
264	276	0.49	0.58	0.52	0.49
265	1027	1.86	2.00	1.33	1.63
252	157	0.08	0.11	0.12	
256	111	0.06	0.12	0.12	
257	38	0.03	0.10	0.10	
260	81	0.03	0.04	0.04	
262	401	0.18	0.27	0.31	
269	339	0.14	0.19	0.15	
407	531	0.46	0.46	0.42	
408	575	0.51	0.51	0.48	
261	35	0.01	0.02		
268	12	0.01	0.01		

4th chromatography (after oxidation), cyclohexane 100: benzene 75: methanol 100: water 25.
5th chromatography, dioxane 100: cyclohexane 100: methanol 50: water 25.
6th chromatography, cyclohexane 100: benzene 50: methanol 100: water 25.
7th chromatography (benzhydrazide derivative), dioxane 100: cyclohexane 100: methanol 50: water 25.

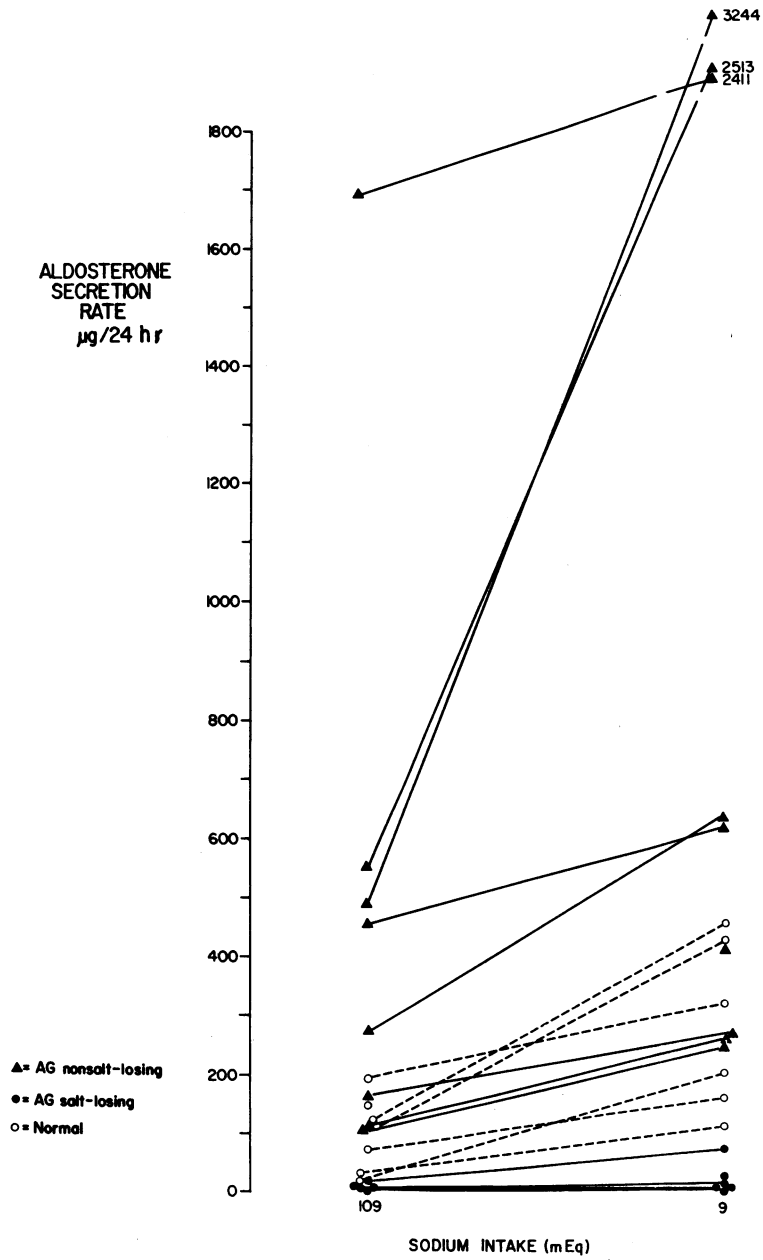


FIGURE 5 Summary of all data on patients studied without steroid suppression. AG non-salt-losing refers to patients on the present study. Normal subjects are normal volunteers studied while receiving 109 mEq of sodium a day, followed by 9 mEq of sodium for at least 4 days after mercurial diuretic. *AG* salt-losing indicates data from patients with sodium-losing adrenogenital syndrome from *J. Clin. Invest.* 1965. **44**: 957.

cretion rates reached values as high as 1697 µg/day in the unsuppressed state despite the ingestion of average amounts of sodium, all patients remained consistently normotensive, and no patient developed hypokalemia or alkalosis. Thus, it appears clear on clinical grounds that one of two situations must obtain: either the actual production rate of aldosterone is not accurately reflected by the aldosterone secretion rate procedure based upon a urinary metabolite, or the *action* of aldosterone is inhibited in these patients. Increased excretion of urinary estriol is well documented in patients with congenital adrenal hyperplasia (1). Increased binding of aldosterone to plasma protein has been demonstrated in patients receiving Enovid (G. D. Searle & Co., Chicago, Ill.) (98.5% norethynodrel and 1.5% mestranol) (10), and slight increases in urinary acid-labile conjugate of aldosterone have been demonstrated during treatment with estrogen (11). It is unlikely that the in-

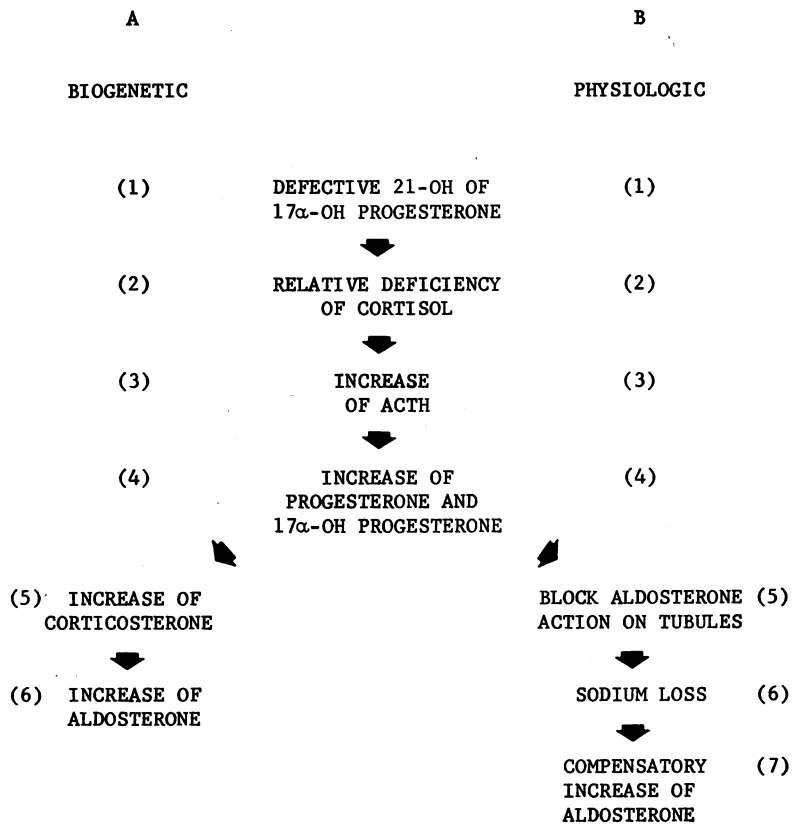


FIGURE 6 Alternative sequences of events to explain the findings in non-salt-losing adrenogenital syndrome. Steps 1, 2, 3, and 4 are common to both hypotheses. For discussion, see text.

creased estrogen secretion in these patients is enough to alter the binding of aldosterone to plasma protein significantly, although it may be enough to change the percentage of metabolite excreted as the acid-labile conjugate.

It is generally agreed that C-21 hydroxylation of steroids is impaired in the most common form of this syndrome (12). This defect results in a limitation in the production of cortisol sufficient to allow an increase in ACTH secretion. The ACTH

TABLE VI
Effect of ACTH 40 U/day on Aldosterone Secretion Rate

Patient	Control aldosterone secretion rate	ACTH			Days*
		Aldosterone secretion rate day 2	Aldosterone secretion rate day 4	Aldosterone secretion rate after ACTH	
	μg/day	*μg/day*	*μg/day*	*μg/day*	
R. H.	554	1098	1466	517	3
D. H.	492	1351	1717	717	3
E. B.	1697	1233			
D. M.	460	410	276	150	2
B. M.	117	155	111	81	2
S. M.	lost	315	303	201	2
B. H.	277	388			

ACTH, adrenocorticotropic hormone.
* Number of days after ACTH.

in turn leads to an increase in the production of progesterone and of 17-hydroxyprogesterone as demonstrated by the excretion of pregnanediol (13), pregnanetriol, and pregnanetriolone in increased quantities (1). The sequence of events in the "non-salt-losing" form of the adrenogenital syndrome might be explained in two different ways (Fig. 6); the clinical consequences may allow a choice between these sequences.

In the first place, overproduction of progesterone might induce overproduction of deoxycorticosterone, of corticosterone, of 18-hydroxycorticosterone, and finally of aldosterone as a consequence of the overabundance of precursor. As noted above, it is clear that the pathways between progesterone and aldosterone are not impaired in these patients, and thus there is no reason a priori to reject this hypothetical sequence (Fig. 6 A). Alternatively, progesterone and 17-hydroxyprogesterone, produced in excess by the same mechanism, might produce loss of sodium (14–16) by antagonizing the effects of endogenous aldosterone. This, by leading to a relative sodium depletion, might lead to the overproduction of aldosterone as a compensatory phenomenon.

It is of interest to examine the consequences of these two alternative sequences to explain the overproduction of aldosterone in the "non-salt-losing" adrenogenital syndrome (Fig. 6). According to sequence A ("Biogenetic"), the production of aldosterone is a direct biogenetic consequence of the overproduction of precursors. Accordingly, the effect of this aldosterone should be apparent from its actions on the organism or on the renal tubules. It would be reasonable to anticipate hypokalemic alkalosis, hypertension, a fixed rate of aldosterone secretion, or a combination of all three, if this were the sole explanation for the aldosteronism.

Alternatively, according to sequence B ("Physiologic") overproduction of aldosterone represents an attempt to compensate for the sodium loss which results in turn from the overproduction of weak sodium-losing steroids such as progesterone and 17-hydroxyprogesterone. As physiologic compensation is virtually never "complete," it would be reasonable to suppose that the amounts of aldosterone produced might just fail to compensate for the loss of sodium; despite the excessive quantities of aldosterone presented to the tubules the sodium-losing steroids would also block sodium-for-hydrogen and sodium-for-potassium exchange and thus prevent the hypokalemic alkalosis that one would anticipate with an "autonomous" aldosteronism of the degree found in these patients.

In all patients an increase in sodium intake decreased aldosterone secretion (or sodium deprivation stimulated it) even when there was no suppression of adrenocortical activity by carbohydrate-active steroids. If sequence A were responsible for the overproduction of aldosterone, an increase in dietary sodium alone should have little effect on the overabundance of precursors to the biogenesis of aldosterone and thus should not decrease its production so effectively. Accordingly, both lines of evidence favor sequence B; these results lend indirect support to the hypothesis that "sodium-losing" steroids (such as progesterone) play an important role in the increased aldosterone production in the "non-salt-losing" adrenogenital syndrome as previously suggested (8).

These results showing excessive production of aldosterone in the "non-salt-losing" variety of the adrenogenital syndrome stand in striking contrast to those in the "salt-losing" variety of the syndrome, in which aldosterone secretion is very low and shows little response to sodium deprivation (4). Furthermore, it appears clear that the two syndromes are genetically distinct from each other (17) although there are reports which tend to question this conclusion (18). It has been suggested that the difference between the two syndromes is one of degree of block in 21-hydroxylation, the sodium-losing variety representing the more severe block (2, 8). This explanation appears unlikely in view of the excessive production of aldosterone in "non-salt-losing" variety. Earlier results suggested that secretion of cortisol was defective in the sodium losers and normal, albeit limited, in the "non-salt-losers" (2). Subsequent studies have demonstrated normal cortisol secretion in the "non-salt-losing" form with limited response to ACTH and low or normal cortisol secretion in the salt-losing form with minimal response to ACTH (19, 20).

In view of all the evidence it appears not unlikely that there are two separate isozymes of the 21-hydroxylase enzyme for C_{21} steroids. Thus the "non-salt-losing" patients may produce an isozyme defective in 21-hydroxylation of 17α-hy-

droxyprogesterone alone, whereas those with the salt-losing form of the disease may produce an isozyme possessing a defect which prevents the 21-hydroxylation of both progesterone and 17α-hydroxyprogesterone. This hypothesis would explain the presently available genetic and clinical data.

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