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Impaired Aldosterone Production in "Salt-losing" Congenital Adrenal Hyperplasia *

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"Salt-losing" congenital adrenal hyperplasia is characterized by a genetically determined defect in the synthesis of cortisol and by excessive urinary sodium loss. The defect in cortisol synthesis was shown by Bongiovanni and Eberlein to result from a block in 21-hydroxylation (1). The role of aldosterone has remained controversial. Whereas Prader, Spahr, and Neher, using chemical assay, reported that urinary aldosterone values were abnormally high (2), Blizzard, Liddle, Migeon, and Wilkins, using bioassay and radioisotopic assay, reported that they were abnormally low (3).

The disorder of steroid metabolism in congenital adrenal hyperplasia has three components: [1] *1*) a block in secretion of cortisol, *2*) an increase in secretion of adrenocorticotropic hormone (ACTH), lacking the normal regulatory inhibition, and *3*) an increase in secretion of cortisol precursors, for which urinary 17-ketosteroids provide a useful index (4). Salt-losing congenital adrenal hyperplasia has each of the above components, with the addition of excessive urinary sodium loss. Since aldosterone is the most active naturally occurring sodium-retaining steroid, it is logical to question its role in this particular disorder. An abnormal increase of aldosterone excretion in patients with the sodium-losing form of the syndrome suggested an action on the renal tubules of unknown steroids promoting sodium excretion (2). The hypersecretion of aldosterone was thought to be compensatory, and the sodium-losing compounds were sought among the steroids or degradation products of steroids appearing on the biosynthetic pathway before the site of the defect (5–8). If such steroids are indeed responsible for the sodium loss, it follows that the sodium loss should disappear when abnormal steroid production is prevented by suppression of ACTH secretion.

An abnormal decrease of aldosterone secretion in the syndrome, on the other hand, provides an immediate explanation for the sodium loss. In the present investigation, carbohydrate-active steroids were administered to assure inhibition of ACTH secretion. Aldosterone production was assessed by radioisotopic measurement of secretion and urinary excretion.

Methods

Patients. Eight patients with salt-losing congenital adrenal hyperplasia were included in this study. The criteria for this diagnosis were *1*) abnormally high urinary excretion of 17-ketosteroids (17-KS) and pregnanetriol before treatment, *2*) low or normal urinary excretion of 17-hydroxycorticosteroids (17-OHCS), showing little or no increase with ACTH given over 5-day periods, *3*) hyperkalemia and hyponatremia before treatment, *4*) restoration of urinary 17-KS to normal with carbohydrate-active steroids, and *5*) restoration of serum potassium and sodium to normal with desoxycorticosterone acetate (DOCA). The patients ranged in age from 2 months to 5½ years. The clinical and laboratory findings are outlined in Table I. The chromatin sex was determined by examination of buccal smears. B.L. had a phallic urethra, and the sex was confirmed by bone marrow karyotype.[2] G.S. and B.S.

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[1] The following abbreviations have been used for the steroids discussed: pregnanetriol = pregnane-3α, 17α, 20α-triol; pregnanetriolone = 3α, 17α, 20α-trihydroxypregnane-11-one; progesterone = Δ^4-pregnene-3,20-dione; 17α-hydroxy progesterone = Δ^4-pregnene-17α-ol-3,20-dione; tetrahydrocortisone = 3α, 17α, 21-trihydroxypregnane-11,20-dione.

[2] Kindly performed by Drs. J. H. Tjio and J. Whang.

TABLE I

Age, sex, pigmentation, and urinary steroids of eight children with "salt losing" adrenogenital syndrome

Patient	Age		Hyperpigmentation	Chromatin sex*	Urinary 17-KS† No treatment	Urinary 17-KS† Treatment	Urinary Pregnanetriol‡ No treatment
	yrs	*mos*			*mg/day*		*mg/day*
R.S.	5	6	No	M	4.0	1.0	1.7
G.S.	4	0	No	F	6.0	1.2	0.4
B.L.	2	2	No	F	1.4	0.7	3.0
B.S.	2	5	No	F	3.5	1.6	0.3
E.J.	1		No	M	4.0	0.9	4.3
W.G.		3	Yes	M	5.4	0.7	0.9
K.M.		3	Yes	M	2.0	0.3	1.3
J.D.		2	Yes	M	6.5	0.4	0.2

* As determined by buccal smear examination.
† 17-KS = 17-ketosteroids. Normal children less than 2 years of age excrete less than 1 mg. The data reported here were obtained before 2 years of age.
‡ Children without adrenal disorder show no detectable pregnanetriol by this method.

had separate vaginal and urethral openings at the introitus, and clitoral hypertrophy. Testes were palpable in the scrotum of each of the other cases. Hyperpigmentation of nipples and scrotum was present in three cases. All of the patients had received treatment with steroids before admission to the Clinical Center. Elevated quantities of pregnanetriol were found in the urine of all patients. Seven patients without adrenal dysfunction were included as controls (Table II).

Procedures. Cortisol, prednisone, or prednisolone was given to the patients in constant amounts throughout each study except that on E.J. (Table III). The control patients had been shown to excrete normal amounts of 17-OHCS and did not receive exogenous steroids. The patients received no DOCA during the study and had received no desoxycorticosterone trimethyl acetate for more than 4 weeks before hospitalization. Sodium chloride was added to a constant, low-sodium diet in an amount sufficient to maintain sodium balance during the "control" periods. The sodium intake was then decreased and kept constant until signs of sodium depletion were evident. Twenty-four hour urine collections were made during initial observations and as frequently as possible during the period of sodium deprivation. E.J. received no exogenous steroids during the study reported here. During another study, however, when he received 0.2 mg of dexamethasone and 20 mEq of sodium a day, he was excreting sodium at the intake level 8 days later and developed vomiting as his serum sodium fell to 122 mEq per L. His urinary 17-KS on the eighth day were 0.9 mg per 24 hours confirming ACTH suppression.

Blood was obtained at 2- to 7-day intervals from a femoral or antecubital vein without stasis and analyzed for hematocrit and for serum sodium, potassium, chloride, bicarbonate, and urea nitrogen concentrations. A sample diet and 24-hour pools of urine were analyzed for sodium and potassium. Stools from B.L., W.G., K.M., and J.D. were also analyzed for sodium and potassium. Body weight was measured to the nearest gram at the same time each day. The control patients were studied by identical procedures, except that the initial sodium intake was less and that no exogenous steroids were given.

Aldosterone secretion was determined by a modification

TABLE II

Age, sex, urinary steroids, and diagnosis of seven children without adrenal dysfunction

Patient	Age		Sex	Urinary 17-KS	Urinary 17-OHCS*	Diagnosis
	yrs	*mos*		*mg/24 hours*		
S.L.	6	4	F	1.2	3.4	Essential hypertension
A.S.	6	8	M		2.1	Lowe's syndrome
B.B.	1	7	M	0.3	2.0	Hemihypertrophy
B.D.	1	11	M	1.1	2.3	Normal with adrenal calcification
T.G.	2	3	F	1.0	1.0	Casein sensitive enteropathy
L.T.	0	10	F †			Turner's syndrome
C.K.	0	4	M	0.4	0.8	*Escherichia coli* enteritis

* 17-OHCS = 17-hydroxycorticosteroids.
† Chromatin negative and presumed to be "XO."

TABLE III

Patients without adrenal dysfunction

Patient	Lowest sodium intake	Duration	Minimal sodium output
	mEq/day	*days*	*mEq/day*
B.B.	4	5	3
L.T.	2	7	0
S.L.	9	6	3
A.S.	7	6	3
B.D.	9	5	2
C.K.	2	6	1

Patients with adrenogenital syndrome

Patient	Treatment compound		Sodium intake producing symptoms	Duration	Minimal sodium output
		mg/day	*mEq/day*	*days*	*mEq/day*
W.G.	Prednisolone	0.9	31	11	16
K.M.	Prednisolone	4.0	13	6	16
R.S.	Prednisone	3.0	110	2	142
G.S.	Hydrocortisone	15	174	3	161
B.L.	Prednisolone	3.0	25	11	18
E.J.*			5	6	11
J.D.	Prednisone	4.0	33	5	28

* See text.

of the double isotope derivative dilution method of Peterson (9). Tracer doses of 1.0 μc 7-H^3-aldosterone [3] were given intravenously over a 2-minute period. The time of urine collection, usually 24 hours, was accurately measured. Approximately 200 ml of urine was extracted with 5 vol of dichloromethane, acidified to pH 1 with concentrated hydrochloric acid, and incubated at 24° C for 18 to 24 hours. The acid-released aldosterone was extracted with 5 vol dichloromethane and washed with one-tenth vol of 0.05 N sodium hydroxide and of 0.1 N acetic acid. After evaporation, the residue was chromatographed for 6 hours in the Bush C system of toluene-ethyl acetate-methanol-water (9:2:5:5 by vol), between spots of authentic *d*-aldosterone.[4] These aldosterone spots were located by ultraviolet absorption, and the corresponding area from the urine extract was eluted with ethanol, evaporated to dryness, and acetylated with acetic-1-C^{14} anhydride (specific activity about 1 mc per mmole) [5] in pyridine for 48 hours at 37° C. After acetylation, nonradioactive aldosterone-18,21-diacetate was added to allow subsequent location on paper by ultraviolet light absorption. The residue was then chromatographed in system II of Kliman and Peterson (10) (cyclohexane-dioxane-methanol-water, 4:4:2:1 by vol) for 16 hours, eluted, dried, and oxidized to a monoacetate derivative with 0.5% (wt/vol) chromium trioxide in glacial acetic acid. The derivative was chromatographed in system II and finally in system I of Kliman and Peterson. The final spot was eluted, and the content of carbon^{14} and tritium was measured in a liquid scintillation spectrometer.[6] The adequacy of purification by four chromatographic separations was demonstrated by three secretion rate determinations in patients with Addison's disease. The net yields of carbon^{14} were 0, 1, and 3 cpm above background, and the yields of tritium were 3,370, 6,870, and 4,980 cpm, respectively. The calculated secretion rates were 0, 0.3, and 1.4 μg per 24 hours. As shown in Table IV, there are significant decreases in the final secretion rate as calculated after the fourth, as compared to the third, chromatographic separation. These data (Table IV) were obtained by removing a sample of the aldosterone monoacetate after the third chromatography.

Specific activity of the acetic-1-C^{14} anhydride was determined as described by Kliman and Peterson (10), and the results were expressed as disintegrations per minute per micromole of acetic anhydride. Calculations were as follows: *1*) aldosterone in final eluate (μg) = [dpm C^{14} in final eluate/SA C^{14} acetic anhydride (dpm/μmole)] × mol wt of aldosterone (μg/μmole), and *2*) aldosterone secretion (μg) = aldosterone in final eluate × [H^3 in dose administered (dpm)/H^3 in final eluate (dpm)].

Since three patients required specific treatment for their sodium deprivation before a complete 24-hour urine collection could be obtained, the urine collection was stopped in those cases when necessary. The duration of the collection period was accurately timed, and subsequent urine was saved for a period of 24 hours. Tracer excretion was determined on the initial and subsequent specimens by formation of a nonradioactive acetate deriva-

[3] Endocrinology Study Section.

[4] Kindly provided by Dr. R. Gaunt, Ciba Pharmaceuticals, Summit, N. J.

[5] New England Nuclear Corp., Boston, Mass.

[6] Tri-Carb, Packard Instrument Co., LaGrange, Ill.

TABLE IV

Aldosterone secretion rates calculated after three and four chromatographic separations

	Secretion rate		
	Third chromatography	Fourth chromatography	Per cent change
	μg/24 hours		
Below 50	4.8	2.6	−46
	7.7	6.9	−11
	10.3	8.7	−15
	11.6	9.8	−16
	27	26	− 4
			Average −18.4
			SD ± 9.5
50 to 250	71	51	−28
	67	59	−12
	103	70	−30
	114	97	−15
	119	103	−13
	127	107	−16
	131	118	−10
	156	126	−20
	160	149	− 7
	202	205	+1.5
	244	218	−11
			Average −14.6
			SD ± 9.0
Above 250	342	310	− 8
	592	595	+0.6
	692	639	− 8
	707	740	+ 4
			Average − 2.9
			SD ± 6.2

tive. For this, 50 ml of urine was hydrolyzed at pH 1 for 24 hours and extracted with 5 vol of dichloromethane. The extract was dried, and the residue was acetylated with nonradioactive acetic anhydride in pyridine for 36 to 48 hours at 37° C. The reaction was stopped with 20% ethanol. Aldosterone-C^{14}-diacetate was added to measure recovery, and nonradioactive aldosterone diacetate was added to allow identification of the steroid in ultraviolet light after chromatography. After extraction with 5 vol dichloromethane, the mixture was dried in an air stream and chromatographed in systems I and II (10). The final eluate was counted for C^{14} and H^3. The final secretion rate as calculated above was divided by the ratio of total H^3 recovered as aldosterone to the amount recovered in the initial specimen. The excretion of tritium-labeled aldosterone conjugate was consistently greater than 90% during the initial collection period.

Urinary aldosterone excretion was determined by the method of Kliman and Peterson (10) on appropriate 24-hour specimens.

Hematocrit was determined on venous blood by the micromethod. Serum and urinary sodium and potassium were measured by flame photometry. Blood urea nitrogen was measured by the autoanalyzer. Urinary 17-KS were measured by the Zimmerman reaction (11) and pregnanetriol by the method of Bongiovanni and Eberlein (12).

Results

The results are shown in Figures 1 and 2 and Tables III, V, and VI.

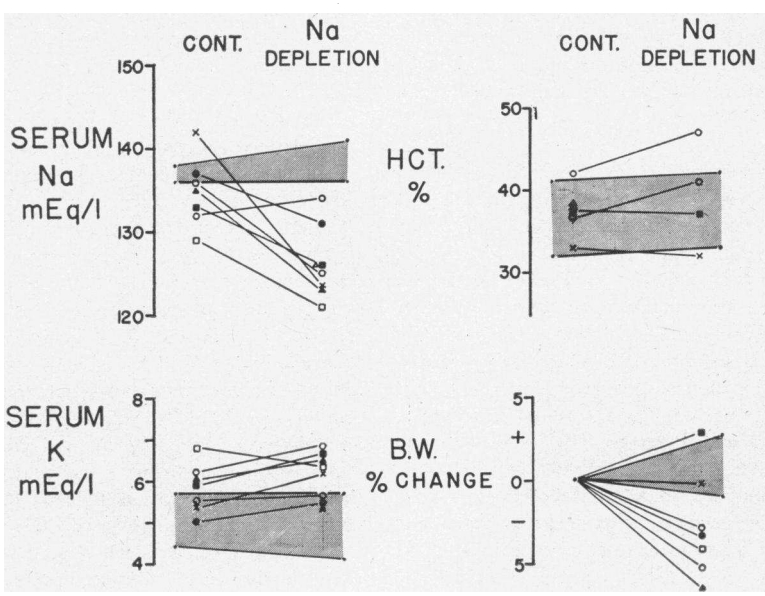

FIG. 1. BODY WEIGHT (B.W.), SERUM SODIUM AND SERUM POTASSIUM, AND HEMATOCRIT (HCT.) BEFORE AND AFTER SODIUM DEPLETION IN PATIENTS WITH THE ADRENOGENITAL SYNDROME. The shaded areas represent the ranges for the control patients.

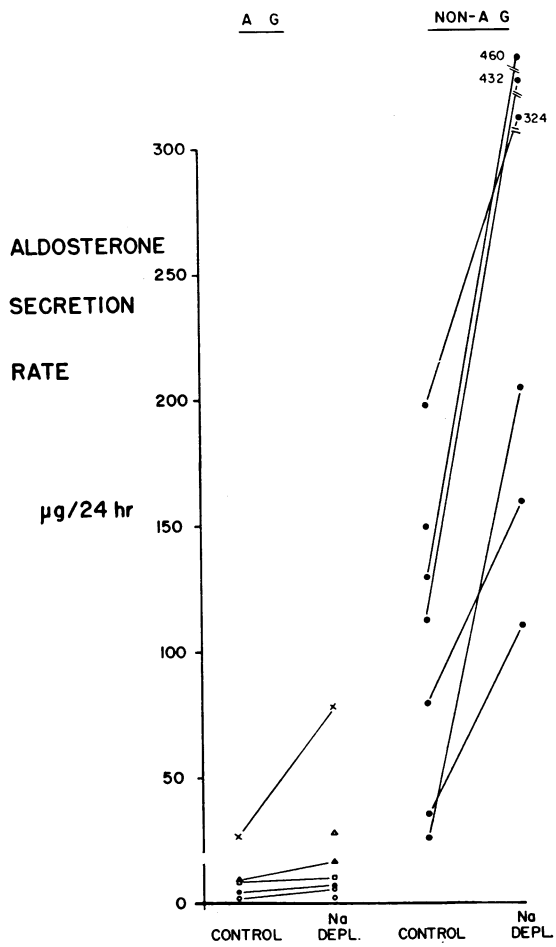

FIG. 2. ALDOSTERONE SECRETION RATES BEFORE AND AFTER SODIUM DEPLETION IN PATIENTS WITH THE ADRENOGENITAL SYNDROME (AG) AND IN PATIENTS OF COMPARABLE AGES WITHOUT ADRENAL DYSFUNCTION (NON-AG.)

A) Clinical and laboratory changes during sodium depletion. All patients with the adrenogenital syndrome developed signs and symptoms of adrenal insufficiency while receiving carbohydrate-active steroids at dosages adequate to suppress 17-KS excretion to normal. These changes included vomiting (six of eight patients), hypotension (two of eight), lethargy (six of eight), loss of normal skin turgor (five of eight), and decrease of body weight (six of eight). The serum sodium concentration decreased in all patients but one, reaching 126 mEq per L or less in six of the eight patients. Despite the hyponatremia, all eight patients were shown to excrete in the urine large quantities of sodium relative to the daily intake (Table III). Serum potassium increased in all patients except B.S., in whom a single value of 5.4 mEq per L was obtained while she was clinically in severe crisis, and E.J., in whom control levels were already 6.8 mEq per L. Figure 1 shows the changes in serum sodium, serum potassium, body weight, and hematocrit and compares them with corresponding values for the subjects without adrenal dysfunction. A single control patient with gastroenteritis continued to vomit, but none of the controls developed hypotension, lethargy, or loss of skin turgor.

B) Response of aldosterone secretion to sodium depletion. Figure 2 shows the response of aldosterone secretion to sodium deprivation in seven of the patients with salt-losing adrenogenital syndrome. The response of urinary aldosterone in all eight patients is shown in Table V. Values in the children without adrenal dysfunction are shown for comparison in Table VI. In the patients with the adrenogenital syndrome, the 24-hour secretion rates during the control periods ranged from 2 to 27 µg, with a mean of 10 µg. With sodium depletion they ranged from 2 to 78 µg, with a mean of 21 µg. The difference between these two means is not significant ($p > 0.1$).

In the children without adrenal dysfunction the 24-hour secretion rates during the control periods ranged from 26 to 198 µg, with a mean of 104 µg. With sodium deprivation, they ranged from 110 to 460 µg, with a mean of 280 µg. The difference between these two means is significant ($p < 0.02$).

The difference between the final secretion rates in the sodium-depleted patients with the adrenogenital syndrome and the sodium-deprived control

TABLE V

Aldosterone excretion in "salt-losing" adrenogenital syndrome during control and sodium depletion

Patient	Urinary aldosterone	
	Control	Sodium depletion
	µg/24 hours	
R.S.		0.6
G.S.	1.3	1.9
B.L.	0.3	0.8
B.S.		2.5
E.J.	0.4	0.5
W.G.	0.8	2.9
K.Mc.	1.1	1.4
J.D.	3.1	4.3

TABLE VI

Urinary aldosterone excretion in patients without adrenal dysfunction during sodium deprivation

Patient	Urinary aldosterone	
	Control	Sodium deprivation
	μg/24 hours	
S.L.	3.5	8.5
A.S.	9.3	10.8
B.D.	3.8	10.2
T.G.	5.9	
L.T.	3.4	10.4
C.K.	0.4	9.5

cases is highly significant ($p < 0.001$). In only one of the patients with the adrenogenital syndrome was the aldosterone secretion rate in the control period as high as the lowest value seen in the normal subjects. With sodium depletion, the aldosterone secretion rate in this patient rose to a value only 28% of the mean increase among the normal subjects and only 66% of the increase shown with salt deprivation by the two normal subjects who showed the smallest response.

The urinary aldosterone (Tables V, VI) showed comparable changes. Although aldosterone excretion increased with sodium depletion in all adrenogenital patients, increase was of borderline statistical significance ($t = 2.66$, $p < 0.05$ for the six paired determinations) and probably of no biological significance. In the control children there was a greater increase in aldosterone excretion with sodium deprivation, and the increase is highly significant ($p < 0.01$). The difference between the excretion values for the two groups of subjects deprived of sodium is also highly significant ($p < 0.01$). Patient A.S. did not show an increase in urinary aldosterone. This was probably related to a relatively low potassium intake with resulting diminished aldosterone secretion.

Discussion

Continued loss of sodium, with classical laboratory and clinical signs of sodium depletion, developed in all the patients with salt-losing adrenogenital syndrome despite treatment with carbohydrate-active steroid adequate to suppress abnormal ACTH secretion. This suggests that the sodium loss in this syndrome is not dependent on adrenal steroids produced in excess as a result of the enzymatic defect.

If indeed the sodium loss did result from the action of excessive quantities of sodium-losing steroids (and there were no defects in aldosterone secretion), the sodium loss should lead to hypersecretion of aldosterone. When renal sodium loss is induced pharmacologically, as with mercurial diuretics or aldosterone antagonists, such a compensatory increase in aldosterone secretion is regularly seen (13, 14). In the studies presented here, however, aldosterone secretion was low and remained low despite severe sodium depletion. Whereas such a failure of compensatory hypersecretion of aldosterone provides no support for the concept that "sodium-losing" steroids play an essential part in the syndrome, it does provide in itself an explanation for the continued loss of sodium.

These studies provide further evidence concerning the possible sites of enzymatic defects in the pathogenesis of the sodium-losing form of the adrenogenital syndrome. Current knowledge concerning these sites is outlined in Figure 3. All of the patients excreted excessive amounts of pregnanetriol, a finding that excludes a deficiency of 3β-hydroxy,Δ^5 steroid dehydrogenase (15) (Figure 3, "3β") and provides evidence for 17α-hydroxylation (Figure 3, "17α").

Indirect evidence excludes the possibility of a defect of 11β-hydroxylation alone. Thus, no patient had hypertension, and no patient excreted excessive amounts of steroids giving the Porter-Silber reaction. Both of these abnormalities are seen in patients with a defect of 11β-hydroxylation (16).

A defect of 21-hydroxylation would explain the abnormalities shown by these patients with sodium-losing form of the syndrome (Figure 3, "21"). Thus, 21-hydroxylation of 17α-hydroxyprogesterone is required for normal secretion of cortisol and thus for normal regulation of the secretion of ACTH (4). Furthermore, 21-hydroxylation of progesterone is probably required for the normal secretion of aldosterone (17). A defect of 21-hydroxylation of progesterone would explain the inability of all patients in this study to secrete aldosterone in adequate amounts.

Whereas a defect of 21-hydroxylation of progesterone and of 17α-hydroxyprogesterone will explain the abnormalities of the patients described in the present study, it will not explain the ab-

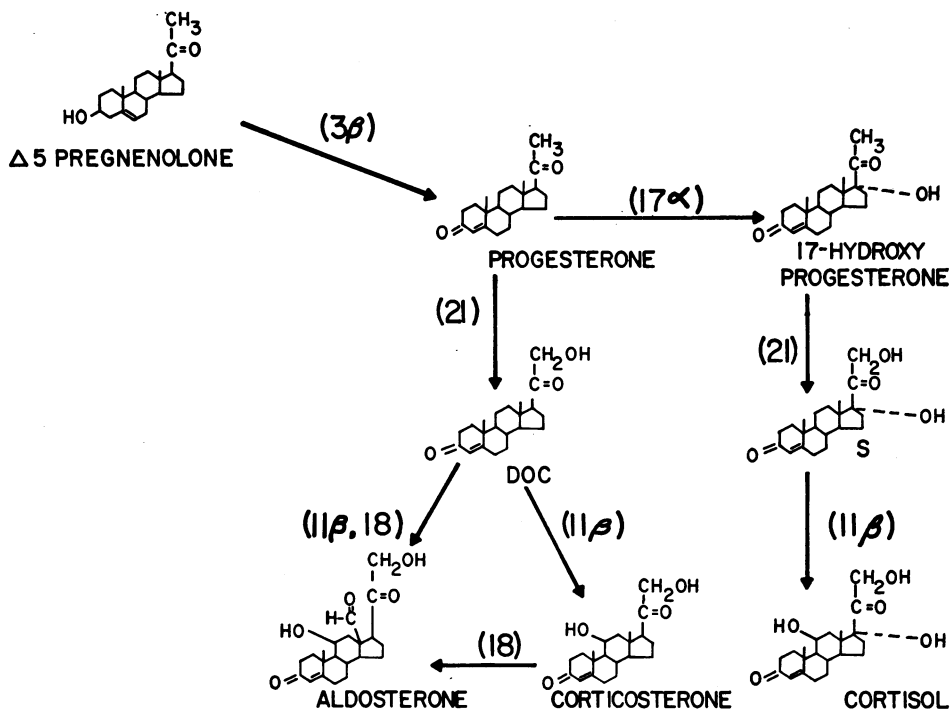

FIG. 3. THE BIOSYNTHETIC PATHWAYS FOR ALDOSTERONE, CORTICOSTERONE, AND CORTISOL. See text for further explanation.

normalities of a similar group of patients who show no tendency to lose sodium, and excrete normal, or indeed excessive, quantities of aldosterone (3, 18). Such patients excrete pregnanetriol (16), providing evidence for 17α-hydroxylation; they do not have hypertension or excrete excessive quantities of Porter-Silber steroids, and they do excrete pregnanetriolone (16), providing evidence for 11β-hydroxylation (*vide supra*). Accordingly, there is presumably a defect of 21-hydroxylation. A defect in the 21-hydroxylation of 17α-hydroxyprogesterone has been shown *in vitro* for two patients with this form of the disorder (19). The 21-hydroxylation of progesterone was not examined. It is not apparent how such patients secrete normal or excessive quantities of aldosterone.

It has been proposed that the block in 21-hydroxylation is more complete in the patients with the sodium-losing form of the disorder than in those with the nonsodium-losing form. Patients with the sodium-losing form of the disorder were shown to excrete little or no tetrahydrocortisone in response to ACTH, whereas those with the nonsodium-losing form excreted small but appreciable amounts (1). In the complete absence of 21-hydroxylation, the adrenal would be unable to secrete even those relatively small quantities of aldosterone required for normal sodium retention (3, 16).

A different hypothesis would explain the results equally well. It may be that 21-hydroxylation in the adrenal cortex is substrate specific and that different 21-hydroxylase enzymes are required for progesterone and 17α-hydroxyprogesterone. In this view, patients with the nonsodium-losing form of the disorder have a deficiency of the 21-hydroxylase whose substrate is 17α-hydroxyprogesterone, but not of that whose substrate is progesterone. A second enzymatic defect is then required to explain the pathogenesis of the sodium-losing form. This defect is presumably not one of 18-hydroxylation or of a dehydrogenase capable of oxidizing the 18-hydroxyl group to an aldehyde. With such a defect, it would be reasonable to expect an increase in secretion of desoxycorticosterone, which should prevent sodium loss. Accordingly, the second enzymatic

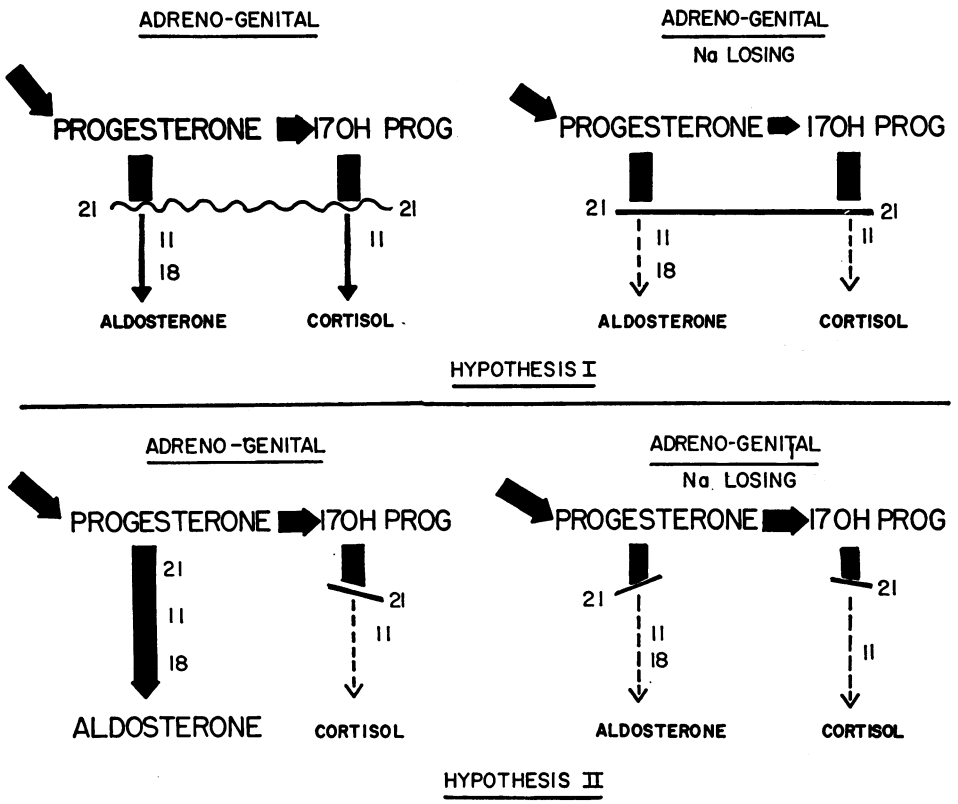

FIG. 4. TWO HYPOTHESIS FOR THE BIOGENESIS OF ALDOSTERONE AND OF CORTISOL IN THE ADRENOGENITAL SYNDROME WITHOUT AND WITH RENAL SODIUM LOSS. I. Hypothesis of Bongiovanni and Eberlein (1). II. Hypothesis proposed in present study. In II, 21-hydroxylases are considered to be substrate specific.

defect presumably involves the 21-hydroxylation of progesterone.

Figure 4 shows both hypotheses in diagrammatic form. There are data which indicate that patients with the nonsodium-losing form of the adrenogenital syndrome may excrete excessive quantities of aldosterone (2, 3, 18). This finding is consistent with hypothesis II, but not with hypothesis I. The question may be resolved if it can be shown that adrenal tissue from patients with the nonsalt-losing form can 21-hydroxylate progesterone, whereas that from patients with the sodium-losing form cannot.

Summary

Eight patients with the "salt-losing" form of virilizing congenital adrenal hyperplasia have been studied and compared to seven children without adrenal dysfunction. Suppression of adrenocorticotropic hormone with exogenous carbohydrate-active steroids did not prevent sodium loss, hyponatremia, or hyperkalemia after 2 to 11 days of sodium restriction in patients with salt-losing adrenogenital syndrome. Aldosterone secretion and excretion were extremely low and differed significantly from those of the control patients. This information offers an explanation for the sodium loss that does not require the mediation of ACTH-dependent, sodium-losing steroids.

A severe defect in the 21-hydroxylation of progesterone and of 17α-hydroxyprogesterone may explain the deficiencies of secretion of aldosterone and cortisol, respectively. It does not explain satisfactorily the nonsodium-losing form of the disorder.

An alternative hypothesis proposes that there is a defect in 21-hydroxylation of 17α-hydroxyprogesterone in all cases, and an additional defect in 21-hydroxylation of progesterone in the sodium-losing form of the disorder.

Acknowledgments

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References

1. Bongiovanni, A. M., and W. R. Eberlein. Defective steroidal biogenesis in congenital adrenal hyperplasia. Pediatrics 1958, **21**, 661.
2. Prader, A., A. Spahr, and R. Neher. Erhohte Aldosteronausscheidung beim kongenitalen adrenogenitalen Syndrom. Schweiz, med. Wschr. 1955, **85**, 1085.
3. Blizzard, R. M., G. W. Liddle, C. J. Migeon, and L. Wilkins. Aldosterone excretion in virilizing adrenal hyperplasia. J. clin. Invest. 1959, **38**, 1442.
4. Bartter, F. C., F. Albright, A. P. Forbes, A. Leaf, E. Dempsey, and E. Carroll. The effects of adrenocorticotropic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia: an attempt to explain and correct its disordered hormonal pattern. J. clin. Invest. 1951, **30**, 237.
5. Neher, R., P. Desaulles, E. Vischer, P. Weiland, and A. Wettstein. Isolierung, Konstitution und Synthese eines neuen Steroides aus Nebennieren. Helv. chim. Acta 1958, **41**, 1667.
6. Neher, R., C. Meystre, and A. Wettstein. Neue 16α-Hydroxysteroide aus menschlichem Urin und aus Schweine-Nebennieren. Isolierung. Konstitution, Synthesen. Helv. chim. Acta 1959, **42**, 132.
7. Klein, R., P. Taylor, C. Papadatos, Z. Laron, D. Kelle, J. Fortunato, C. Byers, and C. Billings. Sodium losing material in human urine. Proc. Soc. exp. Biol. (N. Y.) 1958, **98**, 863.
8. Rosemberg, E., F. X. Dufault, Jr., E. Bloch, E. Budnitz, P. Butler, and J. Brem. The effects of progressive reduction of sodium intake on adrenal steroid excretion and electrolyte balance in a case of congenital adrenal hyperplasia of the salt-losing type. J. clin. Endocr. 1960, **20**, 214.
9. Peterson, R. E. The miscible pool and turnover rate of adrenocortical steroids in man. Recent Progr. Hormone Res. 1959, **15**, 231.
10. Kliman, B., and R. E. Peterson. Double isotope derivative assay of aldosterone in biological extracts. J. biol. Chem. 1960, **235**, 1639.
11. Zimmerman, W. Erie Farbreaktion der Sexualhormone und ihre Anivendrung zur quantitativen colorinetrischen Bestimmung. Hoppe-Seylers Z. physiol. Chem. 1935, **233**, 257.
12. Bongiovanni, A. M., and W. R. Eberlein. Critical analysis of methods for measurement of pregnane-3-alpha, 17-alpha, 20-alpha-triol in human urine. Analyt. Chem. 1958, **30**, 388.
13. Bartter, F. C., D. S. Gann, and J. P. Thomas. On the mechanisms of sodium retention in cardiac and hepatic disease *in* The Human Adrenal Cortex. Edinburgh, E. & S. Livingstone, 1962, p. 294.
14. Thomas, J. P., and F. C. Bartter. Relation between diuretic agents and aldosterone in cardiac and cirrhotic patients with sodium retention. Brit. med. J. 1961, **1**, 1134.
15. Bongiovanni, A. M. The adrenogenital syndrome with deficiency of 3β-hydroxysteroid dehydrogenase. J. clin. Invest. 1962, **41**, 2086.
16. Bongiovanni, A. M., and W. R. Eberlein. Defects in steroidal metabolism of subjects with adrenogenital syndrome. Metabolism 1961, **10**, 917.
17. Ayrers, P. J., A. Eichhorn, O. Hechter, N. Saba, J. F. Tait, and S. A. S. Tait. Some studies on the biosynthesis of aldosterone and other adrenal steroids. Acta endocr. (Kbh.) 1960, **33**, 27.
18. Bartter, F. C., I. H. Mills, E. G. Biglieri, and C. Delea. Studies on the control and physiologic action of aldosterone. Recent Progr. Hormone Res. 1959, **15**, 311.
19. Bongiovanni, A. M. *In vitro* hydroxylation of steroids by whole adrenal homogenates of beef, normal man, and patients with the adrenogenital syndrome. J. clin. Invest. 1958, **37**, 1342.