Aldosterone congenital adrenal hyperplasia 21-hydroxylase 11-ketopregnanetriol pregnanetriol sodium metabolism

# Exchangeable Sodium and Aldosterone Secretion in Children with Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency

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#### Extract

Aldosterone secretion rates (ASR) and exchangeable body sodium (Na<sub>e</sub>) have been measured simultaneously in 25 cases of congenital adrenal hyperplasia (CAH) with and without salt loss, both during normal and during low salt intake. The degree of 21-hydroxylase deficiency was assessed from the cortisol secretion rate (CSR) and urinary excretion of pregnanetriol and 11-ketopregnanetriol.

In cases with salt loss, the sodium equilibrium was unstable, even with optimum treatment and increased dietary salt intake. Clinical evidence of salt-losing crisis appeared when 15-25% of Na<sub>e</sub> was lost. In these subjects, a parallel decrease in ASR and CSR usually occurred. The ASR were somewhat elevated in the nonsalt-losing form.

# **Speculation**

A salt-excreting factor is probably present in CAH, even in the nonsalt-losing form, since the ASR is raised, while the Na<sub>e</sub> remains normal.

Salt loss due to 21-hydroxylase deficiency decreases with age. This appears to be due to modifications in the distribution of body sodium and to an absolute increase in the dietary salt intake rather than to modifications in adrenal secretion.

# Introduction

About one-third of all cases of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency present with a salt-losing syndrome [32]. This may become evident by day 7-day 12 of life in the most severe form of the disorder, may occur at a later age, or, in some cases, may only arise during a situation of stress.

Although it has been shown in the salt-losing form of CAH that the secretion of aldosterone is deficient, there has been less investigation of concomitant changes in body sodium content. The present study was undertaken in the hope of delineating the interrelation between aldosterone production and disordered sodium balance. Sodium levels in plasma and sodium excretion in urine, as well as the values for exchangeable sodium, have been measured in different situations, namely, during normal dietary sodium intake, and during the administration of a low salt regime.

The exchangeable sodium  $(Na_e)$  represents the stock of sodium within the organism that can readily be mobilized to maintain electrolytic homeostasis. In the adult, about 30% of the total body sodium is fixed within the integral structure of bone and is not available for this purpose. In the child, however, almost all the body sodium is exchangeable [17]. The Na<sub>e</sub> value in the adult remains fairly constant at about 40 mEq/kg body weight [2]. The value is higher in the child, particularly in the young child. Values obtained for Na<sub>e</sub> in healthy newborn infants are of the order of 80–90 mEq/kg body weight, but by 6 months of age a rapid fall has occurred and the values range from 55 to 65 mEq/kg. Thereafter, the Na<sub>e</sub> values in healthy children show a much more gradual fall until they reach adult levels, at about age 12 or 13 [11, 12, 18, 26] (table I).

 Table I. Values for exchangeable sodium in normal subjects related to age1

Age	Na <sub>e</sub> , mEq/kg	
Newborns	80–90	
6 months	55–65	
2–5 yr	50-60	
5–10 yr	45-55	
12 yr and more	= 40	

<sup>1</sup> From references 11, 12, 18, 26.

Ideally, one would wish to measure the  $Na_e$  in milliequivalents per kilogram of lean body mass since adipose tissue contains very little sodium. This circumstance sometimes makes it difficult to define the normal limits with precision and it is certainly normal for an obese subject to have an  $Na_e$  value per kilogram total body weight that is less than that of lean subjects.

In most of our cases, in addition to measuring aldosterone secretion, we have also measured cortisol secretion, and the excretion of pregnanetriol in the urine. In this way, it has been possible to assess the degree of enzymatic block and to study the correlation between a deficit in cortisol and aldosterone production and a deficit in body sodium.

# Clinical Data and Methodology

Twenty-five children with congenital adrenal hyperplasia (CAH) due to deficiency of 21-hydroxylase were studied. The ages of the subjects varied from 6 days to 14 years, and the clinical features showed all degrees of severity of the defect. In some cases, the salt loss presented as a crisis; in others, the clinical features did not arouse suspicion in the minds of the parents until the children had reached 3–5 years of age.

The clinical data are summarized in table II. In this table, the degree of salt loss has been estimated on clinical criteria only.

Eight children showing salt loss and two children with the benign form of CAH had been studied prior to the institution of any therapy. In the other children, steroid therapy was stopped for from 3 days to 1 month prior to the investigations. The salt-losing cases were maintained in good general health during this period by appropriate supplementation of their diet with salt.

# Sodium

The sodium content of the diet was calculated from standard tables [27] for the older children and, in the case of the infants, the sodium content of the milk was calculated from the values given by the manufacturer. In a number of cases, these values were verified by direct analysis of the dietary intake.

Sodium levels in plasma and urine were measured by flame photometry.

Tritiated aldosterone (2–3  $\mu$ Ci) and 1  $\mu$ Ci/kg of <sup>24</sup>Na were injected simultaneously. Following 24 h of equilibration, during which time the urine was collected for measurement of the secretion rate of aldosterone and the excretion of labeled sodium, the specific activity of the plasma sodium and the value for the exchangeable sodium were measured by the isotope dilution method [18].

The exchangeable sodium was calculated by the formula:

$$Na_e = \frac{{}^{24}Na \text{ injected} - {}^{24}Na \text{ excreted}}{\text{specific activity of plasma Na}}$$

where: specific activity =

<sup>24</sup>Na (in μCi) per 100 ml plasma <sup>23</sup>Na (in mEq) per 100 ml plasma

<sup>23</sup>Na being naturally occurring sodium.

#### Aldosterone

The secretion rate of aldosterone was measured by the double isotope derivative assay [24] modified as follows:

a) Aldosterone, liberated by hydrolysis at pH 1, was chromatographed in the E2B system (isooctane-tertiary butanol-water, 100:50:90) for 20-22 h. If the extract remained pigmented, it was rechromatographed in the Bush 5 system (benzene-methanol-water, 100:50:50) for 7 h.

b) Following acetylation, the diacetate was chromatographed twice in the system cyclohexane-benzenemethanol-water, 4:4:4:1, and once in the system cyclohexane-dioxan-methanol-water, 4:4:2:1. The initial chromatography was repeated to secure the removal of excess <sup>14</sup>C.

c) The oxidation product was chromatographed twice, once in each of the two systems mentioned above.

The purity of the labeled aldosterone that was injected was checked by means of the E2B system.

WELDON et al. [31] found the ASR to be  $72\pm29.5$   $\mu$ g/24 h for 22 normal subjects whose ages ranged from 8 days to 1 year, the salt intake being normal. In 17

Case nos.	Age	Sex	Pseudoherma- phrodism	Clinical salt loss <sup>1</sup>	Observations
			Prader		
1, CS	31/ <sub>12</sub> yr	F	Type III	0	Two affected siblings
2, JC	54/ <sub>12</sub> yr	Μ		0	0
3, SM	$10^{3}/_{12}$ yr	F	Type IV	0	'Salt-craving' quoted by family
4, JMC	$10^{2}/_{12}$ yr	ĥ	1)po1,	Ő	Brother of MC, case no.6
5, PD	$10^{12}$ /12 /12 12 <sup>2</sup> / <sub>12</sub> yr	F	Type II	ů	One affected sibling
6, MC	$14^{4}/_{12}$ yr	F	Type II	Õ	Sister of $\mathcal{F}MC$ , case no.4
7, DC	6 days	F	Type II	++	First signs of salt loss when 10 days old;
	e aujo			1 1	two affected siblings
8, NL	8 days	F	Type III	++	First signs of salt loss when 15 days old; Sister of PL, case no. 12
9, HC	10 days	F	Type IV	++	First signs of salt loss when 8 days old
10, OM	11 days	F M	Type III	++	First signs when 10 days old; crisis at 1 month
11, SD	18 days	IVL		++	Breast fed for 8 days; first signs when
11, 517	10 days			<u>+</u> +	8 days old; crisis at 15 days
12, PL	6 wk	$\mathbf{M}$		++	Breast fed for 15 days; first signs when
					15 days old; crisis at 1 month and
					$3^{6}/_{12}$ during episode of otitis media;
					Doca stopped at $2^{3}/_{12}$
13, MD	9 wk	F	Type III	+	Doca stopped at 7 months
14, SF	4 months	Μ	<i>,</i> ,	++	First signs when 10 days old; crisis at
					3 months during an infectious episode
15, PC	4 months	F	Type III	++	Breast fed for 2 months; first signs of
			71		salt loss very early; Doca stopped at $2^{3}/_{1}$
16, RB	11 months	м		++	First signs when 12 days old; crisis at
					20 days during episode of otitis media
17, DJ	$3^{1}/_{12}$ yr	$\mathbf{F}$	Type III	++	Crisis at 1 and 2 months; crisis at $3^{6}/_{12}$
<i>,</i> 0	112 7		- / 1		during a low salt diet trial; Doca
					stopped at $4^2/_{12}$
18, CF	$4^{6}/_{12} { m yr}$	F	Type IV	++	First signs when 8 days old; crisis at
	/12 /		-71		2 months, 3 months, $2^{6}/_{12}$ during
					infectious episodes; Doca stopped at $4^{6}/_{1}$
19, PP	5 <sup>1</sup> / <sub>12</sub> yr	М		++	Crisis at $2^{4}/_{12}$ during infectious episode;
	112 7			• •	Doca stopped at 4
20, JI	5 <sup>7</sup> / <sub>12</sub> yr	м		++	Crisis at 1 month, 9 months, $2^{10}/_{12}$ , and
	1127-				$3^{9}/_{12}$ without known precipitating factor
					one affected sibling
21, AA	611/12 yr	F	Type V	++	Doca stopped at $3^{9}/_{12}$ ; crisis at $4^{10}/_{12}$
,	- /12 /-	-	-)P		without known precipitating factor
22, MC	7 <sup>9</sup> / <sub>12</sub> yr	F	Type IV	+	Doca stopped at $6^{10}/_{12}$
23, AG	$\frac{8^{2}}{12}$ yr	F	Type IV	+	One affected sibling; first signs of salt
,	- /12 /*	-	-/ Po	I	loss when 1 month old; Doca and salt
					stopped at 4
24, BB	9 yr	м		++	First signs at 1 month; crisis at 3 and
	~ j.	172		1 1	6 months; Doca stopped at 7;
					crisis during a low salt diet trial
25, GR	10 yr	М		+ +	First signs when 15 days old;
	10 J1	144		1	crisis during low salt diet

<sup>1</sup> 0: no clinical evidence of salt loss; +: slight salt loss as witnessed by relatively late presentation of the salt loss, absence of recurrence of salt loss during treatment on the occasion of illness or other stress, a low requirement of deoxycorticosterone acetate (Doca) and of salt supplementation, and tolerance of a low salt regime, either for a few days, or for longer periods with advancing age; ++: tendency to severe salt loss.

normal subjects, in the age range of 1–15 years, the value was  $91\pm30.4 \ \mu g/24$  h. On a low sodium diet, the mean percentage increase of ASR was 319% in infants and children. Some of the results contributing to these values were obtained in this laboratory.

# Cortisol

Cortisol secretion rates were determined as follows: Two-to-four microcuries of 1,2 3H cortisol were injected and estimates were made on a one-fifth portion of the 24-h urine output from which free steroids had been removed by a chloroform extraction. Glucosiduronate conjugates were hydrolyzed by means of  $\beta$ glucuronidase (Ketodase), and the resulting free steroids extracted with chloroform. After washing with 0.1 N NaOH, the extracts were purified by Florisil column chromatography with collection of the 25% methanol fraction in chloroform. The fraction was chromatographed on paper in the Bush system (benzene-methanol-water, 100:55:45) for 20 h and in the E2B system for 8 h. The radioactivity was measured in the three major metabolites THF, allo THF, and THE [33] and quantitative estimation of these compounds was achieved by the PORTER-SILBER [19] technique.

In some cases of CAH, THE remained contaminated even at this stage and gave a specific activity which was less than that found with the other two metabolites. In such cases, the secretion rate was calculated from the specific activities of THF and allo THF only.

The mean normal value obtained in this laboratory is  $11\pm 3 \text{ mg/m}^2/24$  h for all ages, save the neonatal period [5]. Under ACTH stimulation (25 mg perfused over 8 h) the values rise to  $30-40 \text{ mg/m}^2/24$  h.

In all cases, when secretion rates were being measured, the total radioactivity in the urine was estimated to verify that there had not been an appreciable loss of urine.

# Pregnanetriol and 11-Ketopregnanetriol

Pregnanetriol and 11-ketopregnanetriol were measured by gas-liquid chromatography (GLC).

Following hydrolysis with  $\beta$ -glucuronidase, steroids were extracted with toluene and separated by paper chromatography with the system hexane-benzenemethanol-water, 70:30:80:20 for 12 h. Pregnanetriol and 11-ketopregnanetriol were located from reference standards run in parallel and were eluted. The results obtained following GLC were not corrected for losses.

Fig. 3. Aldosterone secretion rates  $(\mu g/24 h)$  in normal children and in cases of CAH aged 2–14 years, on normal  $\blacksquare$  and low salt  $\square$  regimes. Shaded zone, ASR (mean±sD) for normal subjects aged 1–15 years on a normal salt intake (31).

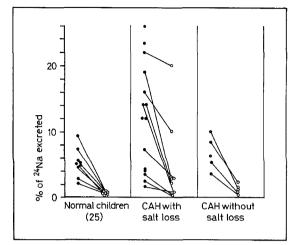


Fig. 1. Percentage of <sup>24</sup>Na excreted in the 24-h urine during normal  $\bullet$  and low salt  $\circ$  regimes.

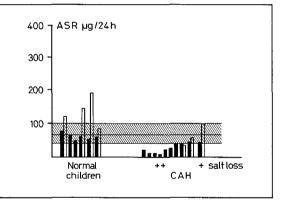
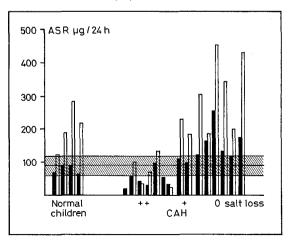


Fig. 2. Aldosterone secretion rates  $(\mu g/24 \text{ h})$  in normal children and in cases of CAH less than 1 year old, on normal  $\blacksquare$  and low salt  $\square$  regimes. Shaded zone, ASR (mean $\pm$ sD) for normal infants aged 8 days-1 year on a normal salt intake (30).



The 17-ketosteroids and the total ketogenic corticoids were measured by the technique of APPLEBY *et al.* [1] and FEW [15].

#### Results

Results are given in table III. Abnormalities of sodium balance in CAH were demonstrated by measurement of the percentage of labeled sodium excreted in the urine of patients (24-h period) (fig. 1). Normal subjects excreted 3–7% sodium on normal salt intake and less than 1% when on a low salt intake [26]. Patients with CAH who had no salt loss responded to a low salt diet by a reduction in their sodium excretion and by a reduction in the percentage of <sup>24</sup>Na excreted in the 24-h urines. Salt losers showed a variable excretion of <sup>24</sup>Na per 24 h, from 1.8 to 25%. In the majority of these subjects, this value does not decrease during administration of a low sodium diet.

In the salt losers, the sodium levels in plasma were frequently low, even when salt supplements were given (case nos. 10, 11, 15, and 21). Low plasma sodium levels also may be found in the simple form of the disorder, on a low salt regime or on a normal salt intake (case nos. 13, 21, and 22), and lowered plasma sodium may even be found in cases who do not show clinical evidence of salt loss (case nos. 3 and 5).

#### Exchangeable Sodium

Patients without salt loss had a Na<sub>e</sub> value that was normal under conditions of normal sodium intake, with little change during administration of a low salt diet. In normal subjects, a reduction of 6-8% is observed on a low salt regime [26]. These children with CAH on a low salt regime, however, although they do not show salt loss frequently show a loss of weight (case nos. 3, 4, and 6) and a reduction in their plasma sodium levels (case nos. 3, 5, and 6).

In all salt losers under 1 year of age, the Na<sub>e</sub> value was below normal whether on normal sodium intake or receiving sodium supplements. A low Na<sub>e</sub> was also found in two older salt losers (*case nos. 20* and 24). In the other salt losers, the Na<sub>e</sub> values were normal or low normal, but these values were likely to have been influenced because they received salt supplements. Typical salt losers cannot conserve sodium during salt restriction; the exceptions were patients with a mild form of the disorder (*case nos. 13* and 22). A clinically evident crisis state of salt losing corresponds to a reduction in the pool of exchangeable sodium of 15–20%.

# Aldosterone Secretion Rates (ASR)

The ASR in salt-losing infants (under 1 year of age) were all low, ranging from 9 to 44  $\mu$ g/24 h. Only one infant (case no. 13) was able to support a significant

reduction in sodium intake; clinically she had a mild form, and was able to double her ASR under a low Na diet. In two infants, reduction of the salt supplements did not give rise to any significant increase in the ASR and this regimen was poorly tolerated (case nos. 15 and 16). The ASR values before and after the salt reduction were 38 and 36  $\mu$ g/24 h in one case and 44 and 56  $\mu$ g/24 h in the other.

In some of the older salt losers (case nos. 18 and 21), the base-line ASR values (44 and 106  $\mu$ g/24 h) were normal; however, during salt restriction, their ASR values were frankly subnormal. In other children, the base-line ASR values were low and there was little or no response to the low salt diet (case nos. 17, 19, 20, 24, and 25).

Children with CAH who were not salt losers showed ASR values that were normal or above normal (124–258  $\mu$ g/24 h) and invariably responded to a low salt regime (188–455  $\mu$ g/24 h) (figs. 2 and 3).

# Pregnanetriol and 11-Ketopregnanetriol

The level of pregnanetriol excreted in the urine was raised in all cases and was further increased following ACTH administration. In the youngest subjects, 11-ketopregnanetriol was excreted in greater quantity than pregnanetriol itself. This was noted in only one of the older subjects (case no. 25) who remained a severe salt loser.

17-Ketosteroids and total ketogenic corticoids were excreted in considerable amounts by all subjects. In two salt losers in whom treatment had been stopped for only 3 and 5 days, respectively (case nos. 23 and 24) at the time of the first estimations, the values were already extremely high and near their maximum. On clinical grounds, however, case no. 23 did not have a severe defect. Almost all the investigations were carried out when the adrenal glands of the patients had returned to what we assumed was their full degree of activity, although, due to the passage of time, this might not necessarily be the degree of activity prior to treatment.

#### Cortisol Secretion Rates (CSR)

Children with the salt-losing form of CAH showed cortisol secretion rates that were widely scattered, with values ranging from immeasurably low to subnormal. The response to ACTH was equally variable, being none (case nos. 12, 16, and 20), inadequate (case no. 23), or even paradoxical with a lowered CSR following stimulation (case nos. 24 and 25). The correlation between a low cortisol secretion rate and a high degree of salt loss is fairly good.

Cases of CAH without salt loss showed CSR values that were low or normal. They showed a response to ACTH with an increase in cortisol secretion, but this response was invariably less than normal. Loras, HAOUR and BERTRAND

17-Ketogenic corticoid, mg/24 h	38.9 84.2 93.4	38.1 87.7 45.0	129.0 161.0 356.0 126.0 7.8	76.6 85.9 109.0 82.6 2.6	45.3 43.5 133.2	45.5 83.6 64.5 1.2	7.8 14.1
17-Ketoste- roid, mg/24 h	13.8 24.6 32.6	12.0 19.4 14.2	27.5 1 34.4 1 74.5 3 34.9 1 5.5	13.7 13.5 14.7 14.0 14.0 2.5	12.1 20.0 21.7 1	10.2 25.1 11.2 5.7	2.6 3.9
11-Ketooxopregnane- triol, mg/24h		8.2 29.1 8.0	15.8 14.5 68.5 12.0	15.7 4.8 20.6 16.0	11.0	5.9 24.7 	1.7
Pregnane- triol, mg/24h		12.7 31.3 2 11.3	48.0 1 51.8 1 90.9 6 54.1 1	46.8 1 39.3 39.3 1 46.6 2 - 1 0.6 -	23.9 1	21.5 41.6 2 	1.4
	5.8 25.3	10.5 3	8.2 5.5 5.2 5.7	8.6 46 39 12.2 46 (	6.6 2: 15.2	10.0 2	5.25
$\mathfrak{mg}/\mathfrak{m}^{24}h$		8.3 10	61				1.0
rate, µg/24 h	3.4 3.4 14.9		10.7 3 33.5 5 33.5	1	T.	) 11.7	
Aldosterone	124	163 188	258 455 409	133 348	i 115	176 436 66	21
mEq/kg diug softwange softwang softwange softwange softwange softwange softw	1 48.7 7 47.9 7 47.1		5 38.3 4 35.3	1,334 50.8	3 38.5 5 36.4	1,553 41.7 1,503 42.0	
	651 637 627		1,595 1,394	1,33	1,498 1,345	1,553 1,503	
Plasma sodium, mEq/liter	135 139 137	136 144 139	140 140 132	138	130 125	140 136	135
F E s% 'vNFS	10.0 1.5 1.3		5.5 0.9	6.3	8.5 2.3	3.7 0.5	
mEq/24 h S U rinary	. 6 <u>1</u> 9 8	80 38 37	110 12	103	133 16	76 11 121	
Sodium intake, mEq/24 h	25-30 25-30 25-30 10 10	50 50 6-10	Normal Normal 6–10 6–10	Normal Normal Normal 6–10 Normal	Normal Normal 35 35	Normal Normal 6–10 Normal	10–12 60
Тћегару	Never treated ACTH ACTH	Never treated ACTH	Stopped for 10 days <sup>3</sup> ACTH Treated for 7 days	Stopped for 20 days <sup>3</sup> ACTH Treated for 6 days	Stopped for 20 days <sup>3</sup> ACTH	Stopped for 20 days <sup>3</sup> ACTH Treated for 15 days	Never treated
Wt, kg	13.3 14.1 13.3 13.3 13.3 13.8	20.5 20.2 20.1	41.7 41.7 41.7 39.5 38.9	26.5 26.7 24.3	38.9 38.4 37.0	37.2 37.2 35.8 37.0	3.13 3.14
Age and degree of clinical salt loss <sup>1</sup>	$\frac{31}{12}$	$5^{4}/_{12}$	10 <sup>3</sup> / <sub>12</sub> 0	10 <sup>2</sup> / <sub>12</sub> 0	$12^{2}/_{12}$ 0	144/12 0	6 days ++
Case nos.	1	~	ŝ	4	S	9	~

Table III. Congenital adrenal hyperplasia: biological data

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Treated for 15 days         100         46         13.5         140         303         71.0         0.2         4.7         1           as         3.37         Never treated         15-20         25         12         132         220         65.0         11         0.2         4.7         1           as         3.37         Never treated         17         1         4.0         134         210         60.0         9         1.4          1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0.2         4.7         1         0         4.8         17.2         1.41         1	mth         Treated for 15 days         00         45         135         140         303         110         022         473         1           pys         3337         Never trasted         15-20         11         02         4.73         1           pys         337         Never trasted         15-20         25         12         132         206         50         11         02         4.73         1           pys         356         Never trasted         157         140         140         141         10         141         02         4.73         11           post         358         Never trasted         157         11         4.0         144         126         166         4.9         141	8 days	3.2	Never treated	10-12	6	4.4	132	171 53.4	11	76	19.9			3.4 8	4α
s       4.3       Treated for 45 days       100       46       13.5       140       303       71.0         a       5.82       Stopped for 15 days       Normal+25       2       12       12       220       65.0       11       0.2       4.7       1         b       5.82       Stopped for 15 days       Normal+25       3       1       4.0       134       210       60.0       9       1.4       -4.7       1.4       -4.7       4.8       4.9       4.9       1.0       4.8       1.1       0.2       5.0       4.9       0.2       5.0       4.9       0.2	s       4.3       Treated for 45 days       100       46       13.5       140       303<71.0	onth		Treated					·			2.4	0.2	0.35	5	> .
3.37 Never treated       15-20       25       12       22       65.0       11 $1.4$ $-1.4$ <t< td=""><td>3.37         Never treated         15-20         5.8         Sopped for 15 days         Normal+-25         2         12         2         0.01         1         <math>0.2</math> <math>4.7</math>         1           hs         480         Treated         17         1         4.0         134         210         60.0         9         <math>0.2</math> <math>4.7</math> <math>0.2</math> <math>4.1</math> <math>0.7</math> <math>4.1</math>         &lt;</td><td>onths</td><td>4.3</td><td></td><td>100</td><td>46</td><td>13.5</td><td>140</td><td></td><td></td><td></td><td></td><td></td><td></td><td>1.2</td><td>3</td></t<>	3.37         Never treated         15-20         5.8         Sopped for 15 days         Normal+-25         2         12         2         0.01         1 $0.2$ $4.7$ 1           hs         480         Treated         17         1         4.0         134         210         60.0         9 $0.2$ $4.7$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.2$ $4.1$ $0.7$ $4.1$ <	onths	4.3		100	46	13.5	140							1.2	3
3.56       Never treated       1       4.0       134       210       60.0       9       1.0       4.8 $\Lambda$ CTH       6       Normal+34       95       24.5       133       331       78.6       10.9       1.0       4.8         10.0       Treated       Normal+34       95       24.5       130       121       503       49.0       16       16.6       4.9         3.3       Never treated       65-70       130       126       53       33.7       4.1         3.3       Never treated       65-70       130       126       0.6       2.4       3.7       4.1         3.35       Never treated       13       9       3.6       134       206       5.3       3.7       4.1         3.35       Never treated       13       9       3.6       134       206       2.3       3.7       4.1       1.9       2.3       3.7       4.1       1.9       3.7       4.1       1.9       2.3       3.7       4.1       1.9       2.3       3.7       4.1       1.9       2.3       3.7       4.1       1.9       2.3       3.6       3.7       4.1       3.7       4.1       1.4 <td>3.56         Never treated         1         4.0         194         210         60.0         9         1.0         4.8           ACTH         6         0.0         1         4.0         14         20         60.0         9         1.0         4.8           10.2         Streated         Normal+34         25         3.0         121         503         49.0         16         66.6         4.9           3.3         Never treated         65-70         130         126         64.9         2.3         2.3         4.1           3.3         ACTH         85         1.3         1.3         2.6         2.3         2.3         2.3         2.3         2.4         1.1         1.9         2.3         2.4         1.1         1.9         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.6         2.3         2.3         2.6         2.3         3.3         3.4         1.3         3.3</td> <td>lays onths</td> <td>5.93 20</td> <td><ul><li>7 Never treated</li><li>2 Stopped for 15 days</li></ul></td> <td>15–20 Normal+25</td> <td>25</td> <td>12</td> <td>132</td> <td>220 65.0</td> <td>11 11</td> <td></td> <td></td> <td><math>1.4 \\ 0.2</math></td> <td>4.7</td> <td><math>13.1 \\ 2.9</math></td> <td>- 6</td>	3.56         Never treated         1         4.0         194         210         60.0         9         1.0         4.8           ACTH         6         0.0         1         4.0         14         20         60.0         9         1.0         4.8           10.2         Streated         Normal+34         25         3.0         121         503         49.0         16         66.6         4.9           3.3         Never treated         65-70         130         126         64.9         2.3         2.3         4.1           3.3         ACTH         85         1.3         1.3         2.6         2.3         2.3         2.3         2.3         2.4         1.1         1.9         2.3         2.4         1.1         1.9         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.6         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.3         2.6         2.3         2.3         2.6         2.3         3.3         3.4         1.3         3.3	lays onths	5.93 20	<ul><li>7 Never treated</li><li>2 Stopped for 15 days</li></ul>	15–20 Normal+25	25	12	132	220 65.0	11 11			$1.4 \\ 0.2$	4.7	$13.1 \\ 2.9$	- 6
3.56 Never treated       17       1       4.0       134       210       0.00       Treated       10       4.8         10.0 Treated       Normal+34       25       3.0       121       503       90.0       16       6.6       4.9         10.1 Stopped for 15 days       Normal+34       25       3.0       121       503       90.0       16       16       6.6       4.9         3.3 Never treated       65-70       130       126       130       130       19       2.3       2.4         3.3 Nover treated       65-70       130       126       0.7       2.4       3.7       4.1         3.3 ACTH       65-70       130       126       0.6       2.4       1.4       1.9         3.3 ACTH       65-70       133       9       3.6       132       290       5.1       1.1       1.9         3.3 Never treated       13       9       3.6       132       290       5.1       1.1 </td <td>3.56 Never treated       17       1       <math>4.0</math> <math>17eated</math> <math>17</math>       1       <math>4.0</math> <math>17eated</math> <math>100</math> <math>1100</math> <math>4.8</math> <math>1100</math> <math>4.9</math> <math>125</math> <math>3.3</math> <math>100</math> <math>17eated</math> <math>100</math> <math>1200</math> <math>120</math> <math>120</math> <math>120</math> <math>120</math> <math>120</math> <math>1102</math> <math>141</math> <math>120</math> <math>123</math> <math>4.9</math> <math>111</math> <math>110</math> <td< td=""><td></td><td></td><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>,</td></td<></td>	3.56 Never treated       17       1 $4.0$ $17eated$ $17$ 1 $4.0$ $17eated$ $100$ $1100$ $4.8$ $1100$ $4.9$ $125$ $3.3$ $100$ $17eated$ $100$ $1200$ $120$ $120$ $120$ $120$ $120$ $1102$ $141$ $120$ $123$ $4.9$ $111$ $110$ <td< td=""><td></td><td></td><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>,</td></td<>				-											,
hs         4.80         Treated         Normal+34         25         3.1         78.6           hs         10.2         Stopped for 15 days         Normal+34         25         3.0         121         503         4.9         16         16.6         4.9           3.3         Never treated         65-70         130         126         2.3         4.9           3.3         Never treated         65-70         130         126         2.3         4.9           3.3         Never treated         65-70         130         126         2.3         4.1           3.5         Never treated         85         134         0         126         2.3         4.1           3.55         Never treated         13         9         3.6         134         26         0.5         2.4         1.9           3.55         Never treated         13         9         3.6         132         2.96         5.3         1.4         1.9           5.4         ACTH         13         9         3.6         3.25         3.8         1.5         6.3         2.2         3.8           5.4         ACTH         13         3.9         0.7 <t< td=""><td>hs         4.80         Treated         Normal+34         25         3.41         301         366         4.9           10.25         Stopped for 15 days<sup>3</sup>         Normal+34         25         3.0         12         503         4.0         16         16.6         4.9           3.3         Never treated         65-70         55-70         130         139         2.3         4.9         2.3         4.9           3.3         ACTH         65-70         134         134         26         0.6         2.4         137         4.1           3.5         Never treated         13         134         24         2.3         3.7         4.1         1.9         2.3         2.4         1.9         2.3         2.4         1.9         2.3         3.7         4.1         1.9         2.3         2.6         0.5         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.5         1.6         1.6         &lt;</td><td>ays</td><td>3.5</td><td>6 Never treated ACTH</td><td>17 68</td><td>-</td><td>4.0</td><td>134</td><td>210 60.0</td><td>6</td><td>1.0</td><td>4.8</td><td></td><td></td><td>4.0 7.8</td><td>0 00</td></t<>	hs         4.80         Treated         Normal+34         25         3.41         301         366         4.9           10.25         Stopped for 15 days <sup>3</sup> Normal+34         25         3.0         12         503         4.0         16         16.6         4.9           3.3         Never treated         65-70         55-70         130         139         2.3         4.9         2.3         4.9           3.3         ACTH         65-70         134         134         26         0.6         2.4         137         4.1           3.5         Never treated         13         134         24         2.3         3.7         4.1         1.9         2.3         2.4         1.9         2.3         2.4         1.9         2.3         3.7         4.1         1.9         2.3         2.6         0.5         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.3         2.6         3.7         4.1         1.9         2.5         1.6         1.6         <	ays	3.5	6 Never treated ACTH	17 68	-	4.0	134	210 60.0	6	1.0	4.8			4.0 7.8	0 00
Ins       10.9       Treated       Normal+34       25       3.0       121       503       49.0       166       4.9         10.2       Stopped for 15 days       Normal+34       130       19       2.3       4.9         3.3       Never treated $65-70$ 126       130       19       2.3       4.9         3.3       Never treated $65-70$ 126       133       2.0       5.4       141         3.3       Never treated $85$ $65-70$ 126       13       2.0       2.1       4.1         3.5       Never treated $85$ $65-70$ 126       13       2.0       5.4       1.1       1.9         5.3       Never treated $13$ 9       3.6       132       2.99       5.4       1.1       1.9         5.4       ACTH $13$ 9       3.6       132       2.99       5.4       3.9       1.5       6.3         5.4       ACTH $13$ 9 $3.5$ 144 $246$ $6.3$ 1.5       6.3       3.8       1.5       6.3       2.2       3.8         5.4 $47$ $68$ <td>Ins         10.9         Treated         Normal+34         25         3.0         121         503         49.0         166         4.9           10.2         Stopped for 15 days         Normal+34         25         3.0         121         503         4.9         2.3         2.4           3.3         Never treated         <math>65-70</math>         130         126         0.6         2.4         2.3         2.6           3.3         Never treated         <math>85</math>         143         2.6         0.6         2.4         1.1           3.3         Never treated         <math>85</math>         134         2.6         0.6         2.4         1.1           5.3         Never treated         <math>85</math>         134         2.9         5.4         1.4         1.9           5.4         ACTH         13         9         3.6         132         2.99         5.4         1.5         6.3           5.4         ACTH         13         9         3.6         1.4         1.9         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         1.5<td>aonths</td><td>4.8</td><td>0 Treated</td><td>Normal + 34</td><td>95</td><td>24.5</td><td>153</td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.1</td><td><del>, -</del></td></td>	Ins         10.9         Treated         Normal+34         25         3.0         121         503         49.0         166         4.9           10.2         Stopped for 15 days         Normal+34         25         3.0         121         503         4.9         2.3         2.4           3.3         Never treated $65-70$ 130         126         0.6         2.4         2.3         2.6           3.3         Never treated $85$ 143         2.6         0.6         2.4         1.1           3.3         Never treated $85$ 134         2.6         0.6         2.4         1.1           5.3         Never treated $85$ 134         2.9         5.4         1.4         1.9           5.4         ACTH         13         9         3.6         132         2.99         5.4         1.5         6.3           5.4         ACTH         13         9         3.6         1.4         1.9         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         1.5 <td>aonths</td> <td>4.8</td> <td>0 Treated</td> <td>Normal + 34</td> <td>95</td> <td>24.5</td> <td>153</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>0.1</td> <td><del>, -</del></td>	aonths	4.8	0 Treated	Normal + 34	95	24.5	153							0.1	<del>, -</del>
		aonths	10.9		Normal + 34	25	3.0	121							Ŀ.	4
3.3       Never treated $65-70$ $130$ $19$ $2.3$ $4.9$ 3.3       ACTH $65-70$ $165-70$ $126$ $2.3$ $2.6$ 3.3       ACTH $65-70$ $126$ $2.3$ $2.6$ $2.3$ $2.16$ 3.5       Never treated $85$ $134$ $26$ $0.5$ $2.4$ $114$ $1.9$ 5.3       Never treated $13$ $9$ $3.6$ $128$ $0.7$ $294$ $54.7$ $1.4$ $1.9$ 5.3       Never treated $13$ $9$ $3.6$ $132$ $296$ $54.7$ $39$ $4.1$ $1.9$ $5.3$ Never treated $13$ $9$ $56$ $52.5$ $190$ $140$ $325$ $56.3$ $15.5$ $1.5$ $1.5$ $6.3$ $5.4$ ACTH $13$ $9$ $56$ $52.5$ $190$ $102$ $126$ $56$ $22$ $33$ $4.1$ $56$ $56$ $56.3$ $15.6$ $15.6$ $15.6$ $15.6$ $35.5$ $440$ <td>3.3       Never treated       <math>65-70</math> <math>130</math> <math>19</math> <math>2.3</math> <math>4.9</math>         3.3       ACTH       <math>65-70</math> <math>126</math> <math>3.7</math> <math>4.1</math>         3.3       ACTH       <math>65-70</math> <math>126</math> <math>3.7</math> <math>4.1</math>         3.55       Never treated       <math>85</math> <math>143</math> <math>26</math> <math>0.6</math> <math>2.4</math>         3.55       Never treated       <math>85</math> <math>134</math> <math>205</math> <math>5.4</math> <math>101</math> <math>126</math>         5.3       Never treated       <math>13</math> <math>9</math> <math>56</math> <math>125</math> <math>101</math> <math>10</math>         5.3       Never treated       <math>13</math> <math>9</math> <math>56</math> <math>128</math> <math>2295</math> <math>54</math> <math>112</math> <math>144</math> <math>112</math> <math>5.4</math>       ACTH       <math>13</math> <math>9</math> <math>56</math> <math>22.5</math> <math>38</math> <math>115</math> <math>115</math> <math>63</math> <math>5.4</math>       ACTH       <math>13</math> <math>9</math> <math>51</math> <math>201</math> <math>2285</math> <math>38</math> <math>115</math> <math>115</math> <math>115</math> <math>115</math> <math>63</math> <math>115</math> <math>63</math> <math>115</math> <math>63</math> <math>115</math> <math>115</math> <math>63</math> <math>115</math> <math>115</math> <math>115</math> <math>115</math> <math>115</math> <math>115</math></td> <td></td> <td>10.2</td> <td></td> <td>Normal + 34</td> <td></td> <td></td> <td></td> <td></td> <td>16</td> <td></td> <td></td> <td>16.6</td> <td>4.9</td> <td><u>с</u></td> <td>2</td>	3.3       Never treated $65-70$ $130$ $19$ $2.3$ $4.9$ 3.3       ACTH $65-70$ $126$ $3.7$ $4.1$ 3.3       ACTH $65-70$ $126$ $3.7$ $4.1$ 3.55       Never treated $85$ $143$ $26$ $0.6$ $2.4$ 3.55       Never treated $85$ $134$ $205$ $5.4$ $101$ $126$ 5.3       Never treated $13$ $9$ $56$ $125$ $101$ $10$ 5.3       Never treated $13$ $9$ $56$ $128$ $2295$ $54$ $112$ $144$ $112$ $5.4$ ACTH $13$ $9$ $56$ $22.5$ $38$ $115$ $115$ $63$ $5.4$ ACTH $13$ $9$ $51$ $201$ $2285$ $38$ $115$ $115$ $115$ $115$ $63$ $115$ $63$ $115$ $63$ $115$ $115$ $63$ $115$ $115$ $115$ $115$ $115$ $115$		10.2		Normal + 34					16			16.6	4.9	<u>с</u>	2
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	lays	3.3		65-70			130		19			2.3	4.9	8.0	0
3.3       ACTH $65-70$ 126       3.7       4.1         3.55       Never treated       85       143       26       0.6       2.4         ACTH       85       134       26       0.6       2.4       1.9         5.3       Never treated       85       134       29       3.6       122       3.8         5.4       ACTH       13       9       3.6       122       294       54.5       96       2.2       3.8         5.4       ACTH       13       9       3.6       123       294       54.5       96       2.2       3.8         5.4       ACTH       13       9       3.6       132       299       54.7       39       1.5       6.3       3.8         3.40       ACTH       85       55       19.0       140       325       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5	3.3       ACTH       65-70       126       37       4.1         3.55       Never treated       85       143       26       0.6       24         ACTH       85       143       26       0.6       24       14       1.9         5.3       Never treated       13       9       3.6       13       294       54.7       14       1.9         5.4       ACTH       13       9       3.6       13       294       54.7       14       1.9         5.4       ACTH       13       9       3.6       132       294       54.8       1.4       1.9         5.4       ACTH       13       9       3.6       23.5       144       244       6.3       3.8       1.5       6.3         3.40       ACTH       13       6.8       200       140       325       6.3       1.5       1.4       1.9         5.5       Treated       85       55       190       140       325       6.3       1.5       1.4       1.5       6.3       3.8         5.5       Treated       85       55       190       140       325       8.1       2.6       2.3 <td></td> <td>3.3</td> <td></td> <td>65-70</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>2.3</td> <td>2.6</td> <td>ω̈́</td> <td>6</td>		3.3		65-70								2.3	2.6	ω̈́	6
3.55 Never treated       85       143       26       0.6       24 $\Lambda CTH$ 85       134       9       3.6       134       not measurable         5.3 Never treated       13       9       3.6       132       290       54.7       39         5.4 $\Lambda CTH$ 13       9       3.6       132       294       54.7       14       1.9         5.4 $\Lambda CTH$ 13       9       3.6       122       299       54.7       39       222       3.8         5.4 $\Lambda CTH$ 13       9       3.6       134       244       5.5       1.6       81       1.5       6.3         3.40 $\Lambda CTH$ 85       55       19.0       140       325       63.9       1.5       6.3       1.5       6.3         3.40 $\Lambda CTH$ 85       55       19.0       140       325       8.8       8.2       2.2       3.8         5.25       Treated       85       55       19.0       120       228       46.6       36       2.0       7.3       1.15       6.3       2.0       7.3       1.5       6.3       5.0       6.1 <td>3.55 Never treated       85       143       26       0.6       2.4         <math>\Lambda GTH</math>       85       134       134       134       134       134       134       134       134       134       134       13       134       13       134       13       134       13       134       134       134       13       134       13       134       13       134       135       148       172       14       13         5.4       <math>\Lambda GTH</math>       13       9       3.5       144       244       53       3.8       1.5       6.3       3.8         5.4       <math>\Lambda GTH</math>       13       0       3.5       144       244       6.5       38       1.5       6.3       3.8         5.25       Treated       85       55       190       140       325       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.4       6.3</td> <td></td> <td>3.3</td> <td></td> <td>65 - 70</td> <td></td> <td></td> <td>126</td> <td></td> <td></td> <td></td> <td></td> <td>3.7</td> <td>4.1</td> <td>ω.</td> <td>2</td>	3.55 Never treated       85       143       26       0.6       2.4 $\Lambda GTH$ 85       134       134       134       134       134       134       134       134       134       134       13       134       13       134       13       134       13       134       134       134       13       134       13       134       13       134       135       148       172       14       13         5.4 $\Lambda GTH$ 13       9       3.5       144       244       53       3.8       1.5       6.3       3.8         5.4 $\Lambda GTH$ 13       0       3.5       144       244       6.5       38       1.5       6.3       3.8         5.25       Treated       85       55       190       140       325       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.4       6.3		3.3		65 - 70			126					3.7	4.1	ω.	2
ACTH         85         134         not measurable           5.3         Never treated         13         134         not measurable           5.4         ACTH         13         9         36         132         294         54.1         39           5.4         ACTH         13         9         3.6         132         294         54.5         96         2.2         3.8           5.4         ACTH         13         9         3.6         132         144         244         6.5         96         2.2         3.8           3.40         ACTH         85         55         19.0         140         325         6.3         1.5         6.3           5.25         Treated         85         55         19.0         140         325         6.3         1.5         6.3           5.25         Treated         85         51         2.00         120         228         46.6         36         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5	ACTH         85         134         not measurable           5.3         Never treated         13         9         36         132         144         1.9           5.4         ACTH         13         9         3.6         132         299         54.7         39           5.4         ACTH         13         9         3.6         132         294         54.7         39           5.4         ACTH         13         9         3.6         132         144         245         36         2.2         3.8           5.3         Teated         85         55         190         140         325         3.8         1.5         6.3         1.5         6.3           8         5.25         Treated         85         55         190         140         325         3.8         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         6.3         1.5         1.5         6.3         1.5         1.5         6.3         1.5         1.5         1.5         1.5         1.5         1.5         1.5         2.0         1.5 <td>eks</td> <td>3.5</td> <td>5 Never treated</td> <td>85</td> <td></td> <td></td> <td>143</td> <td></td> <td>26</td> <td>0.6</td> <td>2.4</td> <td></td> <td></td> <td>ч.</td> <td>~</td>	eks	3.5	5 Never treated	85			143		26	0.6	2.4			ч.	~
5.3 Never treated       13       134       13       9       3.6       132       290       5.4       19       1.6       1.1       1.9         5.4       ACTH       13       9       3.6       132       290       5.4.7       39       5.2       1.4       1.9         5.4       ACTH       13       9       3.6       132       294       5.5       144       244       6.5       3.8       1.5       6.3       3.8         5.25       Treated       85       5.5       19.0       140       325       6.3       1.5       6.3       1.5       6.3       3.8       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       1.5       6.3       2.0       7.3       8       4.7       50       7.0       7.3       8.2       2.0       7.3       8.2       2.0       6.4       1.2       1.4       1.9       1.5       6.3       1.2       3.4       1.2       1.4       1.9       1.5       6.3       1.2       2.2       2.3       3.4       4.7       2.4       2.4       6.3       5.0       7.3       7.3	5.3Never treated13134 $4.5$ 1393.61322.9054.7394.817.21.41.95.4ACTH1393.61322.9454.5962.23.85.4ACTH1393.51442.446.5382.23.85.33.40ACTH855519.014032553.91.68.15.57reated855519.014032553.91.68.15.27reated85505120.012022846.6362.07.35.4.27705120.012022846.6362.07.34.2505120.012022846.6366.13.53.46.4Stopped for 7 days 75689.440764.66.13.53.46.3Doca731.22.384605.06.13.56.3Doca731.23.86015.01.14077.36.3Doca731.23.86015.01.41.01.46.3Doca731.23.86015.01.41.46.3Doca10.010.010.01203144076.66.11.46.3Stopped for 15 days Norma			ACTH	85			134			not m	easurat	ole		<b>ئ</b>	3
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	teks	5.3		13			134			4.8	17.2	1.4	1.9	5	ŝ
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$					13	6	3.6	132		39					5.	-
ACTH         13         ACTH         13         2.2         3.8           anths $3.74$ Stopped for 10 days <sup>3</sup> $85$ $68$ $2.5$ $144$ $2.5$ $34$ $2.15$ $6.3$ $3.40$ ACTH $85$ $55$ $19.0$ $140$ $325$ $63.9$ $1.5$ $6.3$ $3.40$ ACTH $85$ $55$ $19.0$ $140$ $325$ $63.9$ $1.5$ $6.3$ $4.7$ $5.25$ Treated $85$ $55$ $19.0$ $140$ $325$ $63.9$ $8.1$ $4.7$ $5.6$ $51$ $20.0$ $120$ $228$ $46.5$ $8.2$ $2.2$ $4.7$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $6.3$ $2.0$ $7.3$ $4.7$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $6.3$ $2.0$ $7.3$ $6.4$ $50$ $50$ $51$ $20.0$	ACTH         13 $2.2$ 3.8           anths         3.74         Stopped for 10 days <sup>8</sup> 85         68         23.5         144         244         62.5         38         1.5         6.3           atths         5.25         Treated         85         55         19.0         140         325         63.9         1.5         6.3           atths         5.25         Treated         85         50         51         20.0         120         235         6.3           4.7         50         51         20.0         120         228         4.6         36         2.0         7.3           4.7         50         51         20.0         120         228         4.6         36         2.0         7.3           4.7         Normal+72         60         21.0         120         214         37         2.0         6.1           6.3         Doca, ACTH         Normal+72         68         9.4         407         6.7         3.3           6.3         Doca, ACTH         Normal+72         61         10.0         120         2.14         4.07         6.1         2.0         6.1           6.3		5.4		45		0.7			96					÷	4
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			ACTH	13								2.2	3.8	3.	7
3.40       AULIN $33$ $5$ $19.0$ $140$ $325$ $63.9$ $1.0$ $6.1$ arths $4.9$ Never treated $85$ $55$ $51$ $20.0$ $120$ $228$ $56.3.9$ $8.2$ $2.2$ $4.7$ $50$ $51$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $36$ $2.0$ $73$ $4.7$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $36$ $2.0$ $73$ $6.4$ Stopped for $25$ days <sup>3</sup> Normal+ $72$ $61$ $40.7$ $64.6$ $2.0$ $73$ $6.3$ Doca $75$ $68$ $9.4$ $407$ $64.6$ $6.3$ $13.2$ $2.0$ $6.1$ $6.3$ Doca $75$ $68$ $9.4$ $407$ $64.6$ $6.3$ $13.4$ $6.3$ Doca $75$ $61$ $10.0$ $120$ $314$ $9.6$ $6.3$ $13.4$ $6.3$ Doca       Normal+ $54$ $80$ $14.0$ $133$	3.40         ACULA         63         55         19.0         140         325         1.0         6.1           arths $4.2$ Never treated $85$ $56$ $51$ $200$ $126$ $36$ $2.2$ $4.2$ $8.2$ $2.2$ $4.7$ $50$ $51$ $200$ $120$ $228$ $46.6$ $36$ $2.0$ $73$ $4.7$ $70$ $51$ $200$ $120$ $228$ $46.6$ $36$ $2.0$ $73$ $6.4$ $Doca$ $77$ $8.2$ $51$ $200$ $12$ $35$ $2.0$ $73$ $6.3$ $Doca$ $77$ $68$ $9.4$ $407$ $64.6$ $6.3$ $2.0$ $73$ $6.3$ $Doca$ $77$ $80$ $116.0$ $139$ $801$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$ $6.3$	onths	3.7		85 65	68	23.5	144		38	( +	•	1.5	6.3	ς, r	5
Inths $5.25$ Treated $85$ $55$ $19.0$ $140$ $325$ $63.9$ $8.2$ $2.22$ Inths $4.7$ $50$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $36$ $8.2$ $2.2$ $2.0$ $7.3$ $4.7$ $50$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $36$ $2.0$ $7.3$ $4.2$ $70$ $50$ $51$ $20.0$ $120$ $228$ $46.6$ $36$ $2.0$ $7.3$ $6.4$ $Doca, ACTH$ $Normal+72$ $68$ $9.4$ $407$ $64.6$ $36$ $2.0$ $7.3$ $6.3$ $Doca, ACTH$ $Normal+72$ $68$ $9.4$ $407$ $64.6$ $36$ $6.1$ $40$ $6.1$ $407$ $64.6$ $6.3$ $1120$ $324$ $112$ $324$ $112$ $324$ $112$ $326$ $661$ $601$ $50$ $611$ $601$ $601$ $500$ $50$ $611$ $407$ $646$ $654$ $5$	Indust $5.25$ Treated $85$ $55$ $19.0$ $140$ $325$ $63.9$ $8.2$		3.4		65						1.0	8.1			Ċ.	ה
unths       4.9       Never treated       85       60       22.0       136       286       58.4       38       8.2       2.2         4.7       50       50       51       20.0       120       228       46.6       36       2.0       7.3         4.7       6.4       Stopped for 25 days <sup>3</sup> Normal+72       2.0       7.3       2.0       7.3         6.4       Doca, ACTH       Normal+72       2.0       1.2       3.5       2.0       6.1       3.5	anths         4.9         Never treated         85         60         22.0         136         286         58.4         38         8.2         2.0         7.3           4.7         70         70         50         51         20.0         120         228         46.6         36         2.0         7.3           6.4         Stopped for 25 days         Normal+72 $2.0$ $2.0$ $7.3$ $2.0$ $7.3$ 6.4         Doca, ACTH         Normal+72 $6.8$ $9.4$ $407$ $6.1$ $2.0$ $6.1$ $3.5$ 6.3         Doca         75 $68$ $9.4$ $407$ $6.4$ $407$ $6.1$ $40$ $6.1$ $1.2$ $2.0$ $6.3$ $3.5$ 6.3         Doca         Toca stopped for 7 days 75 $61$ $120$ $3.4$ $407$ $6.1$ $407$ $6.1$ $6.3$ $3.4$ $6.3$ $3.6$ $6.3$ $5.6$ $6.3$ $13.4$ $6.3$ Doca         Normal+54 $80$ $14.0$ $6.1$ $10.1$ $10.$	onths	5.2		85	55	19.0	140							-	ŝ
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	onths	4.9	Never tr	85	60	22.0	136		38			8.2	2.2	7	9
4.2       70       2.0       7.3         onths $6.4$ Stopped for 25 days <sup>3</sup> Normal+72       1.2       3.5 $6.4$ Doca, ACTH       Normal+72       2.0       6.1 $6.4$ Doca, ACTH       Normal+72       2.0       6.1 $6.3$ Doca, ACTH       Normal+72       2.0       6.1 $6.3$ Doca       75       68       9.4       407       6.4 $6.3$ Doca stopped for 7 days 75       61       16.0       139       384       61.0       44 $6.3$ Doca stopped for 15 days <sup>3</sup> Normal+54       80       14.0       133       601       56.0       19       10.1       4.0 $10.9$ Stopped for 15 days <sup>3</sup> Normal+54       80       14.0       133       601       56.0       19       10.1       4.0 $10.8$ Normal+54       19.0       146       694       50       60       19.1       4.0 $13.35$ Stopped for 15 days <sup>3</sup> Normal+85       149       19.0       146       694       50       60       14.0       14.0 $12.35$ 12.525	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		4.7		50	51	20.0	120		36					4.5	ŝ
onths $6.4$ Stopped for 25 days <sup>3</sup> Normal+72 $1.2$ $3.5$ $6.4$ Doca, ACTHNormal+72 $2.0$ $6.1$ $2.0$ $6.1$ $6.3$ Doca, ACTHNormal+72 $2.0$ $6.1$ $2.0$ $6.1$ $6.3$ Doca $75$ $68$ $9.4$ $407$ $64.6$ $2.0$ $6.3$ Doca stopped for 7 days 75 $61$ $16.0$ $139$ $384$ $61.0$ $44$ $6.3$ Doca stopped for 15 days <sup>3</sup> Normal+54 $80$ $14.0$ $133$ $601$ $56.0$ $19$ $10.8$ Normal+54 $80$ $14.0$ $133$ $601$ $56.0$ $19$ $10.1$ $4.0$ $13.35$ Stopped for 15 days <sup>3</sup> Normal+85 $149$ $19.0$ $146$ $694$ $52.0$ $60$ $13.35$ Stopped for 15 days <sup>3</sup> Normal+85 $19$ $2.9$ $143$ $637$ $51.6$ $14.2$ $8.4$ $13.6$ Stopped for 15 days <sup>3</sup> Normal+85 $19$ $2.9$ $131$ $712$ $51.0$ $44$ $61.1$ $6.4$			4.2		70						2.0	7.3			4.0	0
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ase nos.	ge and egree of inical salt sa1	۷۴, لاع	herapy	ntake, Diake, Diake,	rEq/24 h so Urinary	A <sup>g°</sup> % <sup>°</sup> N	lasma, mEq/liter lasma	בהת Exchange able sodium בית Exchange		ate, µg/24 h	ıg/m²/24 h ng/24 h ng/24 h		regnane- iol, mg/24 h	l-Ketooxopregnane- iol, mg/24 h	124 h 7-Ketosteroid,	7-Ketogenic prticoid, mg/24 h
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<sup>1</sup> Sec <sup>2</sup> Per <sup>3</sup> Let	centage of <sup>24</sup> Na	lote 1 for a excreted	<sup>1</sup> See table II, footnote 1 for description of clinical salt loss <sup>2</sup> Percentage of <sup>24</sup> Na excreted in the urine in 24 h.	lt loss.	ş											
, Int	erval between (	cessation	<sup>a</sup> Interval between cessation of treatment and the start of the investigation.	urt of the investigation	on.											

Table III. (Continued)

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#### Discussion

# Pregnanetriol and 11-Ketopregnanetriol

The predominance of 11-ketopregnanetriol over pregnanetriol in infants under 1 year of age [14, 15] is probably a manifestation of a more general phenomenon that is also found in the 17-ketosteroids of children with CAH [16, 17].

It was of interest to note that this infantile pattern of adrenal secretion persisted in *case no. 25* and, to a limited extent, in *case no. 20* who showed an increased proportion of 11-ketopregnanetriol during stimulation with ACTH and during salt restriction. It may be significant that these subjects were two of the three children in the series who remained severe salt losers despite the passage of time.

In general, the older patients continued to excrete 11-ketopregnanetriol, but in proportionately smaller amounts. FINKELSTEIN [16] found measurable excretion of 11-ketopregnanetriol in 44 of 50 cases of CAH, and the 6 subjects not excreting this compound probably suffered from the form of CAH due to deficiency of 11  $\beta$ -hydroxylase. 11-Ketopregnanetriol is not excreted by normal subjects in measurable quantities and would appear to arise only during intense stimulation of the adrenals with ACTH. FINKELSTEIN found this compound in the urine of patients suffering from Cushing's syndrome associated with adrenal hyperplasia but not in cases of adrenal tumor.

In CAH, it is possible that  $11 \beta$ -hydroxylase deprived of its normal 21-hydroxylated substrates, acts upon those steroids formed in great quantity, particularly 17-hydroxyprogesterone.

The abnormal excretion of pregnanetriol is reduced rapidly by therapy. Case nos. 4, 21, and 22 show that after only 4–6 days of treatment the urinary levels are low. It is emphasized, however, that in subjects with CAH under 1 year of age, assay of urinary pregnanetriol alone is inadequate because of the importance of the 11-keto form, and could lead to serious underestimation of the quantity of adrenal precursor compounds secreted.

#### Sodium

The four newborns that were studied before clinical evidence indicated salt losing (case nos.7,  $\vartheta$ ,  $\vartheta$ , and 10) showed low sodium levels in plasma, and in the three cases in which it was studied, the Na<sub>e</sub> showed a severe deficit. The negative sodium balance in these infants would, therefore, appear to arise very early and, probably, as suggested by BONGIOVANNI *et al.* [8] dates from the time of birth.

Case no.13 is of interest in that she showed a value for Na<sub>e</sub> that was less than normal during normal salt intake despite a capacity for further aldosterone production, demonstrable on restricting salt. Although the  $Na_e$  was below normal and the plasma sodium was on the low side, the clinical condition at the time was satisfactory and the excretion of sodium in the urine was less than the dietary intake.

In the newborns (case nos. 8, 9, and 10), and in the other subjects during salt restriction (case nos. 15, 16, 21, and 25), clinical signs of salt loss appeared. In all of these cases, the loss of sodium reached or surpassed 15-20% of the Nae value of the child in optimum condition. Salt loss in the urine is indicated by the insufficient reduction in urinary <sup>24</sup>Na. In many cases, the institution of a low salt diet led to increased excretion of total ketogenic corticoid (case nos. 16, 18, 21, 22, and 23), and the increased excretion of pregnanetriol itself was indicated in case no. 22. Thus, a low salt intake, even though it does not lead to a clinically evident saltlosing crisis, may still create a situation of stress for these subjects and provoke an increased secretion of endogenous ACTH. In contrast, in case nos. 20 and 25 in whom the introduction of a low salt regime had led to a frank salt-losing crisis, the steroid excretion was diminished. A probable interpretation of this result is that the adrenals had become exhausted.

In children with the nonsalt-losing form of CAH, in the absence of treatment the Na<sub>e</sub> is seen to be normal for the age and body build, although in *case no.1* the value was rather low. Likewise, the percentage excretion of <sup>24</sup>Na was normal for dietary conditions, but these normal values were associated with aldosterone secretion rates that tended to be higher than those of normal children.

During treatment capable of maintaining the salt losers in good general health the Na<sub>e</sub> values were found to be normal (case nos. 8, 10, and 14) or still low (case no. 10). These findings and the great variation in sodium excretion suggest that sodium equilibrium remains rather unstable even during optimum therapeutic conditions.

# Factors Involved in Salt Loss

Three hypotheses have been put forward to explain the presence or absence of salt loss in CAH due to 21hydroxylase deficiency [8]. Firstly, it has been suggested that there may be two adrenal 21-hydroxylases acting on two types of substrates, steroids with an hydroxyl group at C-17, and precursors of aldosterone. In the simple form of the disorder only the first enzyme would be involved, and in the salt-losing form both would be deficient.

Secondly, some authors hold that the limited capacity for aldosterone secretion is in itself sufficient to explain the salt loss and that the degree of deficiency of a single enzyme is responsible for the varying degrees of severity of the syndrome. Finally, the presence of a factor promoting salt excretion has been suggested, a factor which is probably found among the precursors of cortisol and which is secreted in considerable quantity as a result of the enzymatic block. This factor is believed to act by competitive inhibition with aldosterone at the level of the renal tubule and not as a primary agent prompting sodium excretion. This theory suggests that some affected children compensate for the tendency to salt loss by increasing their secretion of aldosterone and these are the nonsalt losers. The others, in whom the capacity for aldosterone secretion is limited, cannot overcome the effects of the factor and these cases are, clinically, the salt losers.

The investigations described here do not permit us to differentiate between a one- or a two-enzyme defect. The most that can be said is that, in general, the severity of the defect in cortisol secretion corresponds with that in aldosterone secretion. This does not support the idea of two enzymes or of two separate genes being involved.

Among the factors concerned in salt loss there is no doubt that the limited capacity to secrete aldosterone is important. Our observations support those already reported [10, 14, 21, 25, 29].

The most severely affected salt losers have the lowest CSR. This relation is already known [7] but it does not explain the interrelation between sodium and cortisol. These children also have a very low ASR; thus, although the cortisol insufficiency associated with the salt loss could be a causative factor due to lack of the 'permissive' effect of cortisol [8], and although the presence of a salt-excreting factor in the precursors could play a role, the major factor may well be, quite simply, the inadequate ASR which is a constant association.

There are several arguments in support of the idea of a salt-losing factor. 1) Steroid therapy brings a fall in the aldosterone secretion rate (3, 21, 25, and case nos. 3 and 6). 2) A positive relation between the excretion of pregnanetriol in urine and that of aldosterone has been described by HALL and HOKFELT [22] and by MATTOX et al. [28] in both types of CAH. We have found the same relation between ASR and either urinary pregnanetriol or urinary total 17-ketogenic corticoids in nonsalt-losing patients, although the relation has not been defined on a mathematical basis. The existence of this positive relation is strongly in favor of the presence of a salt-losing factor since even if inexact, urinary pregnanetriol is a good index of the quantity of adrenal precursor steroids that have been formed. 3) Cases of CAH without salt loss tend to have elevated ASR values when untreated. The ASR values of our six children can be regarded as on the high side. Above normal values for ASR in nonsalt losers have

also been found in two of three cases by JAILER *et al.* [23], and high values have also been reported by Ko-WARSKI *et al.* [25] and by BARTTER *et al.* [3].

The evidence of an elevated ASR in the absence of steroid therapy suggests that a salt-losing factor is present but is compensated. An explanation is required, however, for the inconstant nature of this phenomenon. In previous studies [3, 21] a few ASR values were within normal limits or only slightly elevated. The five cases of DEGENHART et al. [14] and the four cases of New et al. [29] have ASR values considered normal and one can find neither correlation between these varying values for aldosterone secretion and the 17-ketosteroid values nor correlation between ASR and the other variables as they are presented, namely presence or absence of a treatment period before investigation, sodium intake, chronological age, sex, and age of the child when treatment was first begun. A possible exception would be urinary pregnanetriol values.

GODARD et al. [21] suggested that the capacity to secrete aldosterone is limited, even in the nonsaltlosing subjects. ULICK [30] was of the same opinion in view of his finding of an abnormally high excretion of 11, 18-dihydroxypregnanetriol, and 18 OH-THA [33], suggesting 'that aldosterone deficiency in the saltlosers was a more severe expression of a biosynthetic defect which could occur, in compensated form, in nonsalt-losers as well'. In our cases of nonsalt losers (case nos. 1-6) the ASR response to a low salt regime was certainly not greater than normal (yet the pool of Na<sub>e</sub> was reasonably well maintained). It is, therefore, difficult to understand why some cases show only a normal or subnormal rise in ASR with a low salt intake while others [3] show a very striking rise (to 3,244  $\mu$ g/24 h).

If patients without salt loss can exhibit reasonable satisfactory aldosterone production and  $Na_e$  conservation, however, they can also show evidence of some minor abnormality such as loss of weight during a low salt regime or even a lowering of the sodium level in plasma. The institution of a low salt regime can therefore be regarded as a stress situation in the nonsalt losers, as in the salt losers, but of much less severity. This may be explained by a limitation in the capacity to secrete aldosterone as well as by the concept of a salt-losing factor.

These two concepts are in no way in opposition and it seems reasonable to presume the coexistence of two factors—the salt-excreting factor and the insufficiency of both cortisol and aldosterone—their respective importance being inversely proportional and varying according to the gravity of the deficit. An increasing maturity of renal function with age and the dietary sodium intake must also be taken into consideration.

The syndrome of salt loss will appear when there is failure of equilibrium between the production of salt-

losing factor (illustrated by the ketogenic corticoid levels) and the production of cortisol and aldosterone. The disequilibrium and the salt loss will be worse when the child is very young and the sodium intake is poor.

# The 'Recovery'

The recovery from salt loss, which is known to occur in the majority of cases, can be explained by allied arguments. This 'recovery' usually occurs between 3 and 6 years of age, and in clinical practice means that one may reduce the frequency and later, stop completely, the administration of deoxycorticosterone acetate (Doca) or other mineralocorticoids. Likewise, it is possible to gradually reduce the salt supplementation during the years after mineralocorticoids have been stopped.

The 'recovery' is not perfect. In situations of stress, the needs for Doca and for salt again arise, and, in the absence of stress, a strict low salt intake is poorly tolerated. Our patients (*case nos. 15, 18, 21*, and 22) went into negative sodium balance on a low salt diet and could not conserve Na<sub>e</sub>. Nevertheless, the improvement on the state occurring in the early months and years of life was quite spectacular.

The mechanism of the recovery is not known, although newborns are known to have a most unstable water and sodium control.

An alteration in renal function with age, which permits improved conservation of sodium under the influence of equivalent amounts of aldosterone, is very unlikely. Cases of CAH respond well to aldosterone from a very early age [20].

The possibility that the mechanism concerned with secretion of cortisol and aldosterone is improved with age has also been considered but this conjecture is highly unlikely. We have, in fact, repeated measurements of secretion of aldosterone following a considerable time interval to see if there were significant modifications and the results in two cases show that there were not (case nos.9 and 10).

The one remaining factor that alters with time is the sodium intake and this shows some relation with ASR in normal subjects. Furthermore, the relative as well as the absolute sodium intake increases as the child grows. It would seem, therefore, that Doca can be stopped when the diet contains sufficient salt to balance or to overcompensate for the limited degree of aldosterone production of which the adrenals are capable, and that this dietary sodium is a factor of vital importance in producing the equilibrium.

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- 33. The following abbreviations have been used in this paper: THF: 3 α, 11 β, 17 α, 21-tetrahydroxy-5 β-pregnane-20-one. allo THF: 3 α, 11 β, 17 α, 21-tetrahydroxy-5 α-pregnane-20-one. THE: 3 α, 17 α, 21-trihydroxy-5 β-pregnane-11, 20-dione.
  18 OH-THA: 3 α, 18, 21-trihydroxy-5 β-pregnane-11, 20-dione.
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