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# MULTIPLE OR SINGLE 21-HYDROXYLASES IN CONGENITAL ADRENAL HYPERPLASIA?

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SUMMARY

The objective of this study was to determine whether there is a single 21-hydroxylase in CAH* that is defective or multiple different 21-hydroxylases that are involved. The activity of the 17-OH progesterone 21-hydroxylase in cortisol biosynthesis was estimated by measuring plasma 17-OH progesterone and cortisol levels simultaneously at frequent intervals throughout the day in two patients with non-salt-losing CAH and three patients with salt-losing CAH. Plasma progesterone and corticosterone concentrations were also measured on the same blood samples as an index to progesterone 21-hydroxylase activity in the corticosterone biosynthetic pathway. During periods of maximal adrenal secretory activity it was found that plasma 17-OH progesterone concentrations were markedly elevated in all patients while cortisol levels were either low normal (in non-salt-losers) or low (in salt-losers). At the same time, plasma progesterone and corticosterone concentrations were normal or elevated in both forms of CAH. Although not conclusive, these results favor the multiple enzyme theory in the pathogenesis of CAH.

## INTRODUCTION

Because he contributed so much to our understanding of steroid hormone biosynthesis, it gives us great pleasure to dedicate this report on 21-hydroxylase activity in CAH to Dr. Leo T. Samuels.

Patients with salt-losing CAH are unable to produce normal amounts of both cortisol and aldosterone, but only cortisol production is impaired in non-salt-losing CAH [1, 2, 3]. Because a defect in 21-hydroxylation occurs in both forms of CAH and because 21-hydroxylation is required for both cortisol and aldosterone biosynthesis, these observations have been difficult to explain. Two theories have been proposed: a "single enzyme" theory [4] and a "multiple enzyme" theory [5]. According to the single enzyme theory there is a single 21-hydroxylase system for both cortisol and aldosterone biosynthesis. In non-salt-losers the enzymatic defect is partial and relatively mild, and steroid precursors from cortisol biosynthesis are diverted into the aldosterone pathway in sufficiently high concentrations to overcome the enzymatic defect in aldosterone biosynthesis. The enzymatic defect is thought to be too severe in salt-losers to be overcome by this compensatory mechanism and inadequate aldosterone production results.

In the multiple enzyme theory it is hypothesized that there are two separate and different 21-hydroxylase systems for cortisol and aldosterone biosynthesis (Fig. 1). According to this theory, salt-losers have an inherited defect in both enzymes while non-salt-losers are defective in only one, the 21-hydroxylase in cortisol biosynthesis.

Both the single and multiple enzyme theories provide a reasonable theoretical explanation for the observations obtained to date but there is little solid evidence to support either one. To obtain more information on the single versus multiple 21-hydroxylase theories we have investigated the activity of a third 21-hydroxylase system in CAH, the one found in the zona fasciculata that is involved in corticosterone biosynthesis (Fig. 1).

## EXPERIMENTAL

Plasma concentrations of 17-OH progesterone, cortisol, progesterone, and corticosterone were measured at 20 min intervals for 24 h under normal conditions in two patients with non-salt-losing CAH and one patient with salt-losing CAH. In two other patients with salt-losing CAH plasma steroid measurements were done at 20 min intervals during the nocturnal period of maximal adrenal secretory activity.

All of the patients classified as salt-losers had been admitted to the hospital upon one or more occasions in adrenal crisis. Corticoid therapy was required for these patients to survive. They had received corticoids daily for several months or years prior to the study. One salt-losing patient was switched from cortisone to DOC 1 week prior to the study. In the other two

* In the paper CAH is used as an abbreviation for that form of congenital adrenal hyperplasia (or adrenogenital syndrome) caused by a defect in 21-hydroxylation.

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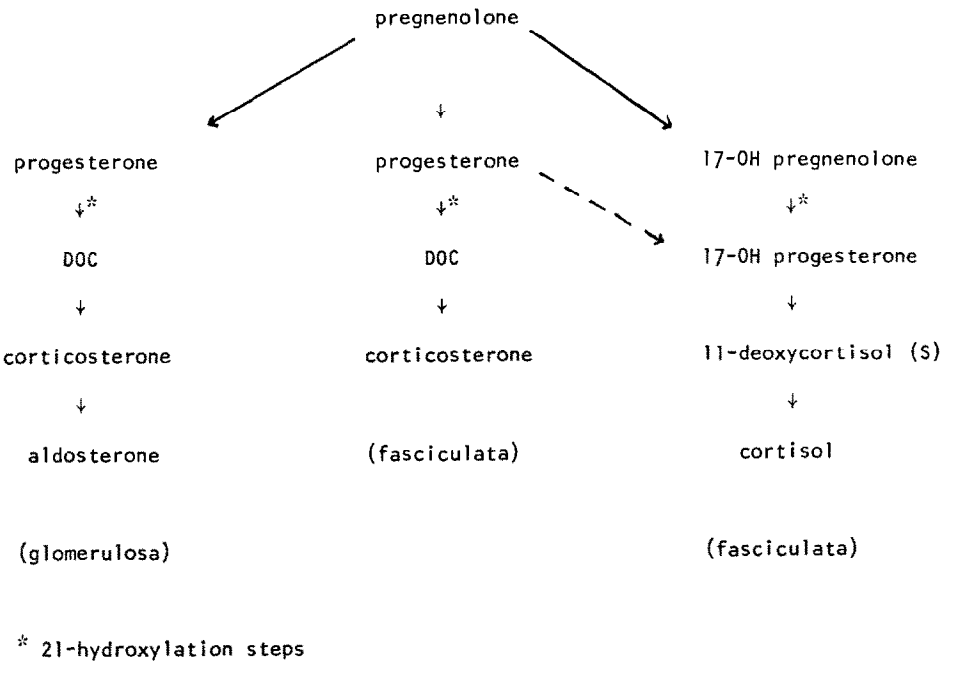


Fig. 1. Steroid biosynthetic pathways in the adrenal cortex that require 21-hydroxylation.

salt-losers the usual schedule for glucocorticoid therapy was not interrupted in order not to jeopardize the patients' health. The studies were done between doses of glucocorticoid from 0200 to 1000 h.

Neither of the two non-salt-losing patients required corticoid therapy. One patient had never been treated and the other had received steroids briefly several years prior to the study.

The normal ranges for steroids were obtained on 10 normal preovulatory menstruating women with assays done at intervals of 15 min over the time periods indicated.

*Laboratory methods*

All four steroids were measured in the same plasma sample by the method previously reported from this laboratory [6]. In this method individual steroids are separated by paper chromatography and measured quantitatively by radioimmunoassay. With the chromatographic purification and the specificity of the antisera there is no significant interference from other known plasma steroids. The coefficients of variation for the interassay precision in measuring these four plasma steroids ranged from 5 to 13% at the levels encountered in this study.

## RESULTS

In all of the figures plasma steroid concentrations are plotted against clock time to show the diurnal variation. The results obtained in the two non-salt-losing CAH patients are shown in Figs 2 and 3; and those for the three salt-losers, in Figs 4, 5 and 6. A comparison of the results shown in the figures reveals the following abnormalities, similarities and differences in plasma steroid concentrations between salt-losing and non-salt-losing CAH patients:

1. *Plasma 17-OH progesterone concentrations.* The most striking abnormality observed in all patients with CAH was the very high level of 17-OH progesterone in blood (Figs 2–6). During the nocturnal period of maximal adrenal secretory activity (from approx. 0400 to 0800 h) plasma 17-OH progesterone levels reached maximum values that were several hundred times normal. Plasma 17-OH progesterone levels were usually modestly elevated during the rest of the day and only rarely descended into the normal range.

With one exception, the patients with salt-losing CAH had higher plasma 17-OH progesterone levels than did the non-salt-losers (Fig. 6.).

2. *Plasma cortisol concentrations.* Plasma cortisol concentrations were consistently lower in the salt-losers (Figs 4–6) than in the non-salt-losers (Figs 2 and 3). The greatest differences in plasma cortisol between the two groups occurred during the nocturnal secretory period at which time the non-salt-losers had plasma cortisol values in the low normal range while the salt-losers consistently had lower than normal levels. Neither form of CAH had the usual marked increase in cortisol secretory activity during the nocturnal secretory period. The salt-losers had very low plasma cortisol levels all day that failed to increase significantly during the nocturnal period

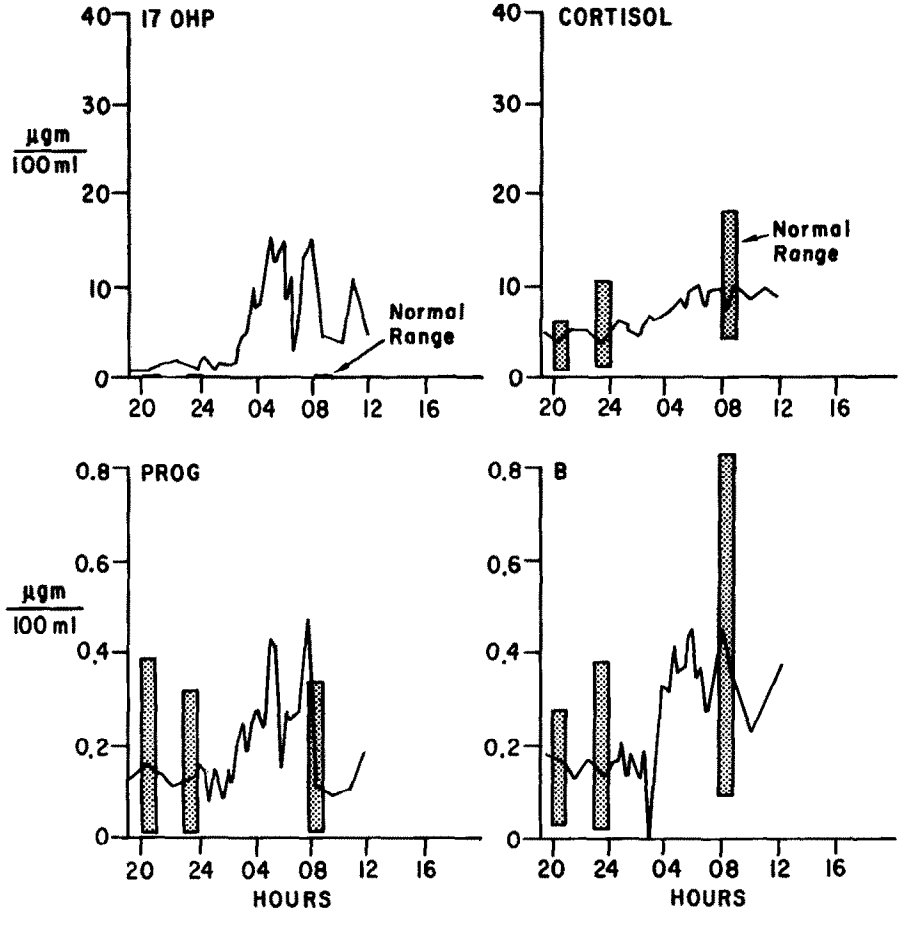


Fig. 2. Diurnal variation in plasma 17-OH progesterone, cortisol, progesterone, and corticosterone concentrations in non-salt-losing CAH. Patient F.P. was a 28-year-old untreated genetic female.

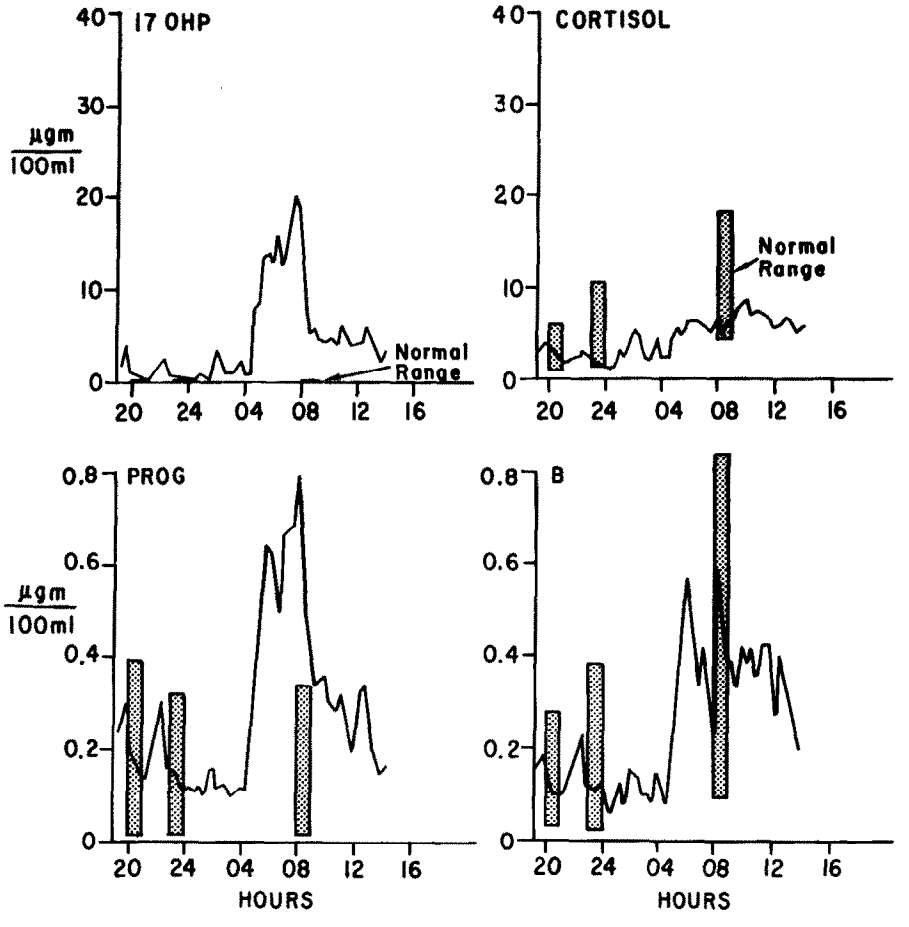


Fig. 3. Diurnal variation in plasma 17-OH progesterone, cortisol, progesterone, and corticosterone concentrations in non-salt-losing CAH. Patient K.P. was a 16-year-old untreated genetic male.

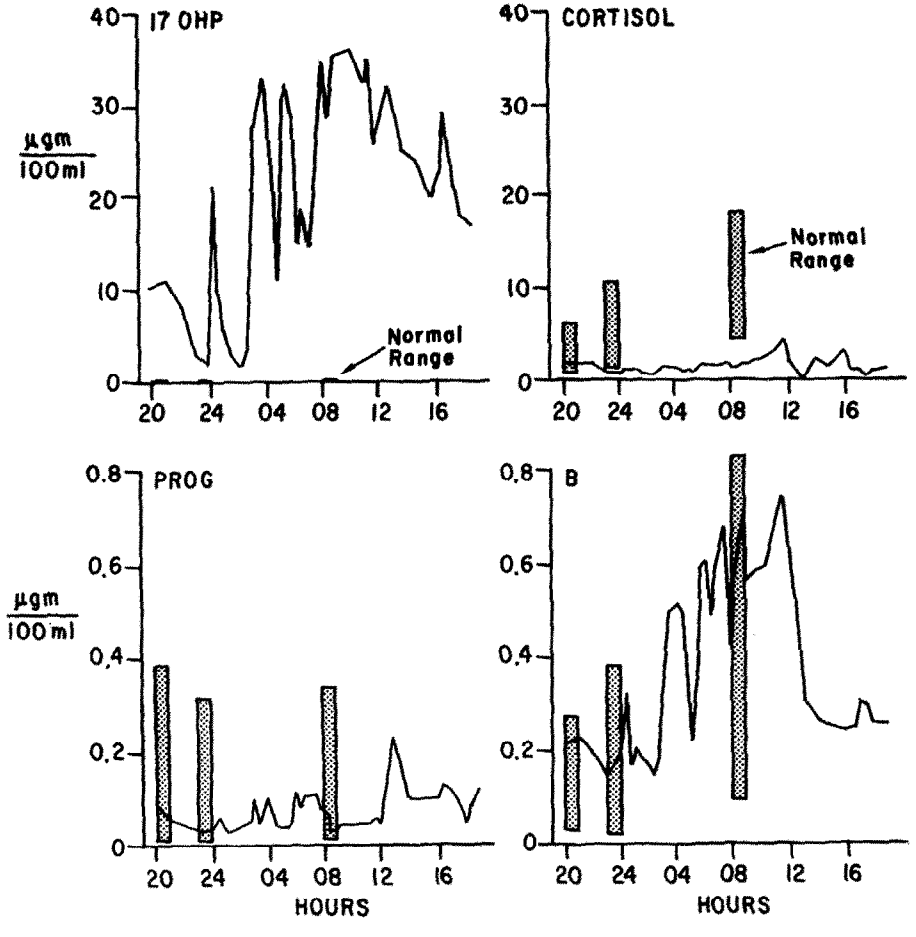


Fig. 4. Diurnal variation in plasma 17-OH progesterone, cortisol, progesterone, and corticosterone concentrations in salt-losing CAH. Patient D.D. was a 13-year-old genetic male who was treated with DOC for 1 week prior to the study.

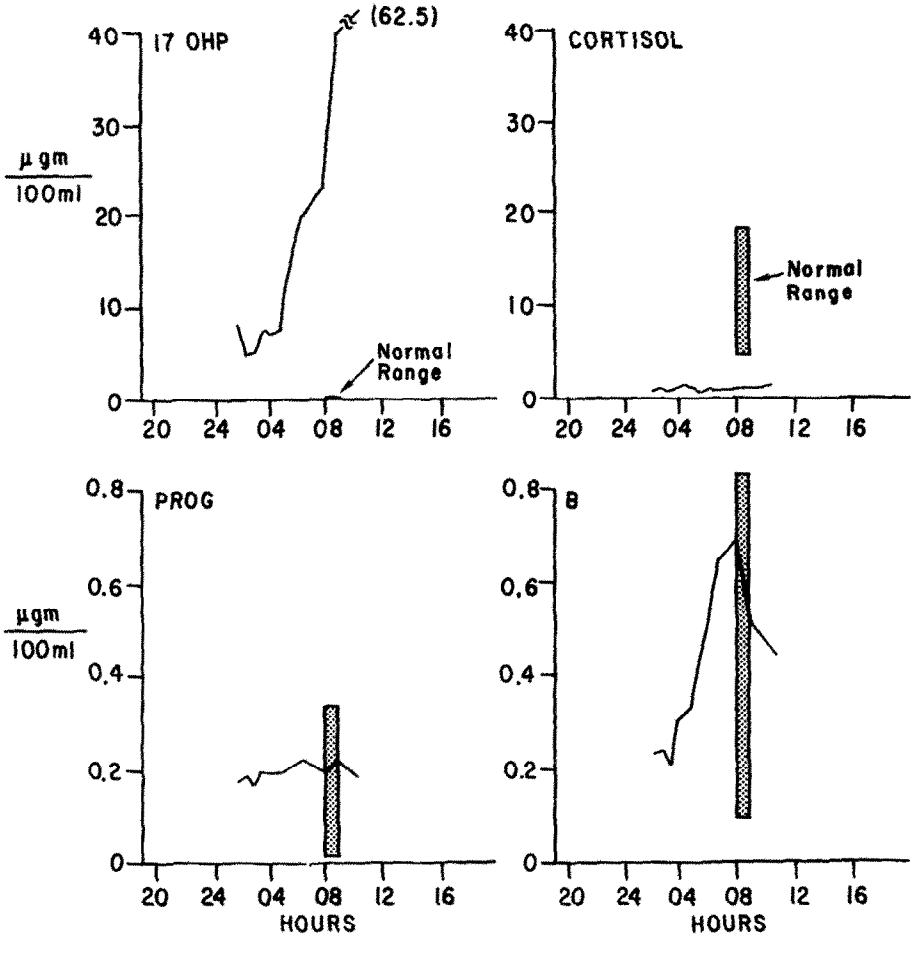


Fig. 5. Diurnal variation in plasma 17-OH progesterone, cortisol, progesterone, and corticosterone concentrations in salt-losing CAH. Patient B.B. was a 10-year-old genetic male who was treated with 12.5 mg of cortisone acetate twice daily at the time of the study.

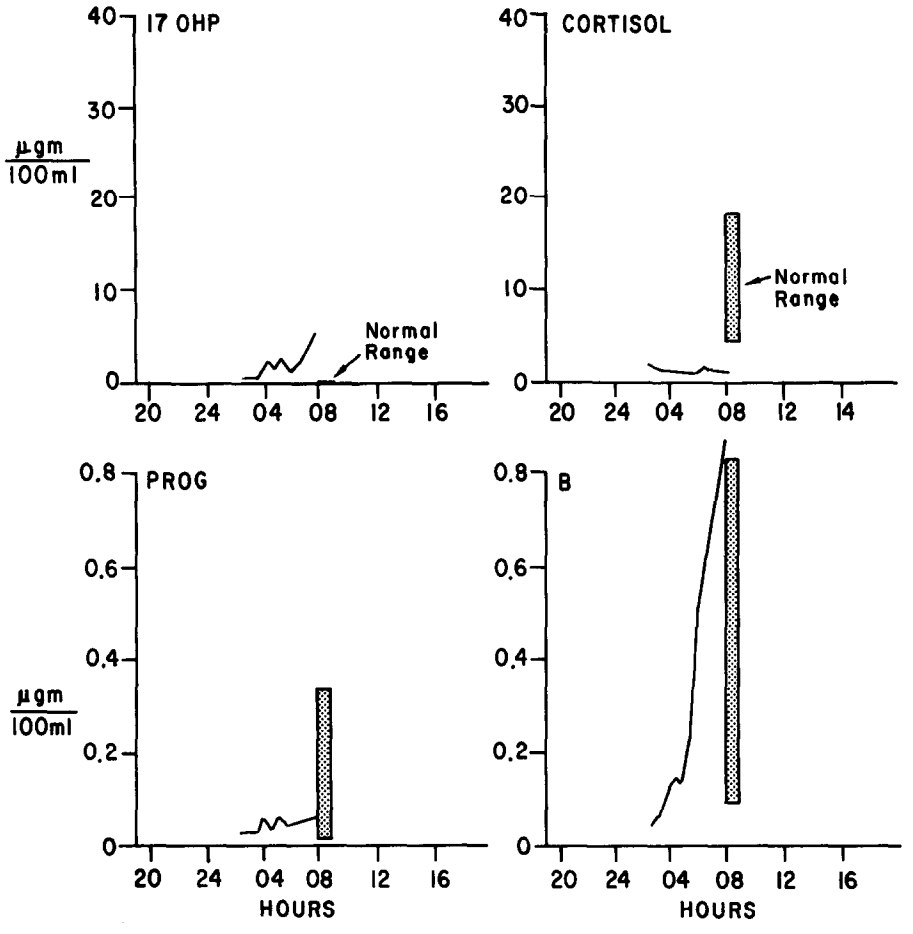


Fig. 6. Diurnal variation in plasma 17-OH progesterone, cortisol, progesterone, and corticosterone concentrations in salt-losing CAH. Patient K.B. was a 12-year-old genetic female who was treated with 12.5 mg of cortisone acetate twice daily at the time of the study.

(Figs 4–6). The non-salt-losers had cortisol levels in the normal range throughout the day that increased gradually during the nocturnal period (Figs 2 and 3). The normal pattern of plasma cortisol levels with many peaks and valleys (caused by episodic secretion) was not observed in any of the CAH patients.

3. *Plasma progesterone concentrations.* Salt-losers differed from non-salt-losers in that the former consistently had lower plasma progesterone levels during the nocturnal secretory period. Furthermore, the salt-losers had no increase in progesterone secretory activity during this period, the plasma levels being approx. the same throughout the day (Figs 4–6.) On the other hand there was a nocturnal increase in plasma progesterone levels in the non-salt-losers with the values rising above the normal range (Figs 2 and 3). During the rest of the day plasma progesterone levels were normal in both forms of CAH.

4. *Plasma corticosterone concentrations.* All patients with both types of CAH had plasma corticosterone levels that were in the normal or high normal range at all times. Furthermore, a definite nocturnal increase in corticosterone levels occurred in all patients but was particularly striking in the salt-losers. During the nocturnal period all patients with salt-losing CAH had plasma corticosterone levels that were near the upper limits of normal (Figs 4–6). The maximum plasma corticosterone values observed in the non-salt-losers were all lower than those in the salt-losers.

## DISCUSSION

Before attempting to interpret the results of this study the validity of the experimental approach should be considered. Our experimental approach is based upon the concept that plasma concentrations of steroids that are substrates and products of adrenal biosynthetic enzymes reflect the activity of those enzymes within the adrenal gland. The concentration of any steroid in blood is determined not only by the rate at which it is synthesized but also by factors that affect its secretion into and clearance from the bloodstream. Since none of our patients or controls had disorders that are known to affect steroid secretion

and clearance rates, it seems reasonable to assume that these factors would not differ significantly between experimental groups and that the major factor that would account for the observed abnormalities in plasma steroid concentrations would be differences in the activity of the adrenal biosynthetic enzyme systems.

As shown in Fig. 1, 21-hydroxylation steps occur in the biosynthetic pathways of all three of the major steroids secreted by the adrenal gland, cortisol, corticosterone and aldosterone. Cortisol and corticosterone biosynthesis occurs primarily in the zona fasciculata and aldosterone is synthesized in the zona glomerulosa. Our demonstration of very high plasma 17-OH progesterone levels in CAH with very low plasma cortisol levels at the same time plasma progesterone and corticosterone levels are normal or elevated suggests that there are two different 21-hydroxylase systems, one for 17-OH progesterone which is markedly impaired while the other for progesterone is unimpaired or much less severely affected.

Can our data be explained on the basis of a defect in a single 21-hydroxylase system for both 17-OH progesterone and progesterone? Since the cortisol biosynthetic pathway is quantitatively by far the most important, a defect in this pathway could conceivably divert progesterone into the corticosterone pathway in sufficient concentrations to overcome the enzymatic defect. This mechanism could possibly account for the normal or elevated corticosterone levels but not for the low normal plasma progesterone levels. The plasma progesterone levels should be very high unless there are other alternate metabolic pathways for the utilization of progesterone.

An essential part of the single enzyme theory is that the 21-hydroxylase defect is more severe in salt-losers than in non-salt-losers. Our finding that cortisol levels were lower and 17-OH progesterone levels were higher in salt-losers than in non-salt-losers is consistent with the theory, but the demonstration that salt-losers had higher plasma corticosterone levels than non-salt-losers is not.

It needs to be emphasized that our data probably do not provide an estimate of progesterone 21-hydroxylase activity in aldosterone biosynthesis. From a quantitative standpoint the amount of corticosterone synthetized in the aldosterone pathway in the zona glomerulosa is much less than that produced during corticosterone biosynthesis in the zona fasciculata (see Fig. 1). Most of the corticosterone in blood originates from the zona fasciculata and is under ACTH control [7]. It is not known whether any of the corticosterone in blood is generated from the aldosterone biosynthetic pathway, but it is known from dexamethasone suppression studies that the amount of corticosterone in blood from this source would have to be very small [7].

Although this study does not relate directly to differences in aldosterone production between salt-losing and non-salt-losing CAH, the evidence for more than one 21-hydroxylase system in the fasciculata suggests that the 21-hydroxylase system in the zona glomerulosa may also be different and in general favors the multiple 21-hydroxylase theory over the single enzyme theory.

In another popular theory it is hypothesized that excessive amounts of intermediate steroids that induce natriuresis are produced by salt-losers but not by non-salt-losers. These hypothetical natriuretic steroids have never been positively identified although several possible candidates have been suggested. Among these is progesterone which is known to cause natriuresis under certain conditions [8]. Our finding that salt-losers had lower plasma progesterone levels than non-salt-losers is against the progesterone-induced natriuresis theory.

It has also been suggested that excessive amounts of 17-OH progesterone might play a role in the pathogensis of salt-losing CAH. It has been demonstrated that 17-OH progesterone is a mild natriuretic hormone [9]. Our finding that salt-losers usually have higher plasma 17-OH progesterone levels is consistent with the 17-OH progesterone-induced natriuresis theory. Possible evidence against this theory is the observation that non-salt-losers may also have very high 17-OH progesterone levels that might be expected to cause salt loss. However, non-salt-losers are able to overproduce aldosterone which may compensate for the excess 17-OH progesterone, whereas salt-losers cannot. The possibility still remains that other unidentified natriuretic hormones are involved.

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