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Adrenogenital Syndrome Due to Enzymatic Defects in Cortisol Synthesis

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CONGENITAL virilizing adrenal hyperplasia is a fascinating disorder, partly because it causes spectacular alterations in sexual development which now can be prevented or dramatically reversed by treatment with cortisone and partly because it is an "experiment of nature," not producible in the laboratory, which helps us to understand the normal biosynthesis and physiology of adrenocortical hormones. It is also one of the best understood of the genetically determined enzymatic defects.

Clinical studies have shown that there are three different types of virilizing adrenal hyperplasia causing (1) simple virilism, (2) virilism with hypertension and (3) virilism with tendency to sodium-loss. Their relative frequency in our clinic is shown in Table I. There is a high familial incidence of the disorder. Each of these types may be due to a different specific defect of enzymes concerned in the biosynthesis of adrenal hormones, since in each family in which the disorder occurs all the children who are affected have the same type of disease. The table suggests, at first glance, that three or four times more females than males are affected. Childs,[1] however, believes that this impression may be biased by the fact that the disease is more apt to pass undiagnosed in the male. Eliminating the so-called "index cases" which present in the clinic, he found that the affected sibs of these patients have an approximately equal sex distribution as shown in Table II.

Bartter and his associates[2] were the first to suggest that in the adrenogenital syndrome the ability of the adrenals to synthesize hydrocortisone might be impaired; Jailer[3] confirmed this by showing that ACTH caused little increase in its output. We[4] illustrated this concept with a diagram indicating that there was a partial defect in the production of glucogenic and possibly sodium-retaining hormones with the formation of abnormal amounts of

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TABLE I
Relative Frequency and Sex Distribution of Different Types of Virilizing Adrenal Hyperplasia in Patients Studied at the Harriet Lane Home of the Johns Hopkins Hospital

Patients	Virilism Alone	Sodium-Losing	Hypertensive	Total
Females...	54*	32	5	91
Males.....	12	8	2	22

* 9 raised as males.

TABLE II
Ratio of Male to Female Patients with Virilizing Adrenal Hyperplasia—Children's Medical Center, Boston. From: CHILDS, B. *Pediatrics*, 25: 565, 1960.

Patients	Males	Females	Total	Sex Ratio M:F
Index cases.	30	84	114	0.36
Affected sibs.....	14	18	32	0.78
Total.....	44	102	146	0.43

TABLE III
Methods of Studying the Adrenogenital Syndrome

1. Effect of ACTH stimulation on { plasma steroids / urinary metabolites }
2. Suppression of adrenals by cortisone and analogues.
3. Administration of precursors to patients.
4. Tissue slice synthesis—C^{14} precursors.
5. SU-4885 (C-11-hydroxylation) block.
6. Aldosterone measurements—under sodium deprivation.

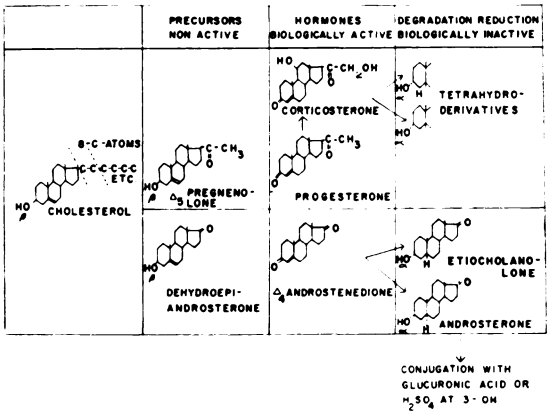

FIG. 1. Chemical structure of active steroidal hormones, their precursors and their degradation products.

androgenic and estrogenic steroids. To compensate for the relative deficiency of hydrocortisone, the activity of the adrenal was whipped up by increased secretion of pituitary ACTH. The latter was demonstrated by Sydnor and his colleagues.[5] It remained, however, to determine more specifically the actual abnormalities of steroidogenesis.

METHODS OF STUDY

The methods which have been applied to the study of virilizing adrenal hyperplasia are listed in Table III. It was recognized early that the concentration of compound F (cortisol) in the plasma and the output of its metabolites tetrahydro-hydrocortisone (THF) and tetrahydrocortisone (THE) in the urine were decreased and that the administration of ACTH did not increase these products to the extent that it does in normal persons. This might be due, of course, to the fact that the adrenals are already working under maximal ACTH stimulation. However, the urine was shown to contain relatively large amounts of steroids not found in significant quantities in normal urine and the amounts of these abnormal steroids were increased by the administration of ACTH indicating that the adrenals were responsive.[6] The question was raised of whether these abnormal metabolites were due to an abnormality in the hepatic degradation of adrenal steroids or whether they are expected degradation products of certain precursors of hydrocortisone which accumulate in excess because of a block in its synthesis. Recent work[7] indicates that, although in the adrenogenital syndrome there may be some minor alterations in hepatic degradation, these are of relatively little importance compared to the block of synthesis of cortisol. The disappearance of the abnormal steroids from the urine when adrenal activity is suppressed by the administration of physiologic amounts of cortisone or its analogues lends further support to this hypothesis.

I shall mention also some interesting studies which have been carried out by administering the chemical SU-4885 which blocks C_{11}-hydroxylation to patients with virilizing adrenal hyperplasia and some studies of aldosterone excretions in patients with the salt-losing type.

PATHWAYS OF STEROIDAL SYNTHESIS AND DEGRADATION

Figure 1 presents the general scheme for the formation in the adrenals or gonads of biologically active steroids from their inactive pre-

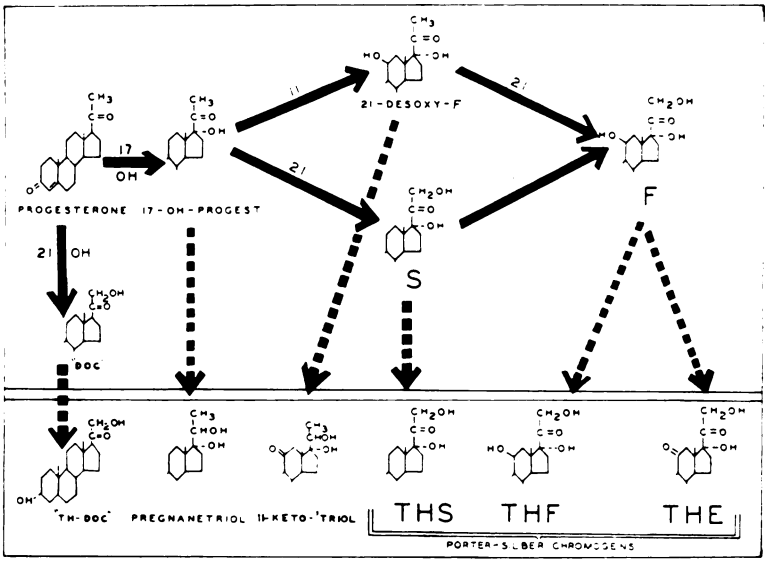

FIG. 2. Pathway of biosynthesis of hydrocortisone showing intermediary precursors and the 21-carbon urinary metabolites derived from them.

cursors followed by their subsequent degradation in the liver to inactive metabolites which are conjugated to soluble compounds readily excreted in the urine. Cholesterol is first converted into 21-carbon or 19-carbon steroids by the removal of six or eight of the carbon atoms attached at C_{17}. Δ_5-pregnenolone and dehydroepiandrosterone are both inactive steroids having, like cholesterol, a double bond at $C_{5,6}$ and a β—OH at C_3. For the formation of biologically active steroids, such as progesterone and Δ_4-androstene-dione, the double bond must be shifted to C_{4-5} and the hydroxyl at C_3 converted to ketone. The degradation of these hormones to inactive metabolites consists of reduction of the A ring with removal of the double bond and the conversion of the C_3 ketone to a 3-α-hydroxyl, which is afterward conjugated with glucuronic or sulfuric acid. Theoretically, a defect of steroidogenesis could be due to a defect of conversion of ΔC_5 to ΔC_4 (since this paper was presented a case of congenital adrenal hyperplasia with this defect has been published by Bongiovanni[19]).

Figure 2 is a simplified scheme showing the synthesis of hydrocortisone from progesterone and the urinary 21-carbon metabolite derived from each of the intermediary precursors. The solid arrows indicate the hydroxylations which occur at the 17-, 11- and 21-carbon atoms. The dotted arrows indicate the corresponding end metabolite derived from each of the precursors of compound F (hydrocortisone). Some of the intermediary compounds in the formation of these metabolites have purposely been omitted. From this chart, one can predict which metabolites one would expect to find when there is a defect of C_{21}-hydroxylation, C_{11}-hydroxylation or both of these hydroxylations.

Less is known about the synthesis of the 19-carbon steroids, or androgens, of the adrenal or the derivation of the 19-carbon metabolites, the 17-ketosteroids. Figure 3 shows (lower section) the probable synthesis of Δ_4-androstenedione and 11-keto-androstenedione from dehydroepiandrosterone. The 19-carbon urinary metabolites (17-ketosteroids) which are principally androsterone and etiocholanolone and their 11-oxy derivations, are shown in the middle section. As indicated by the dotted arrows, a portion of these metabolites can be derived from the 21-carbon steroids (upper line) as well as from the 19-carbon steroids (lower line). However, the 21-carbon steroids make only a minor contribution, probably not over 5 to 10 per cent to the total 19-carbon metabolites.[7]

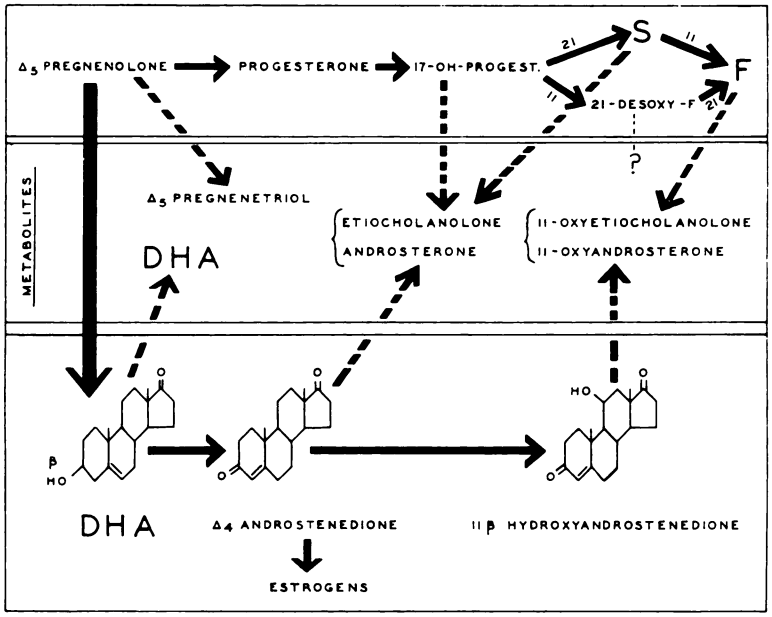

FIG. 3. Urinary 19-carbon metabolites showing their derivation from both 19-carbon and 21-carbon steroids.

THE C-21-HYDROXYLATION DEFECT IN VIRILIZING ADRENAL HYPERPLASIA

The simple virilizing form of adrenal hyperplasia is due primarily to a defect in 21-hydroxylation as shown in Figure 4. With this defect the precursors of cortisol, 17-OH-progesterone and 21-desoxy-F are increased, giving rise to increased urinary output of both pregnanetriol and the 11-oxypregnanetriols.

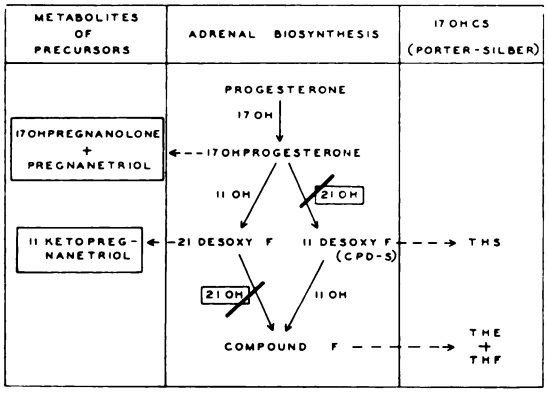

FIG. 4. Steroid synthesis in nonhypertensive adrenal hyperplasia. 21-Hydroxylase defect in simple virilizing and sodium-losing types of congenital adrenal hyperplasia. Note that 11-OH-pregnanolone, pregnanentriol and 11-ketopregnanetriol are increased while THE, THF and THS are decreased.

There is also increased excretion of the 17-ketosteroids and 11-oxy-17-ketosteroids, while the 17-OH-corticosteroids (THE, THF and THS) are decreased.

THE C-11-HYDROXYLATION DEFECT IN THE HYPERTENSIVE FORM OF ADRENAL HYPERPLASIA

Eberlein and Bongiovanni[8] showed that in patients with the hypertensive form of virilizing adrenal hyperplasia, the defect is one of 11-hydroxylation rather than 21-hydroxylation.

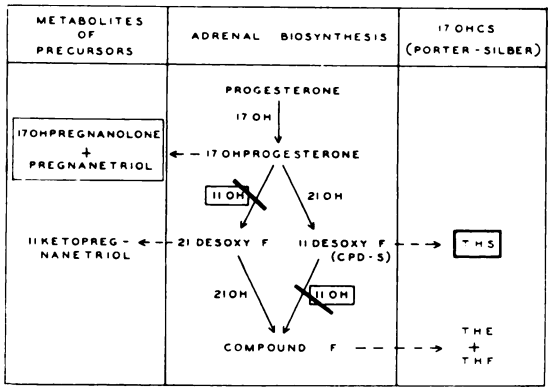

FIG. 5. 11-Hydroxylase defect in hypertensive type of virilizing adrenal hyperplasia. Note that 17-OH-pregnanolone, pregnanetriol and THS are increased while 11-ketopregnanetriol, THE and THF are decreased.

As shown in Figure 5, this gives rise to an entirely different pattern of urinary metabolites. Compound S is formed and tetrahydro-S (THS) is excreted in large amounts. Pregnanetriol is formed but 11-oxygenated pregnanetriol is missing. The 11-oxygenated 17-ketosteroids are also absent. Likewise, desoxycorticosterone (DOC) and tetrahydro-DOC are formed. These are not shown on this chart but are derived from progesterone before hydroxylation at C_{17}. The former is believed to be the cause of hypertension which disappears when the adrenal is suppressed with cortisone. However, a few patients with virilizing adrenal hyperplasia caused by the 11-hydroxylation defect have not had hypertension.

These enzyme defects are probably never entirely complete and vary somewhat in degree from one patient to another. This is illustrated by the urinary steroidal patterns of some patients studied in our laboratory by Dr. Orville Green[9] (Fig. 6 and 7). Although there are minor differences between the patients of each group, it is obvious that there is a very striking difference between the patients who are hypertensive and those who are not.

THE SODIUM-LOSING TYPE OF ADRENAL HYPERPLASIA

There is not sufficient time to discuss the still unsolved problem of the salt loss which occurs in about one-third of the patients with virilizing adrenal hyperplasia. As shown in the previous figure these patients have the C_{21} rather than the C_{11} hydroxylation defect. Bongiovanni and Eberlein[10,11] have shown that in patients with the salt-losing type of adrenal hyperplasia impairment in the formation of hydrocortisone is greater than in those with the simple virilizing type; our observations agree with this. They suggest that the difference between the two types is a quantitative rather than a qualitative one and that hydrocortisone may be necessary or "permissive" for aldosterone to exert its salt-retaining activity. On the other hand it is obvious that a complete absence of 21-hydroxylation would prevent the formation of aldosterone.

Studies of actual aldosterone excretion have been made in only a few laboratories[12–14] and there is a discrepancy in the findings. Studies of our patients[13] made during five-day periods of sodium deprivation failed to show appreciable amounts of urinary aldosterone in the patients with the salt-losing type, whereas there were possibly increased amounts in patients with the simple virilizing type. It is our opinion that possibly in patients with both the simple virilizing and the salt-losing types the ability to reabsorb sodium may be impaired and that the former compensate for this with overproduction of aldosterone while the latter cannot.

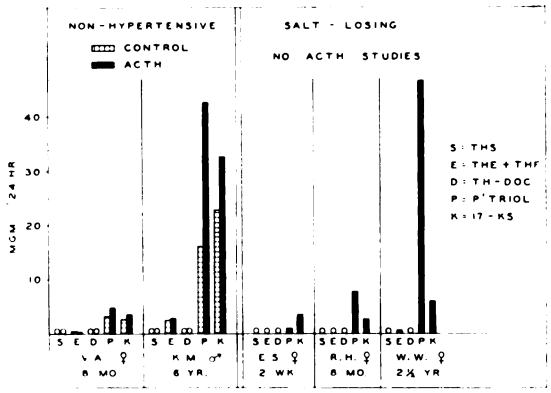

FIG. 6. Variations of urinary steroidal patterns in individual cases of the hypertensive type of virilizing adrenal hyperplasia. From: GREEN, O. C., MIGEON, C. J., and WILKINS, L. *J. Clin. Endocrinol.*, 20: 929, 1960.

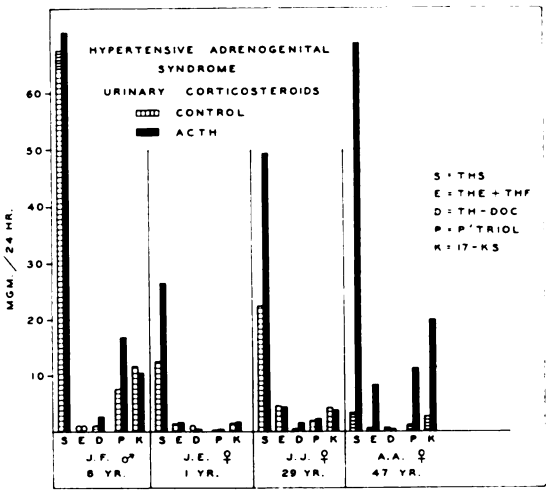

FIG. 7. Variations of urinary steroidal patterns in cases of simple virilizing and sodium-losing adrenal hyperplasia. From: GREEN, O. C., MIGEON, C. J. and WILKINS, L. *J. Clin. Endocrinol.*, 20: 929, 1960.

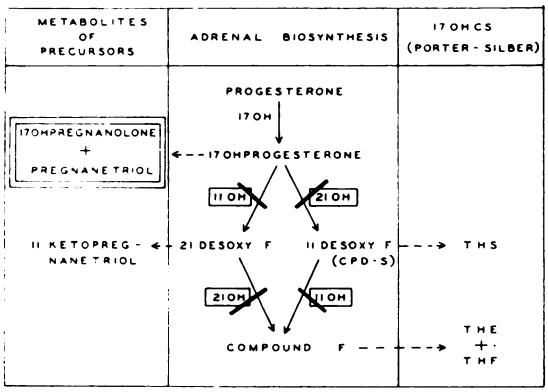

FIG. 8. Effect of SU-4885 block in patients with virlizing adrenal hyperplasia. Note that 17-OH-pregnanolone and pregnanetriol are greatly increased while 11-keto-pregnanetriol, THS, THE and THF are decreased. From: CLEVELAND, W. W. and MIGEON, C. J. *Am. J. Dis. Child.*, 100: 473, 1960.[18]

GENETIC STUDIES

In 1956, Childs and his co-workers[15] reported genetic studies made on our patients. At that time there were seventy-six subjects among 181 sibs in fifty-six families. There were no affected parents, grandparents, uncles, aunts or cousins. There was only one family in which there was consanguinity, the parents being first cousins. As stated previously, Childs[1,15] thought there was no actual difference in the sex ratio of affected patients despite the apparent marked preponderance of females. The apparent difference is probably due to the greater difficulty in recognizing the disorder in males. There was no evidence of deleterious effects on gestation or labor in the mothers nor any evidence of maternal influence on the disorder. He came to the conclusion that the disorder is due to an autosomal recessive gene which manifests itself only in the homozygote.

It would be most valuable to know the frequency of the gene in the population and to be able to detect the heterozygotous carriers of the gene. Childs and his associates,[15] making the unwarranted assumption that all patients in Maryland with the disorder born after 1939 came to our own clinic, estimated the disease occurred once per 67,000 births and calculated that the incidence of heterozygotes was one per 128 individuals of the general population. We suspect now that the incidence of the trait may be 1:50 and 1:100. Prader of Zurich[16] estimates that in Switzerland there is one patient with the adrenogenital syndrome among every 5,000 births, giving an incidence of the gene of 1:37.5 in the general population.

Prior to the treatment of adrenal hyperplasia with cortisone, the patients rarely, if ever, were fertile; with treatment, ovulation or spermatogenesis occurs. Having observed the high incidence of affected sibs in certain families, we were inclined at first to advise our treated patients not to produce children. If the genetic explanation of the disorder is correct, however, the treated patients have only a 1:100 to 1:200 chance of producing affected offspring. If a marriage with a heterozygotous carrier could be avoided, there would be no risk at all.

If the fathers and mothers of the affected subjects are heterozygotous carriers they might be expected to have a minor enzymatic defect which might be demonstrable. Since the defect in the homozygotous patients is well demonstrated by the degree to which urinary pregnanetriol increases after administration of ACTH, Childs, Grumbach and Van Wyk[15] applied this test to the study of ten mothers and ten fathers of our patients. With the exception of one father and one pregnant mother, the increment of pregnanetriol ranged from 0.4 to 3.7 mg. per day, compared with 0.0 to 4.4 mg. per day. The difference of the means was barely significant. It is of interest that in one father the pregnanetriol rose 41.3 mg.

Recently, Jailer[17] and later Cleveland et al.[18] studied the effects of introducing an 11-hydroxylation block with the chemical SU-4885 in patients with simple virilizing adrenal hyperplasia who have the 21-hydroxylation defect. This causes the output of pregnanetriol and its precursor, 17-OH-pregnanolone, to rise as high as 100 to 260 mg. per day compared to the usual output of 40 to 80 mg. per day after stimulation with ACTH. As shown in Figure 8 this is due to the fact that with the SU-4885 block the 11-keto-pregnanetriol (not measured by the usual methods) cannot be formed and all of the precursors appear as

TABLE IV
Ratios of *pregnanes*:17-OHCS in Patients with Virilizing Adrenal Hyperplasia, in Their Parents and in Normal Subjects

Subjects	Average Ratio	Range
5 patients	$\frac{145}{10.4} = 14$	(5.9–26)
6 normal subjects	$\frac{2.6}{21.1} = 0.13$	(0.10–0.18)
9 parents	$\frac{3.3}{28.0} = 0.13$	(0.01–0.20)

NOTE: The higher the ratio the more complete is the 21-hydroxylation defect. From: CLEVELAND, W. W. and MIGEON, C. J. *Am. J. Dis. Child.*, 100: 493, 1960.[18]

pregnanetriol and 17-hydroxypregnanolone. The ratio of the pregnane compounds to the Porter-Silber chromogens can be used as a measurement of degree of the 21-hydroxylation defect.

Cleveland et al.[18] carried out the SU-4885 block in ten parents, five patients and six adult control subjects. Nine of the parents did not respond differently from normal control subjects. The ratios pregnanes:17-OHCS indicating the degree of 21-OH defect are shown in Table IV; disappointingly these do not show any detectable defect in the heterozygotes. It is of interest, however, that one father had the abnormally high ratio of 6 which is in the range of the affected patients. We discovered later that this is the same father who responded with abnormally high pregnanetriol when studied under ACTH stimulation by Childs et al.[15] We wonder whether he might be a homozygote, but have no evidence of this. We are still left without any method for detecting most of the heterozygotes.

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