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Effects of C_{17}-Hydroxy-Steroids on Urinary 17-Ketosteroids in the Rat.* (19620)

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Because all the urinary 17-ketosteroids (17-KS) of females and some part of those excreted by males originate in the adrenal cortex, their determination is one of the means of evaluating adrenocortical function. The association of 17-KS content with androgenic properties, the presence of adrenosterone in the adrenal cortex and the masculinization elicited by some forms of adrenal cortical hyperfunction have supported the concept of a specific androgenic secretion of the adrenal cortex. However, recent evidence(1-6) indicates that 17-KS are formed and excreted during metabolism of corticosteroids, particularly of those hydroxylated at C 17. Relevant studies in human subjects are scanty. Those here reported were undertaken in rats, in which relatively large doses of potential 17-KS precursors could be readily administered.

Methods. Female albino rats (Sprague-Dawley) of 150-250 g body weight were separated into 7 groups, 5 experimental and 2 control; each group contained at least 4 rats. The animals were placed separately in metabolism cages for a period of 13 days; in order to accustom the animals to this environment, 4 days elapsed before real or simulated treatment was begun; during the treatment period, 10 mg of each steroid tested was given by stomach tube daily for 5 days; 4 days were allowed for post-treatment observation. Drinking water was substituted for with 1% NaCl in order to obtain a diuresis; commercial fox chow was given *ad libitum*. A few drops of Merthiolate solution were placed in the collection bottles; the urine was collected and measured daily. The steroids administered were as follows: 11-desoxycorticosterone acetate (DCA); 17-hydroxy-11-desoxycorticosterone acetate (compound S); progesterone; 17-hydroxyprogesterone and Δ^4-pregnene-17 α, 20, 21-triol-3-one diacetate (pregnenetrio-

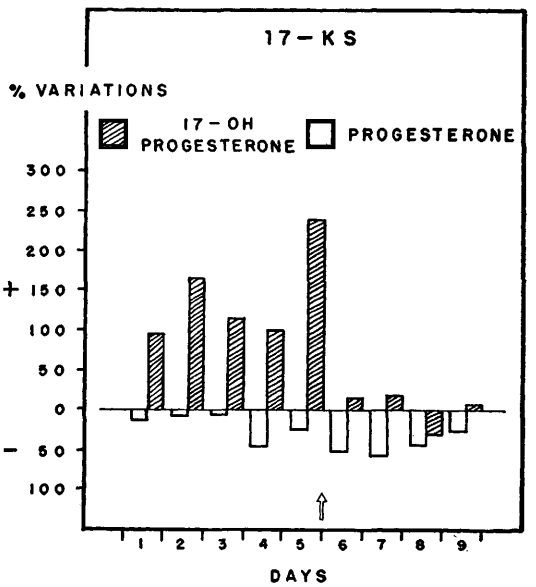

FIG. 1. % variations from control mean of urinary 17-ketosteroids following administration of progesterone and 17-hydroxyprogesterone. Arrow indicates end of treatment.

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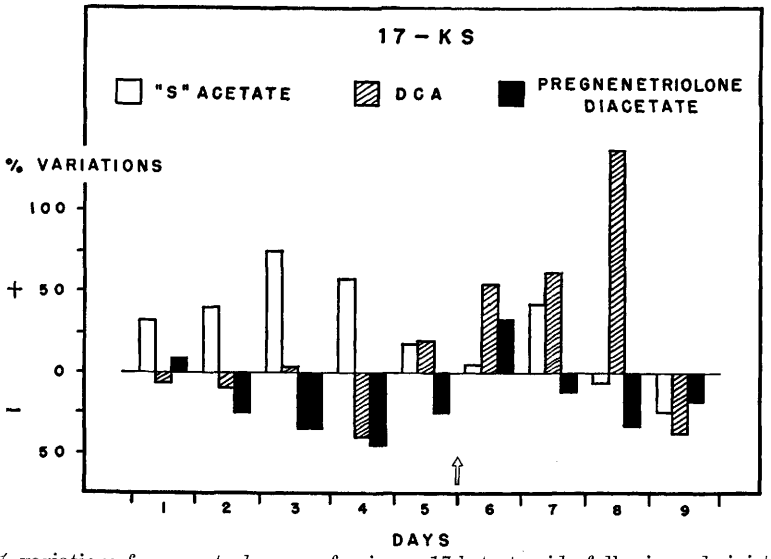

FIG. 2. % variations from control mean of urinary 17-ketosteroids following administration of compound S acetate, desoxycorticosterone acetate and pregnenetriolone diacetate. Arrow indicates the end of treatment.

lone). The steroids were given in water suspensions containing 10 mg per cc; one group of controls was gavaged daily with 1 cc of saline; the other control group was untreated. The method of 17-KS determination was that of Drekter *et al.*(7). It was found advantageous because it can be rapidly completed, requires small amounts of urine and eliminates chromogens which were particularly troublesome in rat urines with other methods. The values obtained are expressed in terms of a dehydroisoandrosterone standard.

Results. In the control groups, administration of saline by gavage had little effect on 17-KS outputs; individually some animals which resisted the procedure showed initially higher outputs which later stabilized at lower levels; similarly, 17-KS outputs of some animals were about 30% higher during the first day or two than they were after they had become accustomed to the metabolism cages.

From 55 determinations in normal females, obtained after achievement of stable levels in this and other experiments, the 17-KS output of the rat was found to average 198 μg per 24 hours (standard error of the mean ± 7.9 μg). Individuals differed fairly widely in their outputs; these were relatively constant in undisturbed animals.

Results from the experimental groups are summarized in Fig. 1 and 2; changes caused by treatment are expressed as percentage variation from the means of the last 3 days of the control periods. DCA and progesterone, which are devoid of a C-17 hydroxyl, do not increase 17-KS output; on the contrary both seemed to decrease slightly the output of 17-KS during treatment. That this decrease may be real in the case of DCA is suggested by the immediate rebound above control level observed during the post-treatment period. The 17-hydroxylated steroids, compound S and, to a greater extent, 17-hydroxyprogesterone increase 17-KS outputs; on the other hand, pregnenetriolone seemed to depress output of endogenous 17-KS without itself being excreted in this form. The significance of the increased outputs is established by the abrupt change in output observed when treatment was discontinued.

Discussion. The 17-KS outputs of normal female rats here reported agree with those of Kowaleski *et al.*(8); their mean of 190 μg per rat per day was obtained by Warren's modification of Callow's method(9). As might be expected, the values are greater than the mean of 30 μg found by Danford and Danford(10) who used the method of Engstrom

and Mason(11) and separation of the ketonic fraction with Girard reagent.

In vitro, periodic acid oxidation of the 3 17-hydroxylated compounds tested in this study can yield the respective 17-ketosteroids (12,13); however, in the case of pregnenetriolone, the initial product of molar oxidation is a 17-hydroxyaldehyde(13). Similarly, *in vivo* in rats, 17-KS appear in urine as metabolic products of compound S and 17-hydroxyprogesterone, but not of pregnenetriolone. The marked effectiveness of 17-hydroxyprogesterone in augmenting urinary 17-KS output is of interest in view of the androgenic property of this steroid in castrate rats(14); it may be that its metabolism results in formation of biologically active 17-KS.

The data obtained from rats accord with observations in some human beings; in these, compound S(1,5) and 17-hydroxyprogesterone (1) increase urinary 17-KS while DCA(15) and progesterone(16) do not. Data are available from only 2 patients given pregnenetriolone(1); in one, the compound had no effect and in the other, the output of 17-KS seemed to double. The observations in rats suggest that this change may have been an artefact.

Technically, the procedure here described has definite advantages in the evaluation of 17-KS precursors. As noted above, it permits the administration of small amounts of steroids with observations in a sufficient number of animals; especially, its advantage lies in its relative independence of endogenous adrenocortical metabolism; thus, in human beings, cortisone has been found to have disparate effects of urinary 17-KS(17), apparently because in smaller dosage it suppresses endogenous adrenocortical function to a greater degree than it contributes to 17-KS output.

In brief, these observations in rats support the view that the adrenal precursors of urinary 17-KS are 17-hydroxylated compounds. Current unpublished observations show that compound F, which seems to constitute the bulk of adrenocortical hormone is a 17-KS precursor; in view of this and of the androgenic properties which seem to be associated with the metabolic products of 17-hydroxyprogesterone, it may be that the masculinizing effects of some forms of adrenocortical hyperactivity should be attributed to the secondary products of hypersecretion rather than to a primary secretion of specific androgens. Indeed, the evidence for androgenic secretion by the adrenal cortex is not substantial. It is based in large part on the presence of adrenosterone in extracts; this is considered by some to be artefactual(3).

Summary. A method is described for detecting precursors of urinary 17-KS in normal female rats. Using it, 2 pairs of compounds which differed in hydroxylation at C-17 were tested and those 2 possessing this grouping were found to be eliminated in part as urinary 17-KS. One 17-hydroxylated steroid, pregnenetriolone did not act as a 17-KS precursor. These observations bring into question the assumed secretion of adrenal androgens, since they are consistent with the view that such might arise from the degradation of non-androgenic hormones.

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Separate Identities of the Donath-Landsteiner Hemolysin (PCH Antibody) and Treponemal Immobilizing Antibody.* (19621)

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Paroxysmal cold hemoglobinuria (PCH) due to the presence of an abnormal serum hemolysin is recognized as a manifestation of syphilis, for nearly all patients with positive Donath-Landsteiner reactions have historical, clinical or serological evidence of syphilitic infection(1-3). The hemolysin is distinct from the Wassermann reagin(4,5), and surveys of non-hemoglobinuric patients with syphilis have detected only a few with positive Donath-Landsteiner reactions(6-8).

Since demonstration of the PCH hemolysin sometimes requires more complement than was used in the tests employed for these surveys (9), it was felt that a more sensitive test might reveal that many patients with syphilis have a detectable, but clinically inactive, hemolysin. Furthermore, because both the PCH antibody (10) and the treponemal immobilizing (TPI) antibody(11,12) are heat stable globulins that require complement for their activities, and because TPI antibody is a specific indicator of syphilitic infection, the relationship of these antibodies was investigated in an effort to clarify further the mechanism of production of syphilitic cold hemoglobinuria.

Materials and methods. Sera. For survey purposes, sera were obtained from hospitalized and clinic patients with strongly positive Wassermann and Kline reactions. The sera used as controls in the absorption experiment were obtained from patients who were not receiving penicillin or other antibiotics. The case histories of the 2 patients with paroxysmal cold hemoglobinuria have been summarized elsewhere(8). *Antibody tests.* The methods used for the following procedures were those previously described(8): 1) hemolysin titration, 2) indirect Coombs tests (a) and (b). The technic of Nelson and Diesendruck(13) was employed for the treponemal immobilization test.† *Absorption of sera.* Sera from the 2 patients with positive Donath-Landsteiner reactions and from 2 other patients with syphilis (controls), but with negative hemolysin reactions, were treated as follows:

Three ml of serum, 0.75 ml of freshly reconstituted lyophilized guinea pig serum, and 1.5 ml of fresh, thrice washed packed group O erythrocytes were added to a tube. This mixture was chilled in an ice bath for an hour, centrifuged in the cold, and the supernatant removed for testing. The erythrocytes used for absorption were washed 3 times with cold saline and tested for agglutination by antiglobulin serum in the indirect Coombs test (a). For each serum, 3 specimens, identified

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