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# GONADAL STEROID SULFATES AND SULFATASE

## V. HUMAN TESTICULAR STEROID SULFATASE: PARTIAL CHARACTERIZATION AND POSSIBLE REGULATION BY FREE STEROIDS

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SUMMARY

To elucidate the role of steroid sulfates as precursors of biologically active hormone in the human testis, cleavage of pregnenolone sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate by microsomal preparations was studied. The respective apparent $K_m$ values were 0.73 $\mu$M, 3.85 $\mu$M and 3.13 $\mu$M. Evidence is presented that the three steroid sulfates are cleaved by the same enzyme.

A number of free steroids were found to inhibit the steroid sulfatase activity. Among 14 $C_{21}$ steroids investigated, 5-pregnen-3$\beta$,21-diol-20-one and 5-pregnene-3$\beta$,-20$\alpha$-diol were the most potent inhibitors. The inhibitory effect of $C_{21}$ steroids was decreased by structural alterations, *e.g.* a $\Delta^4$-3-keto for a $\Delta^5$-3$\beta$-hydroxy configuration, 5$\alpha$-reduction of the A ring, substitution of a 20$\beta$ for a 20$\alpha$-hydroxyl group, or a 3$\alpha$- for a 3$\beta$-hydroxyl group. Among 9 $C_{19}$ steroids investigated, 5$\alpha$-androstane-3$\alpha$,-17$\beta$-diol was the most potent inhibitor. 5$\alpha$ reduction of ring A of $C_{19}$ steroids either increased or did not change their inhibitory effect.

The kinetics of inhibition of the testicular steroid sulfatase by free steroids is consistent with partial competitive inhibition, and suggests that modulation of this sulfatase activity by free steroids may regulate release of essential free steroid precursors of testosterone.

## INTRODUCTION

Previous studies in rat testes[1–4] suggested that testicular steroid sulfatase may act as a control enzyme providing a regulatory mechanism for the release of free steroids which can then be further metabolized to active hormones.

It has recently been reported by LAATIKAINEN *et al.*[5,6] that pregnenolone

Trivial names used: Pregnenolone sulfate, 20-oxo-5-pregnen-3$\beta$-yl sulfate; dehydroepiandrosterone sulfate, 17-oxo-5-androsten-3$\beta$-yl sulfate; androstenediol-3-sulfate, 17$\beta$-hydroxy-5-androstene-3$\beta$-yl sulfate.

sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate are synthesized and secreted by the human testis. To gain information on the possible role of these steroid sulfates as precursors of biologically active hormone in the human testis, the rate of cleavage of these three steroid sulfates by microsomal fractions obtained from the testes of a number of patients undergoing orchiectomy was investigated. The degree and type of inhibition effected by various $C_{21}$ and $C_{19}$ free steroids was also studied.

## MATERIALS AND METHODS

### *Tissue preparation*

Human testes obtained at the time of orchiectomy were processed and submitted to subcellular fractionation as described previously[1,7]. The resulting microsomal fraction (105 000 × *g* pellet) was lyophilized and stored at —10° until assayed for enzyme activity.

### *Steroids*

[7-$^3$H]Dehydroepiandrosterone sulfate (25 C/mmole) and [7-$^3$H]pregnenolone sulfate (25 C/mmole) were purchased from New England Nuclear Corporation. [7-$^3$H]Androstenediol-3-sulfate was prepared and purified from [$^3$H]dehydroepiandrosterone sulfate as described previously[7]. Prior to incubation the $^3$H-labeled compounds were submitted to column partition chromatography and checked for purity by recrystallization of aliquots to constant specific activity[1,7].

### *Incubation*

The appropriate substrate was dissolved in 0.5 ml of 0.05 M Tris–HCl buffer, pH 7.2, unless otherwise indicated. When free steroids were added to the incubation mixture, the substrate and unconjugated steroid were dissolved in either dimethylsulfoxide or ethanol as indicated, prior to the addition of buffer solution. Aliquots of 0.5 ml of homogenized microsomal preparation (0.5 mg protein) was added and the mixture was incubated at 37° for 20 min. A 0.5 ml aliquot was removed at the appropriate time and delivered into a culture tube containing 3 ml 0.1 M NaOH and 15 ml toluene scintillation mixture.

### *Assay for steroid sulfate cleavage*

The amount of free steroid product was measured as described by BURSTEIN AND DORFMAN[8]. The method consists of the partitioning of steroid sulfates and free steroids between NaOH solution and toluene. The aqueous phase, containing the steroid sulfates, was frozen by immersing the culture tube in an acetone–dry ice bath. This was followed by pouring the toluene scintillation mixture, containing the free steroid, through Whatman No. 2$^v$ fluted filter paper. A 10-ml aliquot of the toluene mixture was taken for the determination of radioactivity in a Packard Model 3375 Tri Carb Scintillation Spectrometer. Controls lacking microsomal fraction were incubated and processed in an identical manner as the experimental incubations. The amount of radioactivity found in the toluene extract of these controls was subtracted from that of the experimental samples. The problem of non-enzymatic tissue factors rendering the steroid sulfates soluble in organic solvents as described by BURSTEIN

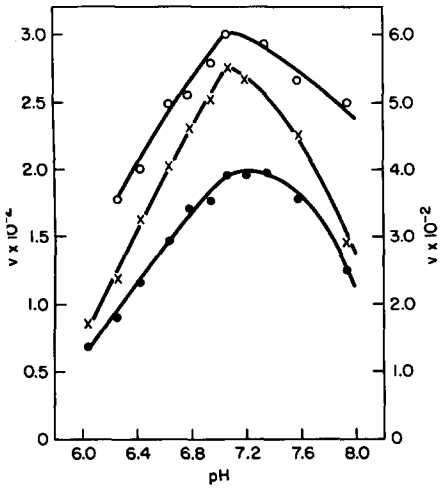


Fig. 1. Effect of pH on the rate of pregnenolone sulfate (○—○), dehydroepiandrosterone sulfate (×—×) and androstenediol-3-sulfate (●—●) cleavage. Scale on left refers to dehydroepiandrosterone sulfate and androstenediol-3-sulfate cleavage; scale on right refers to pregnenolone sulfate cleavage. Testicular microsomal suspension (0.5 mg protein) was incubated for 20 min at 37° with indicated substrate at a concentration of 3.4 μM in 0.05 M Tris–maleate buffer. The specified pH values were measured at the end of the incubation period.

AND DORFMAN[8] was not observed at the concentration of microsomal fraction used in the present study. Steroid sulfatase activity ($v$) is expressed as nmoles of free steroid released per min per mg protein. Protein concentration was measured by the method of LOWRY *et al.*[9] using crystalline bovine serum albumin as a standard.

## RESULTS

### *pH optimum*

Pregnenolone sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate were separately incubated with testicular microsomal fraction at various pH levels. As shown in Fig. 1 the optimal pH for all three substrates is between 7.0 and 7.2.

### *Apparent Michaelis constants*

The effect of increasing concentrations of pregnenolone sulfate, dehydroepiandrosterone sulfate and adrostenediol-3-sulfate, incubated separately, on the testicular steroid sulfatase activity is illustrated in Fig. 2 in the form of a Lineweaver–Burk plot. The apparent $K_m$ values obtained were 0.73 μM for pregnenolone sulfate, 3.13 μM for androstenediol-3-sulfate, and 3.85 μM for dehydroepiandrosterone sulfate with essentially identical $\bar{V}$ values. Inhibition was observed with all three substrates at high substrate concentration. Androstenediol-3-sulfate exhibited substrate inhibition at concentrations greater than 3.4 μM, dehydroepiandrosterone sulfate and pregnenolone sulfate at concentrations greater than 12.8 μM. Similar experiments were conducted in 0.05 M imidazole–HCl buffer at pH 6.8 and comparable results were obtained.

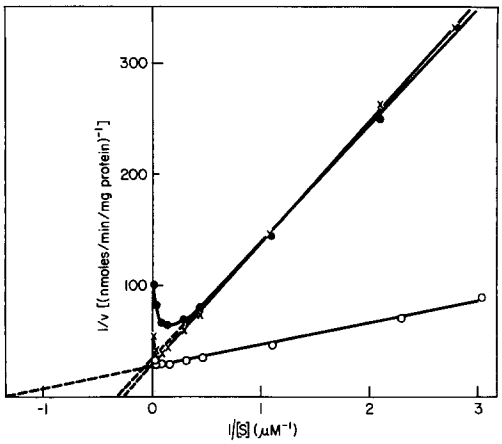


Fig. 2. Lineweaver–Burk plots of the effect of increasing concentration of substrate on rate of free steroid formation. Testicular microsomal suspension (0.5 mg protein) was incubated for 20 min at 37° in 0.05 M Tris–HCl, pH 7.2, with varying concentrations of substrate (0.32–54 $\mu$M). ○—○, pregnenolone sulfate; ×—×, dehydroepiandrosterone sulfate ; ●—●, androstenediol-3-sulfate.

*Competition between pregnenolone sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate*

Testicular microsomal fraction was incubated with [$^3$H]androstenediol-3-sulfate by itself in concentrations ranging from 0.39 $\mu$M–2.3 $\mu$M and in the presence of 2.26 and 4.52 $\mu$M dehydroepiandrosterone sulfate and 1.06 and 2.12 $\mu$M pregnenolone sulfate. Free [$^3$H]androstenediol was measured. Reciprocal plots of the reaction rate against androstenediol-3-sulfate concentration are shown in Fig. 3. The lines intersect

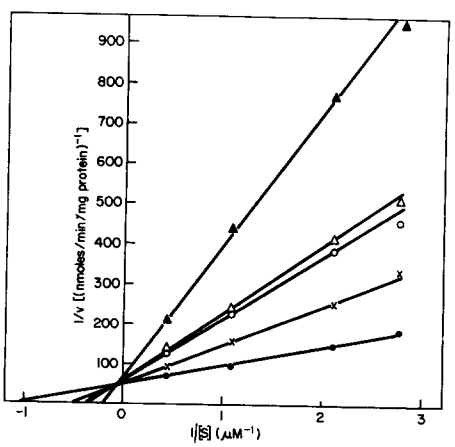


Fig. 3. Lineweaver–Burk plots of the inhibitory effect of pregnenolone sulfate and dehydroepiandrosterone sulfate on the rate of androstenediol formation from androstenediol-3-sulfate. Testicular microsomal suspension (0.5 mg protein) was incubated for 20 min at 37° in 0.05 M imidazole–HCl buffer, pH 6.8, with varying concentrations of [$^3$H]androstenediol-3-sulfate ($1.1 \cdot 10^6$ disint./min, 0.39–2.3 $\mu$M). ●—●, no inhibitor; ×—×, 2.26 $\mu$M dehydroepiandrosterone sulfate; ○—○, 4.52 $\mu$M dehydroepiandrosterone sulfate; △—△, 1.06 $\mu$M pregnenolone sulfate; ▲—▲, 2.12 $\mu$M pregnenolone sulfate.

the ordinate at the same point ($v_{max}$) indicating that the three steroid sulfates are cleaved by the same enzyme. Additional evidence for the same enzyme utilizing all three substrates was obtained by experiments with the mixed substrate method[10]. Equimolar amounts of pregnenolone sulfate, androstenediol-3-sulfate and dehydroepiandrosterone sulfate were incubated either alone or in combination, and the amount of free [$^3$H]steroid product was determined. It was observed that the amount

TABLE I

MUTUAL INHIBITORY EFFECTS OF PREGNENOLONE SULFATE, DEHYDROEPIANDROSTERONE SULFATE AND ANDROSTENEDIOL-3-SULFATE ON STEROID SULFATE CLEAVAGE

Radioactive and nonradioactive steroid sulfates, each at a concentration of 4.4 $\mu$M were incubated either alone or combined as indicated with a testicular microsomal suspension (0.5 mg protein). Incubation conditions are described under MATERIALS AND METHODS.

| *Incubations* | *Substrate* | | *Free [$^3$H]steroid product (nmole/mg protein)* |
|---|---|---|---|
| 1 | [$^3$H]Pregnenolone sulfate | | 0.602 |
| 2 | [$^3$H]Dehydroepiandrosterone sulfate | | 0.354 |
| 3 | [$^3$H]Androstenediol-3-sulfate | | 0.244 |
| 4 | [$^3$H]Pregnenolone sulfate + dehydroepiandrosterone sulfate | 0.514 | |
| 5 | [$^3$H]Dehydroepiandrosterone sulfate + pregnenolone sulfate | 0.076 | 0.590 |
| 6 | [$^3$H]Pregnenolone sulfate + androstenediol-3-sulfate | 0.400 | |
| 7 | [$^3$H]Androstenediol-3-sulfate + pregnenolone sulfate | 0.078 | 0.478 |
| 8 | [$^3$H]Dehydroepiandrosterone sulfate + androstenediol-3-sulfate | 0.156 | |
| 9 | [$^3$H]Androstenediol-3-sulfate + dehydroepiandrosterone sulfate | 0.176 | 0.332 |

of free steroid products, when any two of the substrates were incubated together, never exceeded the amount of product formed by the most reactive of the two substrates when incubated alone (Table I). If there were different enzymes for any of the three substrates, the total activity observed would have been equal to the sum of the activities found for each of the two steroid sulfates when incubated alone.

*Effect of various free steroids on the rate of cleavage of pregnenolone sulfate and dehydroepiandrosterone sulfate*

The effect of various $C_{19}$ and $C_{21}$ free steroids on the rate of cleavage of dehydroepiandrosterone sulfate and pregnenolone sulfate was investigated by the addition of non-radioactive steroids to the incubation mixture. The results are presented in Table II. The most potent inhibitors of the human testicular steroid sulfatase were 5-pregnene-3$\beta$,20$\alpha$-diol and 5-pregnene-3$\beta$,21-diol-20-one. The inhibitory effect of $C_{21}$ steroids was decreased by structural alterations such as substitution of (1) a $\Delta^4$-3-keto for a $\Delta^5$-3$\beta$-hydroxy configuration; (2) 5$\alpha$-reduction of the A ring; (3) substitution of a 3$\alpha$- for a 3$\beta$-hydroxyl group; (4) substitution of a 20$\beta$- for a 20$\alpha$-hydroxyl group; (5) introduction of 17$\beta$-hydroxyl or an 11$\beta$-hydroxyl group. Among the $C_{19}$ steroids investigated, 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol was the most potent inhibitor. In general,

TABLE II

INHIBITION OF STEROID SULFATE CLEAVAGE BY FREE STEROIDS

Testicular microsomal suspension (0.5 mg protein) was incubated in Tris–HCl buffer, pH 7.2, with either 2.28 $\mu$M [$^3$H]dehydroepiandrosterone sulfate or 2.13 $\mu$M [$^3$H]pregnenolone sulfate in the presence of indicated free steroid at a concentration of 22 $\mu$M, added in dimethyl sulfoxide not greater than 4%. Incubation conditions are described under MATERIALS AND METHODS.

| | *Inhibition (%)* | |
|---|---|---|
| | *Dehydroepiandrosterone sulfate cleavage* | *Pregnenolone sulfate cleavage* |
| *$C_{21}$ steroids* | | |
| 5-Pregnen-3$\beta$-ol,20-one (pregnenolone) | 50 | 35 |
| 5-Pregnene-3$\beta$,20$\alpha$-diol | 63 | 47 |
| 5-Pregnene-3$\beta$,21-diol-20-one (21-hydroxypregnenolone) | 65 | 47 |
| 5-Pregnene-3$\beta$,17-diol-20-one (17-hydroxypregnenolone) | 10 | |
| 5$\alpha$-Pregnan-3$\beta$-ol,20-one (allopregnanolone) | 43 | 26 |
| 5$\alpha$-Pregnan-3$\alpha$-ol-20-one | 32 | 10 |
| 5$\alpha$-Pregnane-3$\beta$,20$\beta$-diol | 24 | |
| 5$\alpha$-Pregnane-3$\alpha$,20$\alpha$-diol | 12 | |
| 5$\beta$-Pregnane-3$\alpha$,20$\alpha$-diol (pregnanediol) | 12 | |
| 4-Pregnene-3,20-dione (progesterone) | 21 | 10 |
| 4-Pregnen-3-one,2c$\alpha$-ol | 48 | 33 |
| 4-Pregnen-3-one,20$\beta$-ol | 20 | 11 |
| 4-Pregnene-3,20-dione-21-ol (deoxycorticosterone) | 27 | |
| 4-Pregnene-3,20-dione,11$\beta$-21-diol (corticosterone) | 16 | |
| *$C_{19}$ steroids* | | |
| 5-Androsten-3$\beta$-ol-17-one (dehydroepiandrosterone) | 23 | |
| 5$\alpha$-Androstan-3$\beta$-ol-17-one (epiandrosterone) | 23 | |
| 5$\alpha$-Androstan-3$\alpha$-ol-17-one (androsterone) | 10 | |
| 5-Androstene-3$\beta$,17$\beta$-diol (androstenediol) | 28 | 14 |
| 5$\alpha$-Androstane-3$\beta$,17$\beta$-diol | 27 | 10 |
| 5$\alpha$-Androstane-3$\alpha$,17$\beta$-diol | 40 | 24 |
| 4-Androstene-3,17-dione (androstenedione) | 9 | |
| 4-Androsten-3-one-17$\beta$-ol (testosterone) | 16 | |
| 5$\alpha$-Androstan-3-one-17$\beta$-ol (dihydrotestosterone) | 17 | |

the $C_{19}$ steroids are less inhibitory than the $C_{21}$ steroids. Substitution of the 17$\beta$-hydroxyl group by a $-C-CH_3$ group increases the inhibitory effect as can be seen by comparing the percent inhibition of dehydroepiandrosterone sulfate and pregnenolone sulfate cleavage brought about by androstenediol and pregnenolone. In contrast to the $C_{21}$ steroids, 5$\alpha$ reduction of ring A of $C_{19}$ steroids did not diminish the inhibitory effect. A 5$\alpha$-hydrogen together with a 3$\alpha$-hydroxyl and a 17$\beta$-hydroxyl group (5$\alpha$-androstane-3$\alpha$,17$\beta$-diol) resulted in the greatest percent inhibition of steroid sulfatase activity by any of the $C_{19}$ steroids tested.

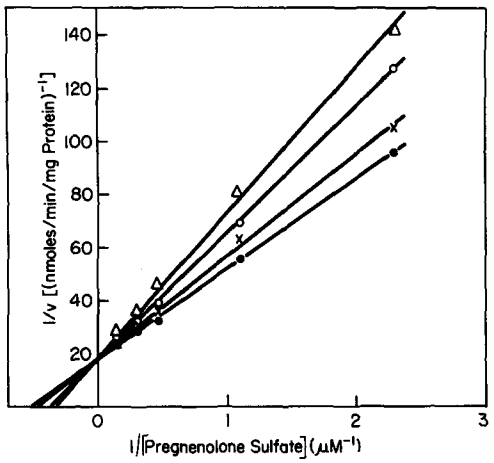


Fig. 4. Lineweaver–Burk plots of the inhibitory effect of 5-pregnene-3$\beta$,20$\alpha$-diol on the rate of formation of pregnenolone from pregnenolone sulfate. Testicular microsomal suspension (0.5 mg protein) was incubated for 20 min at 37° in 0.05 M Tris–HCl, pH 7.2, with varying concentrations of [$^3$H]pregnenolone sulfate ($5.4 \cdot 10^5$ disint./min, 0.43–6.4 $\mu$M) and fixed concentrations of 5-pregnene-3$\beta$,20$\alpha$-diol in 5% ethanol. ●—●, no inhibitor; ×—×, 3.14 $\mu$M; ○—○, 6.28 $\mu$M; △—△, 9.42 $\mu$M.

*Type of inhibition of steroid sulfate cleavage by free steroids*

Testicular microsomal fraction was incubated with varying concentrations of [$^3$H]pregnenolone sulfate (0.43–6.4 $\mu$M) in the presence of three given concentrations of 5-pregnene-3$\beta$,20$\alpha$-diol. Lineweaver–Burk plots (Fig. 4) indicate that 5-pregnene-3$\beta$,20$\alpha$-diol acts as a fully competitive inhibitor[11]. Similar results were obtained when varying concentrations of [$^3$H]pregnenolone sulfate were incubated with three different concentrations of 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol. When [$^3$H]pregnenolone sulfate was incubated at two fixed substrate concentrations in the presence of increasing concen-

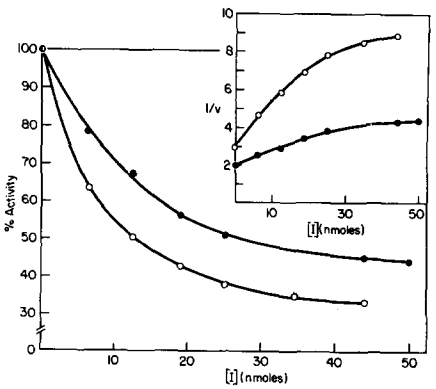


Fig. 5. Inhibition of formation of pregnenolone from pregnenolone sulfate in the presence of increasing concentrations of 5-pregnene-3$\beta$,20$\alpha$-diol. Incubation conditions same as for Fig. 4 except 4% dimethylsulfoxide used instead of 5% ethanol. Insert represents plots of $1/v$ *vs.* inhibitor concentrations. ○—○, 0.86 $\mu$M pregnenolone sulfate; ●—●, 2.13 $\mu$M pregnenolone sulfate.

trations of 5-pregnene-3$\beta$,20$\alpha$-diol, the degree of inhibition did not continue to increase, but appeared to plateau (Fig. 5). This is characteristic of partial competitive inhibition indicating that the free steroid inhibitor combines with the enzyme at a different site than the substrate. This is further illustrated in the plot of $1/v$ *vs.* $[I]$ (Fig. 5, insert) which results in a hyperbolic curve instead of a straight line[12].

*Determination of $K_i$ values for 5-pregnene-3$\beta$,20$\alpha$-diol and 5$\alpha$-androstane-3$\alpha$, 17$\beta$-diol*

These studies were carried out in the presence of 5% ethanol to insure solubility of the free steroid. Ethanol was used in these experiments rather than dimethylsulfoxide because more consistent results were obtained with the use of ethanol at low substrate concentration. In the presence of 5% ethanol, the $K_m$ value for pregnenolone sulfate was 1.9 $\mu$M and the $K_i$ value for 5-pregnene-3$\beta$,20$\alpha$-diol was 15 $\mu$M, and for 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol, 40 $\mu$M.

*Other inhibition studies*

To establish whether the $C_{19}$ and $C_{21}$ free steroids combine with the enzyme at the same site, testicular microsomal fraction was incubated with [$^3$H]pregnenolone sulfate and one of five inhibitory steroids. Equimolar concentrations of the inhibitory steroids were added alone or in combination with 5-pregnen-3$\beta$,20$\alpha$-diol and percent inhibition of pregnenolone sulfate cleavage was determined. Table III illustrates that the inhibitory effects of these free steroids, when used in combination with 5-pregnene-3$\beta$,20$\alpha$-diol are not additive. It is, therefore, concluded that these $C_{19}$ and $C_{21}$ free steroid inhibitors combine with the enzyme at the same site. However, in repeated experiments the percent inhibition by combinations of inhibitors equaled or slightly exceeded the percent inhibition by a 2-fold increase in concentration of 5-pregnene 3$\beta$,20$\alpha$-diol.

*Relationship of $K_m$ and $\bar{V}$ values of human testicular steroid sulfatase with serum testosterone levels*

Apparent $K_m$ and $\bar{V}$ values were determined by incubating various concentrations of [$^3$H]pregnenolone sulfate with testicular microsomal fraction obtained

TABLE III

PERCENT INHIBITION OF PREGNENOLONE SULFATE CLEAVAGE BY COMBINATIONS OF FREE STEROIDS

Testicular microsomal suspension (0.5 mg protein) was incubated with 2.13 $\mu$M [$^3$H]pregnenolone sulfate in the presence of indicated free steroid at a concentration of 25 $\mu$M. Incubation conditions are described under MATERIALS AND METHODS.

| *Steroid inhibitor* | *Inhibition (%)* | | |
|---|---|---|---|
| | *Inhibitor alone* | *Inhibitor plus 5-pregnene-3$\beta$,-20$\alpha$-diol, 25 mM* | *Predicted if additive* |
| 5-Pregnene-3$\beta$,20$\alpha$-diol | 49 | 56 | — |
| 5$\alpha$-Androstane-3$\alpha$,17$\beta$-diol | 31 | 59 | 80 |
| 5-Androstene-3$\beta$,17$\beta$-diol | 12 | 56 | 61 |
| 5$\alpha$-Pregnan-3$\beta$-ol-20-one | 36 | 60 | 85 |
| 5-Pregnen-3$\beta$-ol-20-one | 35 | 64 | 84 |

TABLE IV

RELATIONSHIP OF $K_m$ AND $\bar{V}$ VALUES OF HUMAN TESTICULAR STEROID SULFATASE WITH SERUM TESTOSTERONE LEVELS

Increasing concentrations of [$^3$H]pregnenolone sulfate 0.43–6.4 $\mu$M were incubated with a testicular microsomal suspension (0.5 mg protein) from each patient. $K_m$ and $\bar{V}$ values were determined from reciprocal plots of $1/v$ *vs.* $1/[S]$. Incubation conditions are described under MATERIALS AND METHODS.

| *Patient* | $K_m$ *(μM)* | $\bar{V}$ *(nmoles/min per mg protein* $\times 10^{-2}$*)* | *Serum testosterone* (ng/100 ml)* |
|---|---|---|---|
| C.P. | 1.04 | 4.9 | 262 |
| E.C. | 0.97 | 5.0 | — |
| J.McK. | 1.10 | 6.7 | 312 |
| U.K. | 1.00 | 7.3 | 520 |

* Testosterone + dihydrotestosterone.

from testes of four patients. Preoperative serum testosterone levels were measured in three of these patients. As illustrated in Table IV the $K_m$ values for pregnenolone sulfate obtained with the testicular microsomal preparations from four patients are essentially identical while the $\bar{V}$ values tend to correlate with the serum testosterone levels.

## DISCUSSION

The cleavage of dehydroepiandrosterone sulfate by a homogenate of human testis was first demonstrated by BURSTEIN AND DORFMAN[8]. Recently we determined the rate of cleavage of pregnenolone sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate in the microsomal fraction of testes from 11 patients[7]. A close correlation between the rate of cleavage of the three steroid sulfates and serum testosterone levels was observed. These studies were carried out at only one substrate concentration and no attempt at characterization of the enzyme(s) was attempted. In the present study human testicular steroid sulfatase was demonstrated to have a pH optimum between 7.0 and 7.2. Pregnenolone sulfate, dehydroepiandrosterone and androstenediol-3-sulfate were shown to be cleaved by the same enzyme. Similar results were obtained previously with rat testicular steroid sulfatase[1–3] except that the rat enzyme exhibited a pH optimum of between 6.2 and 6.4.

The inhibitory effect of various free steroids on steroid sulfatase activity has been reported for rat testicular steroid sulfatase[1–3] and for human placental steroid sulfatase[14]. In our previous studies with a microsomal preparation from rat testes[1], among 15 $C_{19}$ and $C_{18}$ free steroids investigated the most potent inhibitor was found to be 5$\alpha$-androstane-3$\beta$,17$\beta$-diol. NOTATION AND UNGAR[4] reported that 5-pregnene-3$\beta$,20$\alpha$-diol was the most effective inhibitor of pregnenolone sulfate cleavage in rat testicular homogenates. TOWNSLEY *et al.*[14] reported that the greatest per cent inhibition of the placental steroid sulfatase was brought about by 5-pregnene-3$\beta$,21-diol-20-one.

The human testicular steroid sulfatase was inhibited to the greatest degree by 5-pregnene-3$\beta$,20$\alpha$-diol and 5-pregnene-3$\beta$,21-diol-20-one. The most potent inhibitor among the $C_{19}$ steroids was found to be 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol. This is in contrast

to our observations with the rat testicular steroid sulfatase in which androstane-3$\beta$,17$\beta$-diol was found to be the most potent inhibitor. In general, structural alterations of the $C_{19}$ steroids did not change their inhibitory effect on the human testicular steroid sulfatase. With the rat enzyme, structural alteration such as a 17$\beta$-hydroxyl for a 17-keto group and a 5$\alpha$ saturated A ring for a $\Delta^5$ bond markedly increased the per cent inhibition of steroid sulfate cleavage.

The type of free steroid producing the greatest degree of inhibition of a steroid sulfatase in cells of a particular species or of a particular organ may relate to the type of steroid products synthesized in those cells. For example, in a previous study[2] in which we compared the metabolism of dehydroepiandrosterone sulfate and androstenediol-3-sulfate in a rat testicular microsomal fraction, various 5$\alpha$ reduced steroids were identified as products. 5$\alpha$-androstane-3$\beta$,17$\beta$-diol, which had been shown to be the most potent inhibitor of the steroid sulfatase activity, was found to be the most abundant free steroid identified other than testosterone and androstenedione.

The free steroid products of pregnenolone sulfate in the human testis have not been identified. However, 20$\alpha$-hydroxysteroid dehydrogenase has been reported in testicular tissue[15] and it is conceivable that 20-keto reduction of pregnenolone would prevent or markedly reduce the further metabolism of this compound to testosterone, as has been observed for 4-pregnene-3-one-20$\alpha$-ol compared to 4-pregnene-3,20-dione in monkey testes[16]. The 20$\alpha$-reduction of pregnenolone yielding 5-pregnene-3$\beta$,20$\alpha$-diol could in turn act on the steroid sulfatase by inhibiting cleavage of pregnenolone sulfate, dehydroepiandrosterone sulfate and/or androstenediol-3-sulfate thus decreasing the amount of free steroid made available as a ready source for conversion to testosterone.

Demonstration of partial competitive inhibition of the human testicular steroid sulfatase by various free steroids and demonstration of substrate inhibition at high substrate concentrations are in agreement with our earlier observations on the rat testicular steroid sulfatase[1] and suggest that the human enzyme also may function as a regulatory enzyme.

Pregnenolone sulfate, dehydroepiandrosterone sulfate and androstenediol-3-sulfate are synthesized and secreted by the human testis[5,6]. Thus, these three steroid sulfates are available as potential inactive precursors for testosterone. Modulation of the activity of the steroid sulfatase by various free steroids in the testis could be a factor controlling the concentration of these free steroids which are the necessary precursors of testosterone.

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