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STEROID METABOLISM IN TESTIS TISSUE: THE METABOLISM OF PREGNENOLONE, PREGNENOLONE SULFATE, DEHYDROEPIANDROSTERONE AND DEHYDROEPIANDROSTERONE SULFATE IN HUMAN AND BOAR TESTES *IN VITRO*

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SUMMARY

The metabolism of pregnenolone, dehydroepiandrosterone and their sulfate conjugates was studied in minced human and boar testis tissue *in vitro*. Unlabelled steroids were usually used as substrates and the steroid metabolites were analyzed by gas–liquid chromatography (g.l.c.) and gas chromatography-mass spectrometry (g.c.–m.s.). Both the unconjugated and sulfated steroids were metabolized to testosterone. Human and boar testis tissue had a very high capacity to synthesize testosterone from dehydroepiandrosterone, even from 1 mg of the substrate boar testis tissue (0.5 g) could metabolize over 500 μg to 5-androstene-3β,17β-diol, androstenedione and testosterone. Testosterone synthesis from dehydroepiandrosterone sulfate but not from pregnenolone sulfate seemed to be mainly dependent on the steroid sulfatase activity in the testis. Human and boar testes also synthesized steroid sulfates, and a direct conversion of one steroid sulfate to another took place in both tissues. In boar testis 16-androstenes were formed both from pregnenolone and pregnenolone sulfate. The conclusion is that active metabolism of sulfated steroids occurs in human and boar testes. The sulfated steroids, which are known to be quantitatively important in the testes of the two species studied, are also used as precursors of testosterone *in vitro*.

INTRODUCTION

There are many similarities in the steroid composition of human and boar testes. In addition to unconjugated steroids, the testes of both species contain large amounts of sulfated steroids[1, 2] and some of these compounds are secreted into the blood circulation[3–5]. In the testes of different species many investigators have demonstrated the formation, hydrolysis and interconversions of several neutral steroid sulfates [6–10].

In human testes unconjugated testosterone, pregnenolone sulfate and dehydroepiandrosterone sulfate are quantitatively the most important steroids[1]. In boar testis, pregnenolone sulfate and dehydroepiandrosterone sulfate are also present but their concentrations are from ten to one hundred times higher than the concentration of unconjugated testosterone[2]. Therefore we decided to investigate how these endogenous steroids are metabolized, and to determine their contribution to testosterone biosynthesis in these two species *in vitro*. As a comparison, the metabolism of unconjugated pregnenolone and dehydroepiandrosterone was also investigated.

The following trivial names have been used: dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one; androstenedione: 4-androstene-3,17-dione; testosterone: 17β-hydroxy-4-androsten-3-one; pregnenolone: 3β-hydroxy-5-pregnen-20-one; 17α-hydroxypregnenolone: 3β,17α-dihydroxy-5-pregnen-20-one; progesterone: 4-pregnene-3,20-dione; 17α-hydroxyprogesterone: 17α-hydroxy-4-pregnene-3,20-dione; stigmasterol: (24S)-24-ethyl-5,22-cholestadien-3β-ol.

EXPERIMENTAL

Tissue samples. Human testis tissue was obtained from patients undergoing orchiectomy because of prostatic carcinoma. These patients did not receive any hormone therapy prior to surgery. Boar testis tissue was taken from fertile Finnish Landrace boars immediately after slaughtering. Tissue samples were kept on crushed ice before incubations, which were started 30–45 min after orchiectomy.

Steroids and chemicals. Non-radioactive reference steroids have been described previously[2]. The purity of these compounds was certified by g.c.–m.s. The [7-^{3}H]-dehydroepiandrosterone sulfate (S.A. 1–5 Ci/mmol) and dehydroepiandrosterone [^{35}S]-sulfate (S.A. 2–5 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, United Kingdom. The purity of the radioactive compounds was verified by thin layer chromatography (t.l.c.). When necessary, Lipidex-5000™ column chromatography or t.l.c. was used for the purification of steroid standards. The doubly labelled steroid [7-^{3}H]-dehydroepiandrosterone [^{35}S]-sulfate was obtained by mixing the two former radioactive standards. ATP, NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase

were purchased from Sigma Chemical Company, Saint Louis, U.S.A. Lipidex-5000™ (hydroxyalkoxypropyl Sephadex) and the scintillation liquid "Insta-Gel" were purchased from Packard-Becker, B.V., Chemical Operations, Groningen, The Netherlands.

Incubation procedure. Minced testis tissue (0.5 g) was incubated in 10 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 containing 0.1 mmol glucose and 22 μmol $MgCl_2$. When the synthesis of steroid sulfates was studied the incubation flask also contained 5 μmol additional ATP. After this addition the synthesis of steroid sulfates could be detected by the analytical methods used. Incubations were performed at 37°C in a Dubnoff metabolic incubator for 4 h using mixture of 95% O_2 and 5% CO_2 as the gas phase. Incubations were terminated by transferring the incubation medium into 50 ml ethanol.

Usually 150 μg of non-radioactive steroid was used as the substrate. When the testosterone synthesizing capacity of boar testis tissue was further tested, incubations were also performed with one mg of dehydroepiandrosterone and dehydroepiandrosterone sulfate. The conversion of dehydroepiandrosterone sulfate to 5-androstene-3β,17β-diol sulfate was also studied using radioactive, doubly labelled [7-^{3}H]-dehydroepiandrosterone [^{35}S]-sulfate as a marker substrate.

No cofactors were usually used. However, in boar testis homogenates, two incubations were performed with unconjugated pregnenolone and dehydroepiandrosterone where the incubation flask also contained the following NADPH-generating system; glucose-6-phosphate (50 μmol), NADP (10 μmol) and glucose-6-phosphate dehydrogenase (5 i.u.).

Control incubations were performed with minced testis tissue but without added steroid substrates, and in the presence of substrates but with boiled testis tissue.

Analytical procedure. The tissue and the medium were analyzed together. The incubation was terminated by the addition of 50 ml ethanol and the tissue sample was homogenized. After filtration, the residue was rehomogenized in chloroform-methanol (1:1, v/v), filtered, and the combined filtrates were evaporated. The residue was chromatographed on a 20-g Sephadex LH-20 column (1.5 × 60.5 cm), and the free and monosulfate steroid fractions were collected as described earlier[11]. The analytical procedure for 16-androstenes has been described earlier[12]. The analysis of other unconjugated and monosulfated steroids was performed essentially as published[1], but the final fractionation of steroids before g.c.–m.s. was achieved by Lipidex column (0.5 × 24 cm) chromatography with petroleum ether:chloroform 95/5 for elution. The following fractions were collected: Fraction 1 (0–30 ml) contained progesterone and androstenedione, Fraction 2 (30–70 ml) contained pregnenolone, dehydroepiandrosterone, testosterone and 17α-hydroxyprogesterone. The solvent was then changed to methanol and Fraction 3 (70–90 ml) was collected. This fraction contained 17α-hydroxypregnenolone and the C_{19}-and C_{21}-diols. G.l.c. of the steroid trimethylsilyl (TMS) and O-methyloxime trimethylsilyl (MO-TMS) derivatives on QF-1 and SE-30 columns was performed as described previously[13]. For quantitation, stigmasterol was used as an internal standard. G.c.–m.s. was carried out on a gas chromatograph (Carlo Erba 2300)-mass spectrometer computer system (Jeol JMS D-100 Mass Data System) using the same steroid derivatives and g.l.c. columns. The identification of steroids was based on their properties identical with those of relevant reference compounds on g.l.c. and g.c.–m.s. The detection limit of the method is about 0.05 μg of steroid per incubation.

Thin layer chromatography (*t.l.c.*) was used for the separation of the sulfated metabolites when the direct conversion of one steroid sulfate to another was studied. After LH-20 chromatography the steroid monosulfate fraction was desalted using Sephadex G-25 chromatography as described by Jänne and Vihko [14]. The fraction of sulfated steroids was evaporated *in vacuo* and O-methyloxime derivatives were formed from the residue. The sample was further extracted with 40 ml ethyl acetate:water (1:1, v/v). The water fraction was re-extracted with 10 ml of ethyl acetate. Finally the water fraction was lyophilized and the residue was transferred with methanol to t.l.c. plates. The separation of steroid sulfates was performed using 20 × 20 cm precoated silica gel F_{254} layers (Merk AG, No 5715, 0.25 mm). The system was a modification of that described by Crépy *et al.*[15]. Five developments were carried out in the solvent system *n*-butylacetate–toluene–4N/NH_4OH–methanol (85:35:35:85, by vol.). The system gave a complete separation of dehydroepiandrosterone sulfate O-methyloxime and 5-androstene-3β,17β-diol monosulfate. The location of steroid sulfates on thin layer plates was performed using an automated two dimensional t.l.c. scanner (Philips PW 4007). The $^3H/^{35}S$ ratio was calculated from each band by the scintillation counter (LKB-Wallac 81000). Identification of the steroids in each radioactive band was performed after solvolysis and trimethylsilylation by g.l.c. and g.c.–m.s.

RESULTS

Methodological losses during the analytical procedure were determined for most steroids analyzed. The mean recovery percentages from three different analysis, carried out by steroid standards, were as follows: Pregnenolone 86, 17α-hydroxypregnenolone 91, dehydroepiandrosterone 86, 5-androstene-3β,17β-diol 86, progesterone 89, 17α-hydroxyprogesterone 87, androstenedione 92, testosterone 90, 5,16-androstadien-3β-ol 89, pregnenolone sulfate 84 and dehydroepiandrosterone sulfate 79. The results given in tables and figures are not corrected for methodological losses.

Table 1. The metabolites of unconjugated pregnenolone (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced human and boar testis tissue. In boar No. 3 additional NADPH-generating system was also used in tissue homogenate (B_3C). Values are expressed as micrograms per incubation

Metabolite	H_1	H_2	H_3	H_4	B_1	B_2	B_3	B_3C
17α-Hydroxypregnenolone	—	0.7	2.6	0.3	0.8	1.0	2.4	6.4
Dehydroepiandrosterone	—	—	0.8	1.2	1.1	0.8	7.2	10.1
5-Androstene-3β,17β-diol	—	—	0.5	0.4	0.5	0.2	2.8	2.0
Progesterone	1.4	0.8	0.8	—	1.2	2.6	2.8	0.5
17α-Hydroxyprogesterone	—	0.3	0.4	—	—	0.2	0.9	0.6
Androstenedione	—	—	—	—	—	—	0,6	0.8
Testosterone	—	—	1.1	0.4	0.3	1.7	3.0	3.1
5-Pregnene-3β,20α-diol	0.2	1.5	1.2	2.8	0.8	0.9	1.9	9.3

H = Human testis. B = Boar testis. C = Additional cofactors. — = Not detected.

When minced human and boar testis tissue were incubated with non-radioactive pregnenolone or dehydroepiandrosterone both 3β-hydroxy-5-ene and 3-keto-4-ene metabolites were formed (Table 1, Figs. 1 and 2). In human and boar testes the amounts of progesterone and other 3-keto-4-ene metabolites formed from pregnenolone were less than the amounts of androstenedione and testosterone formed from dehydroepiandrosterone, indicating the different substrate specificity of the 3β-hydroxysteroid dehyd-

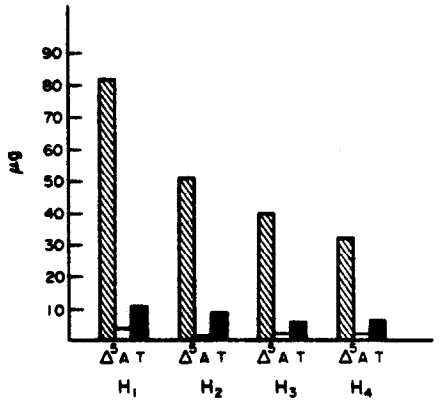


Fig. 1. The metabolites of unconjugated dehydroepiandrosterone (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced human testis tissue. Values are expressed as micrograms per incubation. Δ^5 = 5-androstene-3β,17β-diol, A = androstenedione, T = testosterone, H = human testis.

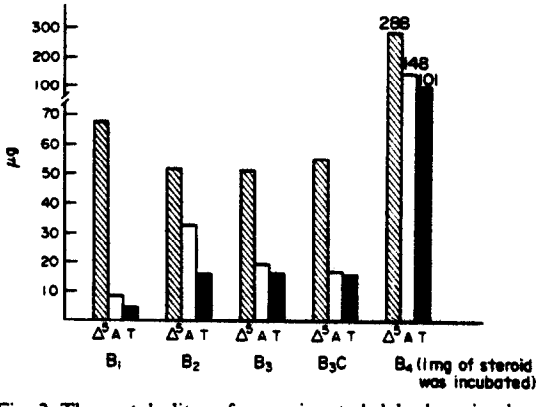


Fig. 2. The metabolites of unconjugated dehydroepiandrosterone (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced boar testis tissue. In boar No. 3 additional NADPH-generating system was used (B_3C). In boar No. 4 1 mg of the substrate was incubated. Values are expressed as micrograms per incubation. Δ^5 = 5-androstene-3β,17β-diol, A = androstenedione, T = testosterone, B = boar testis, C = additional cofactors.

Table 2. The metabolites of pregnenolone sulfate (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced human and boar testis tissue. Values are expressed as micrograms per incubation

Metabolite		H_1	H_2	H_3	H_4	B_1	B_2	B_3
Pregnenolone	F	85	41	47	118	11.6	10.3	6.2
17α-Hydroxypregnenolone	F	—	0.3	1.6	—	0.8	1.6	0.8
	M	—	—	0.3	0.8	0.4	0.2	0.6
Dehydroepiandrosterone	F	—	—	2.2	0.3	0.8	0.6	0.3
	M	—	—	0.8	0.3	0.7	0.2	0.4
5-Androstene-3β,17β-diol	F	—	—	2.6	0.4	0.3	2.1	0.2
	M	—	—	0.5	0.3	1.1	0.5	0.3
Progesterone	F	0.8	—	0.3	0.6	0.6	0.3	0.3
17α-Hydroxyprogesterone	F	—	—	—	—	—	—	—
Androstenedione	F	—	—	—	—	—	—	—
Testosterone	F	—	—	1.5	—	0.2	0.5	0.4
5-Pregnene-3β,20α-diol	F	—	2.3	0.4	0.4	0.9	2.0	1.1
	M	0.5	2.4	0.8	0.9	1.1	0.4	0.5

F = Free steroid. M = Steroid monosulfate. H = Human testis. B = Boar testis. — = Not detected.

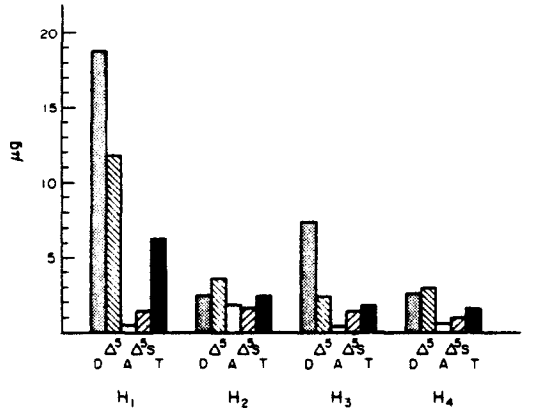


Fig. 3. The metabolites of dehydroepiandrosterone sulfate (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced human testis tissue. Values are expressed as micrograms per incubation. D = dehydroepiandrosterone, Δ^5 = 5-androstene-3β,17β-diol, A = androstenedione, Δ^5S = 5-androstene-3β,17β-diol sulfate, T = testosterone.

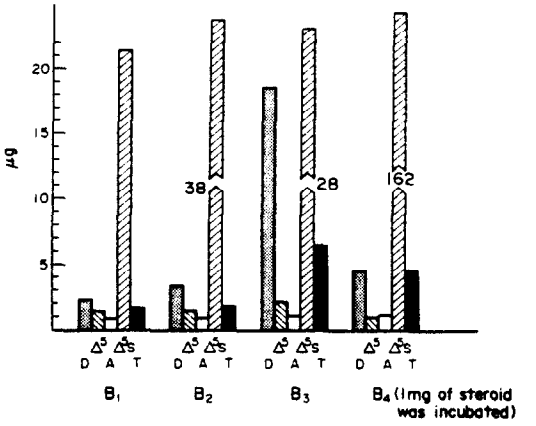


Fig. 4. The metabolites of dehydroepiandrosterone sulfate (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced boar testis tissue. In boar No. 4 1 mg of the substrate was incubated. Values are expressed as micrograms per incubation. D = dehydroepiandrosterone, Δ^5 = 5-androstene-3β,17β-diol, A = androstenedione, Δ^5S = 5-androstene-3β,17β-diol sulfate, T = testosterone.

rogenase-isomerase enzyme complex. In the boar testis homogenate an additional NADPH-generating system modified the metabolism of pregnenolone, but did not markedly increase the production of testosterone (Table 1). When pregnenolone sulfate was used as a substrate there was an increase in the production of some unconjugated and sulfated steroids. The main metabolite was the corresponding unconjugated compound (Table 2). The formation of the sulfated metabolites provides indirect evidence of the operation of the metabolic pathway, which involves only intact steroid sulfates, because in the incubation conditions used no steroid sulfates could be formed from unconjugated steroids. In both human and boar testis tissue pregnenolone and its sulfate conjugate were metabolized to testosterone, but testosterone synthesis from those precursors was much lower than from dehydroepiandrosterone and its sulfate conjugate (Tables 1 and 2, Figs. 1–4).

In all incubations the main metabolite of unconjugated dehydroepiandrosterone was 5-androstene-3β,17β-diol, followed by testosterone, in human, and androstenedione in boar testis (Figs. 1 and 2). Of the 150 μg of unconjugated dehydroepiandrosterone incubated 0.5 g of testis tissue could metabolize 30–75% to 5-androstene-3β,17β-diol, androstenedione and testosterone. In the boar testis homogenate an additional NADPH-generating system did not markedly increase the production of testosterone from unconjugated dehydroepiandrosterone (Fig. 2). When one mg of dehydroepiandrosterone was incubated with minced boar testis tissue, the total amount of metabolites increased over 500 μg per incubation (Fig. 2).

In human testis the main metabolites of dehydroepiandrosterone sulfate were the corresponding free steroid and 5-androstene-3β,17β-diol, followed by testosterone, 5-androstene-3β,17β-diol sulfate and androstenedione (Fig. 3). In boar testis the main metabolite was 5-androstene-3β,17β-diol sulfate followed by unconjugated dehydroepiandrosterone, testosterone, 5-androstene-3β,17β-diol and androstenedione (Fig. 4). Using one mg of dehydroepiandrosterone sulfate as substrate, minced boar testis tissue had the capacity to increase greatly the production of 5-androstene-3β,17β-diol sulfate, but the high substrate concentration did not have any marked effect on the production of testosterone (Fig. 4).

The possible direct conversion of dehydroepiandrosterone sulfate to 5-androstene-3β,17β-diol sulfate was studied with doubly labelled [7-^{3}H]-dehydroepiandrosterone [^{3}S]-sulfate both in human and boar testes. The band having an R_F value of 0.49 on thin layer plates (see "Experimental") had identical g.l.c. properties and mass spectra with those of dehydroepiandrosterone MO-TMS. Thus the compound was

Table 3. c.p.m. Data of the steroid sulfates identified when [7-^{3}H]-dehydroepiandrosterone [^{35}S]-sulfate was incubated with human and boar testes

Steroid	^{3}H c.p.m.	^{35}S c.p.m.	^{3}H/^{35}S
Human testis			
5-Androstene-3β,17β-diol sulfate	1968	338	5.8
Dehydroepiandrosterone sulfate	75204	12922	5.8
Boar testis			
5-Androstene-3β,17β-diol sulfate	2784	460	6.1
Dehydroepiandrosterone sulfate	35904	6088	5.9

Table 4. The unconjugated 16-unsaturated metabolites of pregnenolone (150 μg) and pregnenolone sulfate (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer at 37°C, with 0.5 g of minced boar testis tissue. Values are expressed as micrograms per incubation.

Metabolite	Boar 1 Pregnenolone as a substrate	Boar 2 Pregnenolone sulfate as a substrate
5α-Androst-16-en-3α-ol	2.9	0.1
5α-Androst-16-en-3β-ol	13.3	0.9
5.16-Androstadien-3β-ol	5.4	2.5

Table 5. The sulfated metabolites of unconjugated pregnenolone (150 μg) and dehydroepiandrosterone (150 μg) after 4 h incubation in 10 ml of Krebs–Ringer bicarbonate buffer with additional ATP at 37°C, with 0.5 g of minced human and boar testis tissue. Values are expressed as micrograms per incubation

Metabolite	Substrate:	Pregnenolone			Dehydroepiandrosterone		
	Subject:	H_1	H_2	B_1	H_1	H_2	B_1
Pregnenolone sulfate		0.2	0.2	1.1	—	—	—
Dehydroepiandrosterone sulfate		0.2	—	0.4	0.4	0.3	6.1
5-Androstene-3β,17β-diol sulfate		—	—	—	0.2	0.2	0.3

H = Human testis. B = Boar testis. — = Not detected.

identified as the precursor dehydroepiandrosterone sulfate. Using the same criteria the band having an R_F value of 0.38 was identified as 5-androstene-3β,17β-diol sulfate. The c.p.m. values and the $^3H/^{35}S$ ratios of the identified compounds are given in Table 3. The results indicate that the conversion of dehydroepiandrosterone sulfate to 5-androstene-3β,17β-diol sulfate took place without hydrolysis of the substrate. In boar testis the formation of 16-androstenes was demonstrated from pregnenolone and pregnenolone sulfate (Table 4). When dehydroepiandrosterone and its sulfate conjugate were incubated with boar testis tissue 16-androstenes were also measured, but no increase was noticed in their production. In the presence of additional ATP the formation of sulfated steroids from unconjugated pregnenolone and dehydroepiandrosterone is presented in Table 5. The main metabolites are the corresponding sulfated steroids.

DISCUSSION

There are great species differences in the testicular composition of unconjugated and sulfated steroids[1, 2, 16]. In addition to unconjugated steroids, sulfated steroids seem to play a central role in human and boar testis tissue *in vivo*[1, 2]. Human and boar testes can also secrete sulfated steroids into the blood circulation[3–5] and in human testis this secretion is increased after HCG stimulation[17]. In recent years data has accumulated indicating that these compounds can also be used as precursors of testosterone in testis[7–10]. Among the sulfated steroids present in human and boar testes, pregnenolone sulfate and dehydroepiandrosterone sulfate were chosen for incubation studies because their concentrations are very high in these tissues. For comparison, the metabolism of unconjugated pregnenolone and dehydroepiandrosterone was investigated. Unlabelled steroids were used as substrates in rather high concentrations and the total amount of steroid metabolites was analyzed. Under the conditions used, all four substrates, unconjugated and sulfated, were metabolized to testosterone in human and boar testes *in vitro*.

Both 3β-hydroxy-5-ene and 3-keto-4-ene metabolites were formed in human and boar testis tissue from pregnenolone and pregnenolone sulfate, but their concentrations were considerably lower when compared to the metabolites of dehydroepiandrosterone or its sulfate conjugate. Additional NADPH-generating system did not increase the production of testosterone in boar testis homogenate. It has been shown earlier that in the reaction chain from pregnenolone to testosterone the intermediates 17α-hydroxypregnenolone and 17α-hydroxyprogesterone competitively inhibit the 17α-hydroxylation of both pregnenolone and progesterone[18], and in these reactions the saturation of the binding sites of cytochrome P-450 necessary for both 17α-hydroxylase and C_{19}-C_{21} lyase enzymes is an important inhibiting factor[19]. The inhibition of the metabolism of pregnenolone sulfate by its metabolites has also been demonstrated in the human testis[20]. In these incubations, where steroid metabolites are accumulating, a more effective product inhibition of pregnenolone metabolism may explain why testosterone production from pregnenolone and pregnenolone sulfate is in some case negligible or remarkably lower than from dehydroepiandrosterone or dehydroepiandrosterone sulfate.

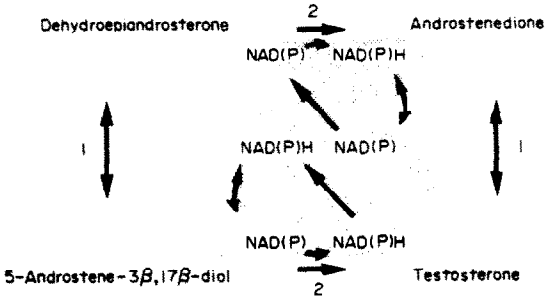
In a kinetic study, Yanaihara and Troen have compared the 3β-hydroxy-5-ene and 3-keto-4-ene metabolic pathways in human testis *in vitro*[21]. They con-

cluded that in human adult testis tissue the 3β-hydroxy-5-ene pathway is favored in the biosynthesis of testosterone. In the present investigation the formation of 3β-hydroxy-5-ene compounds was more evident both in human and boar testes. The major steroids produced by the human testis are known to be effective inhibitors of 3β-hydroxysteroid dehydrogenase for pregnenolone[22]. In this investigation less progesterone and other 3-keto-4-ene metabolites were formed from pregnenolone than androstenedione and testosterone from dehydroepiandrosterone. Therefore dehydroepiandrosterone is a better substrate than pregnenolone for the 3β-hydroxysteroid dehydrogenase–isomerase enzyme complex in both human and boar testes. This difference in substrate specificity may also explain why pregnenolone is more readily metabolized through the 3β-hydroxy-5-ene pathway to testosterone.

The main metabolite of pregnenolone sulfate both in human and boar testes was the corresponding free compound, but sulfated metabolites were also detected. Under the same incubation conditions no noticeable amount of sulfated steroids were formed from unconjugated pregnenolone or dehydroepiandrosterone. This provides indirect evidence of the operation of the sulfated pathway from pregnenolone sulfate through 17α-hydroxypregnenolone sulfate and dehydroepiandrosterone sulfate to 5-androstene-3β,17β-diol sulfate. Recently the direct conversion of pregnenolone sulfate through 17α-hydroxypregnenolone sulfate to dehydroepiandrosterone sulfate was demonstrated in boar testis *in vitro*[23]. In the present work, the direct conversion of dehydroepiandrosterone sulfate to 5-androstene-3β,17β-diol sulfate is demonstrated both in human and boar testes *in vitro*. Thus the sulfated pathway from pregnenolone sulfate as far as 5-androstene-3β,17β-diol sulfate has been demonstrated to operate in boar testis. It has been shown earlier that the adrenals and human fetal testis can also biosynthesize sulfated steroids by a pathway involving intact steroid sulfates[24–26].

Unconjugated dehydroepiandrosterone was metabolized very actively to 5-androstene-3β,17β-diol, androstenedione and testosterone by both human and boar testes *in vitro*. When the substrate concentration was changed from 150 μg to 1 mg per incubation flask, minced boar testis tissue still had the capacity to increase greatly the production of 5-androstene-3β,17β-diol, androstenedione and testosterone. The total amount of the metabolites was over 500 μg after 4 h incubation. It was not further investigated how much testosterone can be synthesized from dehydroepiandrosterone by a small piece of testis tissue. On the basis of these results dehydroepiandrosterone metabolism to testosterone is poorly inhibited in testis.

The following explanation for this high conversion of dehydroepiandrosterone to testosterone is given. Dehydroepiandrosterone can be metabolized to testosterone *via* two pathways (Fig. 5). One goes through



1 17β-Hydroxysteroid dehydrogenase
2 3β-Hydroxysteroid dehydrogenase-isomerase

Fig. 5. The two metabolic pathways from dehydroepiandrosterone to testosterone. Reutilization of pydridine nucleotides may explain the high conversion of dehydroepiandrosterone to testosterone in the reactions, which seem to be poorly inhibited in testis.

androstenedione and another through 5-androstene-3β,17β-diol. In both pathways there is one oxidation and one reduction reaction catalyzed by 3β-hydroxysteroid dehydrogenase-isomerase and 17β-hydroxysteroid dehydrogenase enzymes and if these enzymes can use the same coenzymes there is no consumption of reduced or oxidized formes of pyridine nucleotides, and these nucleotides can be continuously reutilized for the metabolism of dehydroepiandrosterone.

Added NADPH-generating system did not seem to have any great effect on the 3β-hydroxysteroid dehydrogenase-isomerase or 17β-hydroxysteroid dehydrogenase enzyme reactions when unconjugated dehydroepiandrosterone was used as a substrate in the boar testis homogenate. It remains to be elucidated whether these enzyme reactions take place only in the interstitial cells or whether the tubules, as a major compartment, participate in this high conversion of dehydroepiandrosterone to testosterone. On the basis of results published previously[27], the latter is very possible because in the human testis the enzymes necessary for the conversion of dehydroepiandrosterone to testosterone are located both in the interstitial and tubular tissues. In the rat testis the 3β-hydroxysteroid dehydrogenase-isomerase activity has been reported to be mainly located in the interstitial cells [28].

Testosterone production from dehydroepiandrosterone sulfate, but not from pregnenolone sulfate, seems to be mostly dependent on the steroid sulfatase activity in human and boar testes. Both tissues have much higher capacity to convert unconjugated dehydroepiandrosterone to testosterone than is liberated from its sulfate conjugate by testicular steroid sulfatase, but more pregnenolone can be liberated from its sulfate conjugate by this enzyme than can be immediately metabolized to testosterone. When the dehydroepiandrosterone sulfate concentration was increased to one mg per incubation flask there was no effect on the steroid sulfatase activity, or on the production of testosterone in minced boar testis tissue.

The only marked change was the very high production of 5-androstene-3β,17β-diol sulfate. Generally boar testis tissue metabolized the incubated steroids to testosterone more efficiently than the human testis. A clear difference is seen in the metabolism of dehydroepiandrosterone sulfate. This steroid conjugate is mainly metabolized to 5-androstene-3β,17β-diol sulfate by boar testis tissue, but in the human testes obtained from old men, this metabolite is quantitatively of minor importance *in vitro*. In these incubations unconjugated dehydroepiandrosterone was the best immediate precursor of testosterone, followed by dehydroepiandrosterone sulfate, pregnenolone and pregnenolone sulfate.

In human and boar testes unconjugated and sulfated 16-androstenes are present *in vivo* and in the boar they are quantitatively the most important steroids[2, 12]. Some 16-androstenes, also called "boar-taint" steroids, have pheromonal action and are physiologically important for the animal[29]. In human testes these steroids are quantitatively of minor importance and their physiological role is unknown. Therefore, in this investigation the synthesis of unconjugated 5,16-androstadien-3β-ol, 5α-androst-16-en-3β-ol and 5α-androst-16-en-3α-ol was investigated in boar testis. All the three 16-androstenes were synthesized from pregnenolone and pregnenolone sulfate. In support of the earlier findings[29] the 16-androstenes were not formed from dehydroepiandrosterone or its sulfate conjugate. Recently a new metabolic pathway involving intact steroid sulfates from pregnenolone sulfate to 5,16-androstadien-3β-ol sulfate was demonstrated in boar testis *in vitro*[23].

In human and boar testes many of the potential precursor steroids of testosterone are present in sulfate conjugated form and their tissue concentrations are very high. It has been shown that human and boar testes have the ability to actively synthesize, directly metabolize and hydrolyze these sulfated steroids and use them also as precursors of testosterone *in vitro*. The mechanisms in the testis which regulate the synthesis and hydrolysis of steroid sulfates *in vivo* remain to be studied.

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