Free and Sulfate-Conjugated Neutral Steroids in Human Testis Tissue†

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ABSTRACT: Fractions of free, monosulfated, and disulfated neutral steroids were isolated by chromatography on Sephadex LH-20 from human testes taken from cadavers and in connection with orchiectomy for prostatic cancer. The steroids in these fractions were identified by gas–liquid chromatography and gas chromatography–mass spectrometry and were quantified. The following unconjugated steroids were found: testosterone, androstenedione, dehydroepiandrosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol, pregnenolone, and 5-pregnene-3β,20α-diol. The following compounds were present as sulfate conjugates: androsterone, epiandrosterone, dehydroepiandrosterone, testosterone, epitestosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol, 5-androstene-3β,16α,17β-triol, pregnenolone, 17α-hydroxypregnenolone, 5α-pregnane-3α,20α-diol, 5β-pregnane-3α,-20α-diol, and 5-pregnene-3β,20α-diol. The main compounds were dehydroepiandrosterone sulfate, pregnenolone sulfate, testosterone, and pregnenolone. These results strengthen the view that sulfated precursors play an important role in the testicular biosynthesis of testosterone and that steroid sulfatase may therefore be involved in the regulation of this process. In addition, the Δ^5 pathway in testicular steroidogenesis seems to be important in the human testis *in vivo*.

The role of neutral steroid sulfates in the steroid metabolism of the human organism, except during pregnancy, is obscure. However, several observations suggest that these compounds are not mere water-soluble end products of metabolism to be eliminated from the organism but play a more active role. Thus, dehydroepiandrosterone[1] sulfate is excreted in large amounts by the human adrenal cortex (Baulieu, 1962) and metabolic changes in the steroid nucleus are possible without hydrolysis of the conjugate (see Lieberman, 1967). In testis tissue, particularly, many workers have demonstrated the formation, hydrolysis, and interconversion of several neutral steroid sulfates (summarized in Tamm, 1967; Laatikainen *et al.*, 1969) and the human testis has been shown to secrete these conjugates (Laatikainen *et al.*, 1969, 1971). Recent data suggest that testosterone formation may be regulated by testicular sulfatase (Notation and Ungar, 1969a–c; Payne *et al.*, 1969). In these last-mentioned studies the activity of this enzyme in rat testis tissue was found to be inhibited by certain unconjugated steroids, and Notation and Ungar (1969c) found that human chorionic gonadotropin (HCG) lowers this product inhibition. The human testis has also been shown to secrete sulfate-conjugated neutral steroids and the process is dependent on HCG, observations which suggest that steroid sulfates may play a role in testicular steroid biosynthesis (Laatikainen *et al.*, 1969, 1971).

As a step toward a better understanding of the interrelationships between free and sulfate-conjugated neutral steroids in steroid-forming tissues this paper describes their identification and quantification in human testis tissue obtained from cadavers or during orchiectomy of patients with prostatic cancer.

Materials

Testes were obtained from cadavers (7 subjects) or in connection with orchiectomy (6 subjects) performed because of prostatic cancer. Data on the samples are given in Table I. The testicular capsule was removed and tissue specimens of *ca.* 0.5 g were removed for hemoglobin determination. The tissue samples were weighed and stored at −20° until analyzed. No medication was given to the patients with prostatic cancer before operation.

Methods

Both testes of a subject were combined for steroid determinations. The tissue sample was immersed in acetone–ethanol (1:1), 5 ml to 1 g of tissue, homogenized with an Ultra-Turrax homogenizer, and placed in an ultrasonic bath for 10 min. The sample was kept on a water bath at 39° overnight and then filtered, and the precipitate was resuspended in chloroform–methanol (1:1, 5 ml to 1 g of tissue) and again ultrasonicated. After filtration, the combined filtrates were evaporated *in vacuo* and the residue was dissolved in 3- and 1-ml portions of chloroform–methanol (1:1) containing sodium chloride (0.01 mole/l.) and applied to a column of 8 g of Sephadex LH-20 (10 × 445 mm) in the same solvent. Unconjugated steroids were eluted in the fraction at 20–45 ml and steroid monosulfates at 45–110 ml of the effluent. The solvent was changed to methanol and a disulfate fraction was collected at 110–190 ml of the effluent.

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[1] Trivial and systematic nomenclature of the steroids: androsterone, 3α-hydroxy-5α-androstan-17-one; epiandrosterone, 3β-hydroxy-5α-androstan-17-one; dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one; testosterone, 17β-hydroxy-4-androsten-3-one; epitestosterone, 17α-hydroxy-4-androsten-3-one; 5α- (or 5β-) dihydrotestosterone, 17β-hydroxy-5α- (or 5β-) androstan-3-one; androstenedione, 4-androstene-3,17-dione; 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, (24*S*)-24-ethyl-5,22-cholestadien-3β-ol.

TABLE I: Data on Patients Studied.

Cadaver Testes					Testes Obtained during Orchiectomy		
Subject	Age (years)	Time Interval after Death (hr)	Weight of Combined Testes (g)	Cause of Death	Subject	Age	Weight of Combined Testes (g)
VJ	16	4	26.8	Cerebral hemorrhage	SJ	67	19.6
RP	18	2	24.5	Pulmonary fatty embolism	WW	68	6.1
RM	24	5	29.1	Suicide (intoxication with clopenthixol and orphenadrine)	PK	68	47.0
BV	46	15	23.4	Pneumohemothorax	SY	70	28.1
VH	47	18	24.9	Coronary infarction	WH	74	34.7
HO	50	11	30.5	Drowning	SK	80	26.5
LV	76	4	21.3	Cerebral hemorrhage			

TABLE II: Relative Retention Time Values of Me_3Si and MO-Me_3Si Derivatives of Steroids of Testis Tissue and Those of Corresponding Derivatives of Relevant Reference Compounds. Conditions 2.2% SE-30 225° and 3% QF-1 210°. Cholestane = 1.00.

	SE-30				QF-1				
	Compound from Testis		Reference Compound		Compound from Testis		Reference Compound		
Identification	Me_3Si	MO-Me_3Si	Me_3Si	MO-Me_3Si	Me_3Si	MO-Me_3Si	Me_3Si	MO-Me_3Si	Fraction[b]
Androsterone	0.38		0.38	0.46	1.08		1.08	0.53	M
Epiandrosterone	0.49		0.49	0.58	1.46		1.47	0.705	M
Dehydroepiandrosterone	0.47		0.47	0.56	1.31		1.31	0.64	F,M
Epitestosterone	0.54	0.61	0.54	0.61	*a*	0.84; 0.88	2.34	0.84; 0.88	M
Testosterone	0.63	0.71	0.63	0.71	*a*	0.96; 1.00	2.73	0.95; 1.00	F,M
Androstenedione		0.65		0.66		*a*		1.09; 1.13	F
5α-Androstane-3α,17β-diol	0.49		0.49		0.44		0.44		F,M,D
5α-Androstane-3β,17β-diol	0.62		0.62		0.61		0.61		F,M,D
5-Androstene-3β,17α-diol	0.53		0.53		0.49		0.49		F,M,D
5-Androstene-3β,17β-diol	0.61		0.61		0.57		0.57		F,M,D
5-Androstene-3β,16α,17β-triol	1.20		1.18		0.98		0.97		M
Pregnenolone	0.78	1.00	0.78	1.00	1.79	1.02	1.83	1.02	F,M
17α-Hydroxypregnenolone	*a*	1.33	1.13	1.31	*a*	1.06	1.96	1.06	M
5α-Pregnane-3α,20α-diol	0.93		0.93		*a*		0.86		M,D
5β-Pregnane-3α,20α-diol	0.96		0.96		*a*		0.92		M,D
5-Pregnene-3β,20α-diol	1.14		1.14		1.06		1.06		F,M,D
Progesterone				1.16				1.77; 1.81	
17α-Hydroxyprogesterone			1.14	1.49			2.62	1.83	

[a] Impurities or other steroids did not allow the determination of relative retention time values. [b] F = free steroid fraction, M = steroid monosulfate fraction, D = steroid disulfate fraction.

The fraction of *unconjugated steroids* was taken to dryness, dissolved in 100 ml of ethyl acetate and washed with 10 ml of 0.1 N NaOH and twice with 10 ml of water. The ethyl acetate phase was dried with magnesium sulfate and evaporated to dryness. The steroids were fractionated on 3-g silicic acid columns (210 × 6 mm) as described previously (Laatikainen and Vihko, 1969). The column was eluted with 20 ml of 10% ethyl acetate in benzene (fraction I), 20 ml of 27% ethyl acetate in benzene (fraction II), 20 ml of 35% ethyl acetate in benzene (fraction III), 20 ml of ethyl acetate (fraction IV), and 20 ml of methanol. Fractions II and III were further purified by thin-layer chromatography, with five developments in the solvent system ethyl acetate–cyclohexane (30:70, v/v) on 20 × 20 cm precoated silica gel F_{254} layers (Merck AG, No. 5715, 0.25 mm).

The mono- and disulfate fractions were evaporated to dryness and solvolyzed as described by Burstein and Lieberman (1958a). The liberated steroids were fractionated on 3-g silicic acid columns as described above.

Gas–liquid chromatography (*glc*) was performed on 3%

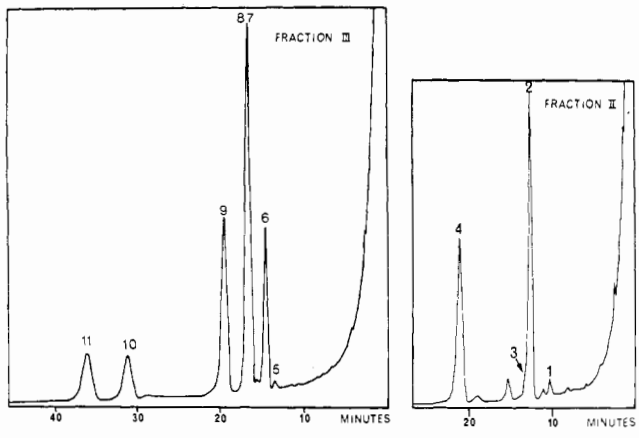

FIGURE 1: Gas chromatographic analysis of the Me_3Si derivatives (fraction II) and MO-Me_3Si derivatives (fraction III) of steroids in the monosulfate fraction of human testis tissue. Fractions were eluted from silicic acid with 27% (fraction II) and 35% (fraction III) ethyl acetate in benzene. 1 = androsterone, 2 = dehydroepiandrosterone, 3 = epiandrosterone, 4 = pregnenolone, 5 = 5α-androstane-3α,17β-diol, 6 = 5-androstene-3β,17α-diol, 7 = 5-androstene-3β,17β-diol, 8 = 5α-androstane-3β,17β-diol, 9 = testosterone, 10 = 5-pregnene-3β,20α-diol, 11 = 17α-hydroxypregnenolone. 2.2% SE-30 on Gas Chrom S 100–120 mesh (2 m × 3.5 mm), 225°. The compounds not separated on this column are resolved on the QF-1 liquid phase.

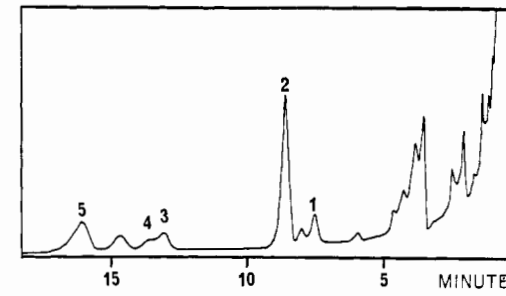

FIGURE 2: Gas chromatographic analysis of the Me_3Si ethers of steroids in the disulfate fraction of human testis tissue. Fraction eluted from silicic acid with 35% ethyl acetate in benzene. 1 = 5-androstene-3β,17α-diol, 2 = 5-androstene-3β,17β-diol, 3 = 5α-pregnane-3α,20α-diol, 4 = 5β-pregnane-3α,20α-diol, 5 = 5-pregnene-3β,20α-diol. Column and conditions as in Figure 1.

QF-1 and 2.2% SE-30 columns as described previously (Vihko, 1966). Before analysis, the steroids were converted to trimethylsilyl (Me_3Si) or *O*-methyloxime trimethylsilyl (MO-Me_3Si) ether derivatives. For quantification a suitable amount of stigmasterol (15–20 μg) was added as an internal standard before glc.

Gas chromatography–mass spectrometry (gc–ms) of the Me_3Si and MO-Me_3Si derivatives was performed with an LKB Model 9000 gas chromatograph–mass spectrometer, using QF-1 and SE-30 columns (see Vihko, 1966). The energy of bombarding electrons was 70 eV and the ionizing current 60 μA.

The *blood content* of the testis tissue was determined essentially by the method of Hohorst *et al.*, 1959, measuring hemoglobin as cyanmethemoglobin.

Results

Identification of Steroids in Testis Tissue. UNCONJUGATED STEROIDS. Preliminary gc–ms analyses showed that the C_{19} and C_{21} steroids present in the fraction of unconjugated steroids were eluted from the silicic acid column in fractions II and III. Because nonsteroidal contaminants were present in both fractions, further purification by tlc was necessary. Reference compounds were chosen on the basis of the preliminary gc–ms studies, and in this way highly purified fractions were obtained. The relative retention time values of the Me_3Si and MO-Me_3Si derivatives of steroids found and those of the corresponding derivatives of relevant reference compounds are seen in Table II. Final identification was carried out by gc–ms, with identical columns and conditions. The following unconjugated steroids were found in testis tissue: testosterone, androstenedione (for mass spectra, see Laatikainen *et al.*, 1969; Huhtaniemi *et al.*, 1970a; Dray and Weliky, 1970), dehydroepiandrosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol, pregnenolone, and 5-pregnene-3β,20α-diol (for mass spectra, see Vihko, 1966; Sjövall and Vihko, 1968). The 5α structure of both androstanediols was confirmed by oxidation of the corresponding tlc subfractions with CrO_3 in acetone (see Laatikainen and Vihko, 1969). 5α-Androstane-3,17-dione was the only product obtained. The configuration of the hydroxyl groups at C_3 and C_{17} was clarified by glc, because 5α-androstanediols are separated on the columns used (Laatikainen *et al.*, 1968).

STEROID MONO- AND DISULFATES. Figures 1 and 2 show glc analyses of steroids in the mono- and disulfate fractions of testis tissue. The following compounds were found in the monosulfate fraction (Table II): androsterone, epiandrosterone, dehydroepiandrosterone, testosterone, epitestosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol, 5-androstene-3β,16α,17β-triol (for the mass spectrum see Laatikainen, 1970), pregnenolone, 3β,17α-dihydroxy-5-pregnen-20-one (for the mass spectrum see Huhtaniemi *et al.*, 1970b), 5α-pregnane-3α,20α-diol, 5β-pregnane-3α,20α-diol (for the mass spectra, see Laatikainen *et al.*, 1968), and 5-pregnene-3β,-20α-diol. Epitestosterone as the Me_3Si and MO-Me_3Si derivatives gave mass spectra closely similar to those of the corresponding derivatives of testosterone (see Laatikainen *et al.*, 1969; Huhtaniemi *et al.*, 1970a). It was well separated from testosterone on both the columns used (Table II).

Quantities of Steroids in Testis Tissue. UNCONJUGATED STEROIDS. After chromatography on tlc the zones corresponding to the mobility of reference steroids were scraped off and, after addition of the internal standard, quantified by glc, as Me_3Si or MO-Me_3Si derivatives on QF-1 and SE-30 columns. The homogeneity of the peaks to be quantified was checked by gc–ms. The concentrations of unconjugated testosterone, dehydroepiandrosterone, 5-androstene-3β,17β-diol, 5α-androstane-3β,17β-diol, pregnenolone, and 5-pregnene-3β,20α-diol are listed in Table III. 5α-Androstane-3α,17β-diol and 5-androstene-3β,17α-diol were present in all samples in concentrations below 2 μg/100 g of tissue. However, nonsteroidal contaminants prevented their exact quantification. Androstenedione was present in trace amounts in four of the samples. As seen in Table III, the main free steroid was testosterone, followed by pregnenolone.

STEROID MONO- AND DISULFATES. The concentrations of these compounds in testis tissue are shown in Table III. Of the monosulfates, dehydroepiandrosterone and pregnenolone were the main compounds, followed by 5-androstene-3β,17β-diol, 5-androstene-3β,17α-diol, and testosterone. Of the other compounds, it might be mentioned that in all the samples studied epitestosterone was present in concentrations

TABLE III: Concentrations of Free Steroids and Steroid Mono- and Disulfates in Testis Tissue. The Values Are Expressed as Micrograms of the Free Steroid in 100 g of Tissue and Are Uncorrected for Methodological Losses.

Compound	e	Cadaver Testes								Testes Obtained during Orchiectomy						
		VJ	RP	RM	BV	VH[a]	HO	LV[a]	Mean	SJ	WW	PK	SY	WH	SK	Mean
Androsterone	M	4.4	12	15	4.8		1.3		7.5	3.6	12	3.6	12	11	4.6	7.8
Epiandrosterone	M	3.4	4.9	12	1.2		<0.5		4.4	2.1	3.5	<0.5	5.2	1.9	<0.5	2.3
Dehydroepiandrosterone	F	*d*		<0.5						<0.5	4.0	<0.5	<0.5	<0.5	1.5	1.3
	M	130	45	210	43		23		90	67	78	10	32	38	19	41
Testosterone	F	13	25	30	51	33	59	9.0	31	41	130	80[c]		14	66	55
	M	24	*b*	70	4.1		1.2		25	10	3.2	5.7	7.7	11	3.1	6.8
5α-Androstane-3α,17β-diol	F	2.2	0.5	2.5	*b*	1.7	*b*	<0.5	1.5	*b*	*b*	*b*	*b*	0.5	*b*	
	M	2.6	0.5	2.6	1.2		0.5		1.5	1.0	5.6	0.5	1.1	0.8	3.6	2.1
	D									<0.5	1.2	0.5	0.5	<0.5	0.5	0.6
5α-Androstane-3β,17β-diol	F	1.7	7.8	*b*	6.4	10	6.4	4.7	6.2	*b*	*b*	*b*	*b*	8.3	*b*	
	M	4.8	2.3	14	2.6		1.8		5.1	2.8	5.8	1.5	2.0	2.1	2.5	2.8
	D									<0.5	1.3	0.5	0.5	<0.5	1.2	0.8
5-Androstene-3β,17α-diol	F	*b*	<0.5	1.2	*b*	<0.5	*b*	<0.5	0.7	*b*	*b*	*b*	*b*	0.5	*b*	
	M	21	19	46	9.5		6.5		20	18	21	10	13	14	11	15
	D	6.6		12	1.3		0.7		5.2	11	12	2.6	2.1	2.9	2.4	5.5
5-Androstene-3β,17β-diol	F	1.2	3.8	*b*	6.2	6.8	3.3	<0.5	3.6	*b*	*b*	*b*	*b*	3.0	*b*	
	M	52	19	120	11		5.0		41	31	31	10	18	13	11	19
	D	6.7		15	2.9		1.7		6.6	7.5	18	2.3	6.5	2.8	2.8	6.7
Pregnenolone	F	36	24	42	19	28	35	16	29	38	43	40[c]		11	20	25
	M	83	42	190	12		13		68	52	160	18	23	26	27	51
3β,17α-Dihydroxy-5-pregnen-20-one	M			30						5.6	5.0	1.3	1.2	3.1	5.0	3.5
5α-Pregnane-3α,20α-diol	M	<0.5	0.8	1.2	0.7		0.7		0.8	1.5	1.0	0.5	1.0	1.8	0.5	1.1
	D				<0.5					1.0	<0.5	0.5	0.8	1.2	<0.5	0.8
5β-Pregnane-3α,20α-diol	M	<0.5	0.5	1.1	0.5		0.5		0.6	1.3	1.2	0.5	0.5	2.1	0.5	1.0
	D				<0.5					<0.5	<0.5	<0.5	<0.5	0.5	<0.5	
5-Pregnene-3β,20α-diol	F	3.4	<0.5	5.7	3.6	8.0	2.2	<0.5	3.4	*b*	*b*	9.3[c]		*b*	3.5	4.3
	M	18	7.3	25	5.1		3.3		12	8.2	9.4	2.9	2.4	2.5	4.1	4.9
	D	4.2		5.1	1.9		1.3		3.1	4.0	8.5	3.1	2.2	3.4	2.0	3.9

[a] Steroid sulfates were not determined. [b] Impurities disturbing the peak. [c] These values were obtained after combination of silicic acid fractions II and III of free steroids from testes PK and SY. [d] Not found. [e] F = free steroid fraction, M = steroid monosulfate fraction, D = steroid disulfate fraction.

below 2 μg/100 g of tissue. Nonsteroidal contaminants or other steroids prevented its exact quantification.

Of the disulfates, 5-androstene-3β,17β-diol, 5-androstene-3β,17α-diol, and 5-pregnene-3β,20α-diol were the main compounds (Table III).

The *blood content* of the testis samples obtained from the patients with prostatic cancer averaged 4.1 ml in 100 g of tissue.

Discussion

It has been demonstrated repeatedly that chromatography on Sephadex LH-20 gives efficient separation of free, glucuronide-conjugated, monosulfated, and disulfated steroids (see Vihko, 1966; Jänne *et al.*, 1969; Laatikainen *et al.*, 1969; Laatikainen and Vihko, 1969). In this study, the free steroid fraction was further purified by solvent partition, silicic acid, and thin-layer chromatography. The sulfate conjugates were cleaved under conditions in which neither steroid glucuronides (Burstein *et al.*, 1960) nor phosphates (Burstein and Lieberman, 1958b) are hydrolyzed. All the steroids found were identified by glc and gc–ms, and quantifications were performed by glc in conditions in which the determinations were specific. For certain compounds occurring at very low concentrations in testis tissue an accurate specific determination was not always possible (see Table III).

Because considerable quantities of steroid sulfates circulate in blood plasma (see Vihko, 1966; Jänne *et al.*, 1969), the possibility that the samples were contaminated with plasma steroids has to be given serious consideration. The blood content of the testis samples obtained from the patients with prostatic cancer averaged 4.1%. The peripheral plasma concentrations of the main sulfated steroid, dehydroepiandrosterone sulfate, in the corresponding age group is 44 μg/100 ml (see Vihko, 1966) and therefore the contribution of plasma dehydroepiandrosterone sulfate would be about 1.8 μg/100 g of testis tissue studied. Because concentrations of 10–78 μg/100 g of tissue were found, the contribution of plasma steroid sulfates to the figures obtained is negligible.

The material of the present study consisted of testicular samples from cadavers and from patients with prostatic cancer. These two groups of samples were similar in steroid composition, which shows that postmortem changes in testicular steroids take place very slowly. In one subject (RM, Tables I and III) most of the steroids determined were present in considerably higher concentrations than in any of the others. This may have been due to interindividual variation in the concentrations of the steroids, which is considerable, or to the exceptional cause of death (intoxication with clopenthixol and orphenadrine). It remains to be shown whether the steroid composition of completely normal testes is the same as that in testes removed from cadavers or from patients suffering from prostatic cancer.

Altogether 9 unconjugated neutral steroids were identified in testis tissue, the main compound being testosterone. Only one additional steroid with a 3-keto-Δ^4 structure was found, androstenedione, and this was present in very low concentrations. Despite a search, progesterone and 17α-hydroxyprogesterone were not found with the present methods. This indicates that the concentrations of these compounds must be low in testis tissue (below 1–2 μg/100 g). In dog testis progesterone was observed to be present in a lower concentration than any of the other steroids found (see Eik-Nes, 1970). On the other hand, several compounds with a 3β-hydroxy-Δ^5 structure (pregnenolone, 5-pregnene-3β,20α-diol, dehydroepiandrosterone, 5-androstene-3β,17α-diol, and 5-androstene-3β,17β-diol) were present in measurable concentrations. The absence of progesterone and 17α-hydroxyprogesterone either might be due to rapid metabolism of these compounds or might suggest that under conditions *in vivo* the Δ^5 pathway leading to testosterone is utilized to a large extent. However, an intermediate, 17α-hydroxypregnenolone, in the pathway pregnenolone → 17α-hydroxypregnenolone → dehydroepiandrosterone → 5-androstene-3β,17β-diol → testosterone, was not found as an unconjugated steroid, but was present as a monosulfate. The normal human testis secretes both 17α-hydroxypregnenolone and 17α-hydroxyprogesterone (Laatikainen *et al.*, 1971).

A quite remarkable feature of the steroid composition of human testis tissue is the presence of a number of sulfate-conjugated C_{19} and C_{21} steroids. It has already been demonstrated that boar testis secretes dehydroepiandrosterone sulfate (Baulieu *et al.*, 1967), that testis with interstitial cell carcinoma secretes the same conjugate (Lipsett *et al.*, 1966) and that normal human testis secretes testosterone, pregnenolone, and possibly 5-androstene-3β,17β-diol sulfates (Laatikainen *et al.*, 1969). Upon stimulation with HCG considerable secretion of dehydroepiandrosterone, 5-androstene-3β,17β-diol, and pregnenolone sulfates was observed, but no increase in the secretion of testosterone sulfate (Laatikainen *et al.*, 1971). It is possible that some of these sulfates serve as precursors in the biosynthesis of testosterone (Dixon *et al.*, 1965; Payne and Mason, 1965; Raheja and Lucis, 1969). If so, testicular steroid sulfatase might play a role in the regulation of this process (see Notation and Ungar, 1969a–c; Payne *et al.*, 1969).

The identification of androsterone, epiandrosterone, and 5α-androstane-3α (and β),17β-diol shows that human testis tissue contains a 5α-hydrogenase active on C_{19} steroids, like that previously found in rat testis homogenates (Stylianou *et al.*, 1961; Nayfeh *et al.*, 1966). Further, the presence of 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol demonstrates that a 5α and a 5β reductase acting on C_{21} steroids is to be found in human testis tissue. Despite a search neither 5α- nor 5β-dihydrotestosterone was found in testis tissue. If present, their concentration must be well below 0.5 μg/100 g of tissue.

In the testes of a number of animal species 20α-hydroxysteroid dehydrogenases acting on progesterone have been found (see Eik-Nes, 1970). The two pregnanediols present in human testis tissue indicate the presence of a corresponding enzyme. In addition, the presence of 5-pregnene-3β,20α-diol demonstrates that a 5-pregnene can serve as a substrate for a 20α-hydroxysteroid dehydrogenase of human testis tissue.

The identification of 5-androstene-3β,16α,17β-triol shows that a 16α-hydroxylase acting on a C_{19} steroid is present in testis tissue. The human testis was already known to contain 16α-hydroxylase specific for progesterone (Oshima *et al.*, 1967).

Epitestosterone sulfate was found in low concentrations in testis tissue, which indicates that it is synthesized in the gland. A possible precursor of epitestosterone, 5-androstene-3β,17α-diol, was present in high concentrations in free and sulfated form. This last-mentioned compound was found in fetal testes (Huhtaniemi *et al.*, 1970) and is secreted as a free compound from normal testes (Laatikainen *et al.*, 1971). These findings demonstrate the presence of 17α-hydroxysteroid dehydrogenase in human testis tissue. It remains to be shown whether epitestosterone or its sulfate or both, are secreted by the human testis.

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^{13}C Nuclear Magnetic Resonance Relaxation Measurements of Synthetic Lecithins and the Effect of Spin-Labeled Lipids†

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ABSTRACT: The ^{13}C spin–lattice relaxation times of dipalmitoyllecithin in bilayers have been measured above the thermal transition, below which the fatty acid chain resonances disappear. The T_1 values above the transition show that molecular motion in the bilayer increases from the glycerol carbons toward both the terminal methyl of the chains and the N^+Me_3 polar head group. The structure is most tightly packed at the glycerol group which probably constitutes the main permeability barrier in the structure. The T_1 values are characteristic of both the chemical structure of the lecithin, and the steric interactions between the molecules in different solvents. The N^+Me_3 resonance from the choline phosphate head group can be observed in the bilayer below the transition, and appears to undergo a conformation change which is coupled to the crystallization of the fatty acid chains. The reversible aggregation of the vesicles which occurs below the transition is attributed to a structure in which the choline phosphate dipoles lie in the plane of the vesicle surface; above the transition the dipoles are in a more extended conformation with the N^+Me_3 groups forming the extreme surface of the vesicle. The effects of nitroxide-labeled lipids incorporated into the bilayer on ^{13}C relaxation times are interpreted qualitatively in terms of the localization of the nitroxide group within the structure.

Preliminary measurements of ^{13}C spin–lattice relaxation times (T_1)[1] of dipalmitoyllecithin (DPL) in sonicated aqueous suspensions have shown that detailed information about the molecular motion of the lipids can be obtained (Metcalfe *et al.*, 1971). Here, the relaxation times in DPL bilayers have been measured for six of the fatty acid chain carbons, and all

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[1] Abbreviations used are: DPL, dipalmitoyllecithin; LSL, lecithin spin label; SASL, stearic acid spin label; T_1 is the spin–lattice relaxation time.