Interconversion of Testosterone and Androstenedione in the Human Testis: A Comparison Between the Activities Displayed by the Interstitium and the Seminiferous Tubules

It had been widely accepted that the interstitium was the only site of androgen biosynthesis in the mammalian testis[1] and almost all of the biochemical investigations into steroidogenesis in the testis have been performed on the whole organ or cell fractions obtained from the entire gland with the assumption that the activity demonstrated by the tissue had originated in the interstitium. However, results obtained by incubating tubules or interstitium from rat testis separately with radioactive precursors have shown that both of these testicular components are capable of steroid metabolism[2–5]. It is probable therefore that there are at least 2 distinct sources of testicular steroids a) the interstitium, which produces androgens for systemic circulation and maintenance of the accessory sex organs[6], and b) the seminiferous tubules, producing locally acting hormones controlling germ cell maturation[7].

In previous work[2,3], results obtained by incubating the separated components of the rat testis with the added radioactively-labelled precursors, pregnenolone[8] and progesterone, indicated that both tissues were capable of producing androgens, but that there was a marked difference in the relative quantities produced by the interstitium and the tubules. Androstenedione and testosterone were formed in nearly equal amounts in the interstitium, whereas in the tubules the major product was testosterone, and the levels of androstenedione formed were consistently very low. It is clear therefore that under identical conditions of incubation, and when provided with identical quantities of radioactive precursors the tubules exhibit a potential to produce a higher testosterone/androstenedione ratio than the interstitium which may be a reflection of the relative activities of the 17β-hydroxysteroid dehydrogenase enzyme in the fractions of the testis.

The present study was undertaken in order to determine whether the separation procedure for obtaining interstitial and tubular fractions, used successfully on the rat testis, could be applied to the human testis, and whether the variations of the 17β-hydroxysteroid dehydrogenase enzyme activities of the separated fractions, demonstrated in the rat, could also be shown to occur in the human testis.

Experimental. Human testes were obtained from 2 patients, aged 72 (Case 1) and 25 (Case 2) years respectively. In both testes, spermatogenesis was proceeding normally judging from the histological appearance of the specimens. The testis tissue was separated into tubule and interstitial fractions by dissection under a binocular microscope as previously reported[2,3]. Each fraction (in range of 50–130 mg wet weight) was incubated in 5 ml Krebs-Ringer bicarbonate buffer (pH 7.4)[9], containing glucose (2 g/l), at 35°C with equimolar concentrations (0.52 μg) of [4-^{14}C)-androstenedione (specific activity 198 μCi/mg) and [7α-^{3}H]-testosterone (specific acitvity 6.6 mCi/mg) under an atmosphere of 95% O_2 and 5% CO_2 without added cofactors. Tissue-free samples were taken from the incubation medium at various time intervals and extracted with ethyl acetate. The steroids were fractionated and identified using paper and thinlayer chromatography[3] and recrystallized to constant specific activity (Table I). The radioactivity of androstenedione and testosterone was measured using a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3380, adjusted for double isotope counting with efficiencies of 58.2% and 28.5% for ^{14}C and ^{3}H respectively, with 6.8% efficiency for ^{14}C in the ^{3}H channel and 0.014% efficiency for ^{3}H in the ^{14}C channel. The results are shown in Table II.

Two cases are reported here, and both show similar patterns of androgen interconversion. In the interstitium, the reduction reaction, androstenedione → testosterone, proceeded at roughly the same rate as the reverse reaction i.e. the oxidation of testosterone to form androstenedione. In the tubule incubations, there was a very rapid conversion of androstenedione to testosterone, such that in Case 1, after 4 h incubation, 91% of the precursor androstenedione was converted to testosterone and in Case 2 there was 61% conversion to testosterone. The oxidation reaction in the tubules proceeded at roughly half the rate of that occurring in the interstitium.

It is interesting to compare the conversion of androstenedione to testosterone in the 2 components of the same testis. In Case 2, the conversion in the tubules was at a rate of 15% and the interstitium was 1% per h. In Case 1, the tubules conversion rate was 22% and the interstitium was 3% per h. It appears therefore that the tubules are

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[8] Abbreviations: pregnenolone, 3β-hydroxy-5-pregnen-20-one; progesterone, 4-pregnene-3,20-dione; androstenedione, 4-androstene-3,17-dione; testosterone, 17β-hydroxy-4-androsten-3-one.
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Table I. Successive recrystallization of [4-^{14}C, 7α-^{3}H]-testosterone and [4-^{14}C, 7α-^{3}H]-androstenedione obtained from incubations of seminiferous tubules and interstitium of human testis tissue following admixture of 30 mg authentic steroid

Case 1. Testis tissue from 72-year-old man

Crystallization	Tubule [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)			Interstitium [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)		
No.	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C
1	16,037	723	22.18	405	69	5.87	14,682	79	185.8	885	837	1.06
2	14,881	693	21.47	390	69	5.65	14,580	80	182.3	856	850	1.01
3	14,596	674	21.66	388	68	5.71	14,219	76	187.1	849	825	1.03
4	14,645	681	21.51	382	65	5.88	14,048	75	187.3	860	845	1.02

Case 2. Testis tissue from 25-year-old man

Crystallization	Tubule [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)			Interstitium [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)		
No.	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C
1	17,757	902	19.69	562	36	15.61	13,485	25	539	251	1287	0.195
2	17,251	891	19.36	488	33	14.79	13,598	27	504	256	1360	0.188
3	17,732	901	19.68	440	31	14.19	13,101	26	504	249	1299	0.192
4	17,098	874	19.56	420	30	14.00	12,240	24	510	250	1286	0.194

Table II. Separated interstitium and seminiferous tubules of human testis tissue incubated with equimolar concentrations of [7α-^{3}H]-testosterone and [4-^{14}C]-androstenedione

Tissue	Time	Case 1. Testis tissue from 72-year-old man: % ^{3}H label recovered as		% ^{14}C label recovered as		Case 2. Testis tissue from 25-year-old man: % ^{3}H label recovered as		% ^{14}C label recovered as	
	(min)	[^{3}H]-A[a]	[^{3}H]-T[b]	[^{14}C]-A	[^{14}C]-T	[^{3}H]-A	[^{3}H]-T	[^{14}C]-A	[^{14}C]-T
Interstitium per 100 mg	5	4.6	88.1	91.4	6.7	4.3	89.4	98.6	0.8
	15	4.7	88.4	89.8	8.1	4.4	87.6	98.3	0.9
	30	5.0	88.5	88.7	8.2	4.5	87.9	97.8	1.1
	60	5.9	86.8	89.4	8.7	4.6	86.6	97.5	1.8
	120	–	–	–	–	4.9	86.3	97.3	2.3
	150	6.5	84.9	85.9	11.9	–	–	–	–
	180	–	–	–	–	4.8	82.6	96.3	3.1
	240	6.1	86.7	84.9	12.4	4.7	82.5	95.0	4.3
Tubule per 100 mg	5	2.1	90.9	83.1	15.0	0.9	90.9	83.7	14.7
	15	2.6	91.2	67.7	30.2	1.0	90.9	73.2	22.9
	30	3.9	90.3	50.7	46.9	1.3	89.7	51.4	42.5
	60	4.1	90.1	35.3	62.0	1.6	89.1	31.0	53.4
	120	–	–	–	–	1.6	88.9	23.7	55.2
	150	4.2	89.4	10.3	87.1	–	–	–	–
	180	–	–	–	–	1.7	88.3	23.5	56.4
	240	4.5	89.6	5.1	90.7	2.1	87.6	22.1	60.5

[a] Denotes androstenedione. [b] Denotes testosterone.

much more efficient than the interstitium in carrying out the enzymic reduction of androstenedione. The difference in the results obtained from the 2 cases may be attributed to the great difference in age between the two individuals, which suggests that, in the older man, higher levels of testosterone are needed to maintain normal spermatogenesis in the tubules.

These results indicate that the human testis shows the same differences in the relative activities of the 17β-hydroxysteroid dehydrogenase enzymes in the interstitium and seminiferous tubules as previously demonstrated in the rat. It is also apparent that the separation technique applied to the human testis in these experiments produces preparations with little or no cross-contamination between the tubules and the interstitium, and, like the rat, the human testis possesses at least two sites of steroid metabolizing enzymes, the interstitium and the seminiferous tubules[10].

Zusammenfassung. Nachweis, dass im menschlichen Hoden sowohl das Interstitium wie auch das tubuläre System befähigt sind, Androstendion-4-^{14}C und Testosteron-7α-^{3}H zu konvertieren.

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On the Use of a Peptidase Inhibitor (Trasylol) for Storage of I^{125}-labelled Peptide Hormones (HGH, TSH, ACTH, Insulin, Glucagon, PTH)

One of the main problems in radioimmunoassay is the degradation of I^{131} or I^{125}-labelled tracer hormones. The degradation may preexist due to extraction or storage of unlabelled preparation. It can occur during iodination (iodination damage) or on subsequent storage of the labelled hormone. Finally the degradation may markedly increase during incubation, especially in medium containing plasma or serum. This form of incubation damage