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[4-^{14}C]-TESTOSTERONE METABOLISM AND STEROID PRODUCTION BY INCUBATED WHOLE TESTES, SEMINIFEROUS TUBULES AND INTERSTITIAL TISSUE FROM RATS

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SUMMARY

In incubated testes of young rats (30 days old), 5α-reduction predominates over 7α-hydroxylation. This 5α-reductase activity appears to be located predominantly in the interstitial tissue. In incubated testes of mature rats (120 days old) 7α-hydroxylation is more important than 5α-reduction. This 7α-hydroxylation mainly occurs in the interstitial tissue, while 5α-reduction predominates in the seminiferous tubules. During long term treatment with HCG, 7α-hydroxylation in the incubation of whole testes and of interstitial tissue decreases to low levels and the steroid metabolism shifts to 5α-reduction.

INTRODUCTION

It is well established that androgen target organs metabolize testosterone according to specific patterns, and that the metabolites produced have different androgenic potencies or can even exert specific effects [1].

Androgen metabolism also occurs in the testes, where it depends mainly upon the activity of a 5α-reductase [2–7], a 7α-hydroxylase [8–10], and NADP-linked oxido-reductases for 3β (or 3α)- and 17β-hydroxysteroids [2, 11–17].

In incubated preparations of whole testes of the rat the activity of the first two enzymes seems to be age-dependent. The 5α-reduction is very high in young animals and decreases progressively during sexual maturation to reach low values in the adult rat [3–6, 18–24]; conversely, the 7α-hydroxylase activity, which is very low in the young animal, gradually increases with age to become very high in the adult rat [10, 23, 25].

Metabolization pattern in adult rat testes is modified by the administration of Human Chorionic Gonadotrophins (HCG). Acute administration of HCG results in an increased formation of testosterone and 7α-hydroxy-testosterone by the incubated testes, and to a smaller extent of 5α-reduced testosterone, mainly 5α-androstanediol [26]. When HCG administration is prolonged for at least 4 days, increased testosterone production is maintained, but the formation of 7α-hydroxy-testosterone is depressed well below the pretreatment levels and the formation of 5α-androstanediol is enhanced [25–28].

It is generally assumed that 5α-reduction in the adult rat testis occurs mainly in the seminiferous tubules [21, 29–32]. In the young animal, the location of 5α-reduction activity in the testes is controversial [6, 20, 21, 24, 30, 33]. No direct measurements are available concerning the location of the 7α-hydroxylase.

In the present experiments, the 7α-hydroxylation and the 5α-reduction capacity of incubated seminiferous tubules and interstitial cells was studied in young and mature rats, and in mature rats submitted to a prolonged treatment with HCG.

EXPERIMENTAL

Material

The experiments were performed on rats from either the inbred laboratory strain or the Wistar strain. Human Chorionic Gonadotrophin (HCG Organon) was injected intraperitoneally and the experiments were carried out 24 h after the last injection. All reagents used for steroid analysis were of analytical grade. Non-labelled steroids, used as reference compounds, were obtained from Ikapharm, Israel; [4-^{14}C]-testosterone (specific activity 56 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England.

Testosterone, 17β-hydroxy-4-androsten-3-one: Androstenedione, 4-androstene-3,17-dione: 5α-Androstanedione, 5α-androstane-3,17-dione: 5α-Androsterone, 3α-hydroxy-5α-androstan-17-one: epi-Androsterone, 3β-hydroxy-5α-androstan-17-one: 5α-Androstanediol-3α, 5α-androstane-3α,17β-diol: 5β-Androstanediol-3α, 5β-androstane-3α,17β-diol: 5α-Androstanediol-3β, 5α-androstane-3β,17β-diol: 5β-Androstanediol-3β, 5β-androstane-3β,17β-diol: 5α-Dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one: 7α-Hydroxytestosterone, 7α,17β-dihydroxy-4-androsten-3-one: 7α-Hydroxyandrostenedione, 7α-hydroxy-4-androstene-3,17-dione.

Methods

a. *Tissue preparation.* For each experiment two rats were sacrificed by decapitation and the testes were excised and decapsulated. Two testes, one from each animal, were cut in six to eight pieces and immediately submitted to the wet dissection technique described by Christensen and Mason[34] to separate the testicular tissue into a seminiferous tubules and an interstitial cell fraction. Seminiferous tubules and interstitial cells were put in separate centrifuge tubes containing 10 ml ice-cooled Krebs–Ringer solution. The tubes were centrifuged at 800 *g* for 5 min, the supernatant aspirated and the remaining tissue weighed. The two remaining testes were kept undissected, decapsulated, and, after weighing, incubated as such.

The separated tissues were controlled by histological examination or by the histochemical method of Wattenberg[35] for demonstration of 3β-hydroxysteroid dehydrogenase activity. Incompletely separated tissues were discarded, but a slight cross-contamination in this isolation procedure cannot be entirely avoided.

b. *Incubations.* The interstitial tissue, the seminiferous tubules and the pair of undissected testes were incubated in separate flasks containing 7 ml Krebs–Ringer buffer, enriched with glucose (6 mmol/l), NADP (1.8 mmol/l) and glucose-6-phosphate (5 mmol/l). Incubation was carried out at 37°C for 2 h under continuous shaking and under a constant stream of carbogen (95% O_2 and 5% CO_2). The incubation was stopped by addition of 50 ml acetone–methanol (1:1, V/V).

Two types of incubation experiments were performed. In the first series (*metabolization experiments*) 25 μl ethanol, containing 1000 ng [4-^{14}C]-testosterone (corresponding to 326,000 c.p.m.), were added to the incubation medium to study the metabolism of testosterone. Testicular material from young rats (30 days), mature rats (120 days) and mature rats treated for ten days with HCG (10 I.U./day intraperitoneally) was used in these experiments.

In the second series (*production experiments*), no [4-^{14}C]-testosterone was added and the amount of testosterone, 7α-hydroxy-testosterone and 5α-androstanediol produced from endogenous precursors was measured. The production experiments were performed with testicular material from mature rats (120 days) of which a number had been treated with HCG (10 I.U./day intraperitoneally) for 10 days.

c. *Endogenous steroid content.* The endogenous testosterone, 7α-hydroxy-testosterone and 5α-androstanediol content of the interstitial tissue, seminiferous tubules and whole testes of untreated and HCG treated mature rats was measured; therefore, the separate tissues or the whole testes were immediately transferred in 50 ml acetone–methanol (1:1, V/V) without incubation.

d. *Measurement of the steroids.* Non-labelled steroids in the testicular preparations were measured by fluorimetry (testosterone, 7α-hydroxy-testosterone), gas chromatography (5α-androstanediol) or radioimmune assay (testosterone) according to methods described previously [23]. The labelled metabolites produced from [4-^{14}C]-testosterone by the incubated tissues were separated by column and paper chromatography and measured by liquid scintillation counting [23].

RESULTS

Weight of testicular tissue (*Table* 1)

The amounts of interstitial and tubular tissue that could be isolated represent respectively about 20% and 40% of the whole testes weight of the young animals of the inbred strain, and about 10% and 50% of the testes weight of the mature animals of both strains.

A 10 day treatment of the mature rats with HCG did not significantly modify the weight of the whole testis and of the separated tubular tissue, but it increased significantly (about 17%) the weight of the isolated interstitial tissue.

Metabolization studies

All metabolites are expressed as percentages of the amounts of [4-^{14}C]-testosterone metabolized after 2 h of incubation.

a. *Young rats.* In the incubation period of 2 h whole testes metabolized [4-^{14}C]-testosterone to a large degree (nearly 80%) (Table 2). Less [4-^{14}C]-testoster-

Table 1. Weights of intact testes and dissected testicular tissues (values obtained with pairs of testes)

Age of the rats (days)	Treatment	*n*	Weight (mg)		
			Intact testes	Seminiferous tubules	Interstitial tissue
30	Controls	3	252 ± 60	100 ± 53	55 ± 31
120	Controls	18	2496 ± 249	1329 ± 209	242 ± 39
	HCG (10 I.U./day for 10 days)	16	2587 ± 255*	1304 ± 219*	284 ± 43†

Results expressed in mg ± S.D. (*n* = number of experiments).
* Not significantly different from controls.
† Significantly different from controls ($P < 0.01$).

Table 2. Metabolism of [4-^{14}C]-testosterone by incubated whole testes, seminiferous tubules and interstitial tissue from young rats (30 days old)

Metabolites	Whole testis	Seminiferous tubules	Interstitial tissue
Metabolized testosterone	77.8 ± 6.0	32.4 ± 3.3	53.2 ± 7.8
Androstenedione	7.0 ± 0.3	35.0 ± 4.5	29.2 ± 1.4
7α-OH-Testosterone	1.3 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
7α-OH-Androstenedione	1.0 ± 0.3	2.5 ± 0.5	1.4 ± 0.2
Sum of 7α-OH-steroids	2.3 ± 0.2	4.1 ± 0.5	2.9 ± 0.3
5α-Androstanedione	9.3 ± 2.7	0.8 ± 0.2	7.5 ± 0.5
5α-Dihydrotestosterone			
5α-Androsterone	37.1 ± 4.0	5.2 ± 0.5	21.2 ± 6.3
Epi-androsterone			
5α-Androstanediol-3α	23.6 ± 6.0	2.7 ± 1.6	9.6 ± 3.3
5α-Androstanediol-3β	0.9 ± 0.7	0.3 ± 0.2	0.4 ± 0.2
Sum of 5α-reduced steroids	70.9 ± 6.1	9.0 ± 2.2	38.6 ± 9.9
5β-Androstanediol-(3α + 3β)	0.6 ± 0.7	0.4 ± 0.2	0.3 ± 0.2

Mean values (±S.D.) of three experiments. Metabolites are expressed as % of metabolized testosterone.

Whole testes and testicular compartments were incubated with 1000 ng [4-^{14}C]-testosterone (corresponding to about 326,000 c.p.m.).

one was metabolized by the interstitial cells (53%) and by the seminiferous tubules (32%). In whole testes, testosterone was mainly metabolized to 5α-reduced steroids (71%). In the dissected testicular tissues, 5α-reduced steroids were formed to a much larger extent in the incubations of Leydig cells (38.6%) than in the incubations of tubules (9.0%).

In both tissues, the relative contribution of the different 5α-reduced metabolites was similar to what was found in whole testes. Besides 5α-reduced metabolites, and in contrast to the whole testes, both isolated tissues also produced important amounts of androstenedione.

Only very small amounts of 7α-hydroxylated and 5β-reduced metabolites were formed in both the whole testes and the separated tissues.

b. *Mature rats.* After 2 h of incubation a much larger fraction of [4-^{14}C]-testosterone was metabolized by the whole testes (80.1%) and by the interstitial cells (80.3%) of normal untreated rats than by the seminiferous tubules (49.8%) (Table 3).

In the incubations of whole testes and interstitial tissue the conversion to 7α-hydroxylated metabolites predominated (41.8 and 61.8% respectively), while the conversion to 5α-reduced metabolites was very small (11.0 and 3.3% respectively). In the incubations of seminiferous tubules, however, the transformation to 5α-reduced metabolites was relatively more important

Table 3. Metabolism of [4-^{14}C]-testosterone by incubated whole testes, seminiferous tubules and interstitial tissues from normal (N) or HCG treated (HCG) mature rats (120 days old)

Isolated metabolites	Whole testes		Seminiferous tubules		Interstitial tissue	
	N	HCG	N	HCG	N	HCG
Metabolized [4-^{14}C]-testosterone	80.1 ± 9.2	57.2 ± 7.8	49.8 ± 3.3	46.3 ± 1.9	80.3 ± 11.4	58.6 ± 1.0
Androstenedione	4.4 ± 0.9	11.9 ± 2.8	9.8 ± 0.9	9.7 ± 0.9	10.7 ± 8.5	47.0 ± 5.5
7α-OH-testosterone	35.2 ± 9.9	6.3 ± 2.4	5.4 ± 3.5	2.9 ± 1.7	35.8 ± 15.7	3.2 ± 0.9
7α-OH-Androstenedione	6.5 ± 0.5	2.7 ± 0.8	3.3 ± 0.8	2.7 ± 0.5	26.0 ± 4.8	4.7 ± 0.4
Sum of 7α-OH-steroids	41.8 ± 10.2	9.0 ± 2.9	8.7 ± 4.7	5.6 ± 2.1	61.8 ± 23.7	7.9 ± 1.6
5α-Androstanedione	1.1 ± 0.9	2.3 ± 1.4	3.1 ± 0.5	2.9 ± 2.0	0.4 ± 0.2	2.5 ± 1.0
5α-Dihydrotestosterone						
5α-Androsterone	3.1 ± 0.9	9.6 ± 2.9	7.0 ± 0.3	7.0 ± 0.9	1.5 ± 0.9	4.8 ± 2.0
Epi-androsterone						
5α-Androstanediol-3α	4.0 ± 0.3	10.7 ± 2.2	18.7 ± 4.7	15.1 ± 2.4	1.1 ± 0.9	1.4 ± 0.5
5α-Androstanediol-3β	2.8 ± 0.7	7.6 ± 0.2	1.3 ± 0.3	1.9 ± 0.5	0.4 ± 0.3	0.4 ± 0.2
Sum of 5α-reduced steroids	11.0 ± 1.7	30.2 ± 2.9	30.0 ± 6.7	26.9 ± 3.8	3.3 ± 1.6	9.2 ± 3.3
5β-Androstanediol-(3α + 3β)	2.8 ± 0.7	1.5 ± 0.7	5.7 ± 2.4	6.7 ± 4.7	0.4 ± 0.2	0.6 ± 0.3

Mean values (±S.D.) of three experiments. Metabolites are expressed as % of metabolized testosterone.

Whole testes and testicular compartments were incubated with 1000 ng [4-^{14}C]-testosterone (corresponding to about 326,000 c.p.m.).

Table 4. Steroid content of, and steroid production by whole testes, seminiferous tubules and interstitial tissue from normal or HCG treated mature rats (120 days old)

		Amounts of steroids (ng) Testosterone		7α-OH-testosterone		5α-Androstanediol	
Control rats							
Whole testes	Content	142 ± 32	(n = 7)	29 ± 12	(n = 7)	27 ± 4	(n = 4)
	Production	384 ± 56*	(n = 8)	739 ± 109*	(n = 8)	112 ± 37*	(n = 6)
Seminiferous tubules	Content	62 ± 13	(n = 7)	<20	(n = 7)	25 ± 7	(n = 4)
	Production	49 ± 5	(n = 8)	<20	(n = 8)	31 ± 8	(n = 6)
Interstitial tissue	Content	56 ± 5	(n = 7)	<20	(n = 7)	<10	(n = 4)
	Production	94 ± 30	(n = 8)	295 ± 61*	(n = 8)	15 ± 9	(n = 6)
HCG treated rats (10 I.U./day for 10 days)							
Whole testes	Content	554 ± 127**	(n = 6)	<20	(n = 6)	23 ± 16	(n = 4)
	Production	1736 ± 144*,**	(n = 7)	185 ± 34*,**	(n = 7)	427 ± 67*,**	(n = 5)
Seminiferous tubules	Content	167 ± 36**	(n = 6)	<20	(n = 6)	35 ± 3	(n = 3)
	Production	159 ± 24**	(n = 6)	<20	(n = 6)	99 ± 21**,†	(n = 4)
Interstitial tissue	Content	236 ± 95**	(n = 6)	<20	(n = 6)	<10	(n = 4)
	Production	1855 ± 296*,**	(n = 7)	110 ± 19*,**	(n = 7)	100 ± 30*,**	(n = 5)

Seminiferous tubules and interstitial tissue were obtained from pairs of testes.
n = number of experiments. Mean values ± standard error of mean (S.E.M.).
* Significant difference between production and content.
** Significant difference between control and HCG treated rats.
† Number of experiments too small to apply statistics between content and production.

(30%) than the conversion to 7α-hydroxylated metabolites (8.7%).

Treatment with HCG for 10 days caused both a considerable reduction of the metabolization rate of [4-^{14}C]-testosterone, and a profound modification of the metabolization pattern in whole testes and interstitial cells. Indeed, the conversion to 7α-hydroxylated steroids decreased from 41.8 to 9% for the whole testes, and from 61.8 to 7.9% for the interstitial cells, while the transformation to 5α-reduced metabolites increased, respectively, from 11 to 30.2% and from 3.3 to 9.2%. Moreover, larger amounts of androstenedione were formed in the incubations of whole testis and interstitial tissue. HCG treatment did not influence neither the rate nor the pattern of testosterone metabolism in the incubations of the seminiferous tubules. Whole testes and (particularly) seminiferous tubules converted [4-^{14}C]-testosterone to 5β-androstanediol to some extent, and this conversion was not influenced by HCG treatment.

Production studies

Two series of experiments were performed, one with rats from the inbred strain and one with Wistar rats. As the results were qualitatively and quantitatively comparable they were pooled into one group (Table 4).

A steroid production is called "net production" when the amounts of a steroid measured in the incubations after 2 h are higher than the endogenous content.

For the whole testes under control conditions, a net production was observed for the three steroids studied, and particularly for 7α-hydroxy-testosterone. In the separated tissues only a net production of 7α-hydroxy-testosterone was observed in the incubations of interstitial cells; the amounts of testosterone and 5α-androstanediol in the incubations of both interstitial and tubular tissues were not statistically different from their endogenous content.

In the whole testes treatment with HCG for 10 days resulted in a marked increase of both the endogenous content and the net production of testosterone. A considerable decrease of the net production of 7α-hydroxy-testosterone was observed, while the net production of 5α-androstanediol was markedly enhanced when compared to controls.

In both the tubular and the interstitial tissues, HCG treatment increased the endogenous content of testosterone, but not that of the other steroids. In the tubular tissue there was a net production of 5α-androstanediol, while the 7α-hydroxy-testosterone content remained below the detection level. In the interstitial tissue, on the contrary, there was a net production of all three steroids; compared to the controls the production of testosterone and 5α-androstanediol was significantly increased, while that of 7α-hydroxy-testosterone was significantly decreased.

DISCUSSION

Most information on compartmental steroid metabolism in the testis has been obtained from studies in which the wet dissection technique, originally described by Christensen and Mason[34], was used. One of the main advantages of this technique is that the integrity of most of the cells is preserved [22].

The weights of the interstitial tissue and the seminiferous tubules, isolated in the present study, are in good agreement with data reported by several authors who found that the isolated interstitial tissue represents about 10% of the testicular mass in adult rats [34, 36–39].

The combined weight of interstitial and tubular tis-

sues is about 40% lower than the weight of the whole testes. This is probably due to procedural losses of tissue and tissue fluid, particularly through the broken ends of the tubules.

The metabolism of [4-^{14}C]-testosterone by incubated testicular tissues of rats leads to the formation of at least 24 metabolites [23]. The quantitatively most important metabolites are androstenedione, 7α-hydroxy-testosterone and 7α-hydroxy-androstenedione (7α-hydroxysteroids), 5α-dihydro-testosterone, 5α-androsterone, 5α-epiandrosterone, 5α-androstanedione, 5α-androstanediol (3α and 3β) (5α-reduced steroids) and 5β-androstanediol. Other metabolites are produced in minimal amounts (less than 0.2% of the incubated [4-^{14}C]-testosterone) and were not further considered in this study.

The metabolization experiments indicate that the high 5α-reductase and the low 7α-hydroxylase activity, which characterizes the metabolic pattern in the incubations of whole testes of young rats [23, 40] is mainly determined by the activity of the interstitial tissue. Indeed, in the separated tissues the metabolism is characterized by a more extensive conversion of [4-^{14}C]-testosterone, and a much higher 5α-reduction activity, in the incubations of interstitial cell preparations than in those of the seminiferous tubules, notwithstanding the lower weight of the incubated interstitial tissue; the 7α-hydroxylase activity of both tissues is, as in the whole testes, very low.

The high 5α-reductase activity observed in the whole testes of young rats is in agreement with the literature [3–6, 18–21, 24, 25]; its predominant location in the interstitial tissue, observed in our study, corroborates the view of most of the investigators [6, 21, 24, 30], but not that of Rivarola *et al.*[20, 33].

In the testes of mature rats important modifications of the steroid metabolism are observed; incubations of whole testes show that during the maturation process the 5α-reduction activity decreases, while that of the 7α-hydroxylase increases progressively to become predominant in the mature testis [10, 23].

Both the metabolization and the production experiments with separated tissues indicate that the 7α-hydroxylation activity is mainly located in the interstitial tissue. The minor conversion of [4-^{14}C]-testosterone to 7α-hydroxylated metabolites by the incubated seminiferous tubules represents either some enzymatic activity of the tubular cells or can be due to slight contamination with interstitial cells. Inano *et al.*[9, 25] have reported that the 7α-hydroxylase activity is higher in testes from irradiated and cryptorchid rats compared to normal animals; however, since both the Sertoli cells and the interstitial cells are more or less radio- and heat-resistant, their data provide no information concerning the location of the 7α-hydroxylase activity.

The remaining 5α-reduction activity observed in the whole testis of the mature rats seems to be located mainly in the seminiferous tubules, at least when the metabolization experiments are considered. The fact that no net production of 5α-androstanediol could be observed by the incubated tubular tissues does not invalidate this view. Indeed, as the tubules form little or no testosterone [41–43], they are unable to form appreciable amounts of 5α-reduced metabolites when they are incubated without substrate.

The predominant location of the remaining 5α-reduction activity in the tubular compartment agrees with the observations of several authors [21, 29–32, 44]. According to some, this reduction could occur in the spermatids [45], according to others, in the Sertoli cells and the spermatocytes [46, 47].

Several investigators [22, 45, 48–50] emphasize that the most characteristic biochemical event of the maturation process of rat testes is the progressive decrease of the 5α-reductase activity in the interstitial cells and its progressive increase in the seminiferous tubules. Our results show that the maturation process of the interstitial cells is characterized not only by a decrease of the 5α-reductase activity, but also by a progressive and important increase of the 7α-hydroxylase activity.

Both the metabolization and the production experiments also show that the drastic changes observed in the metabolization pattern of the whole testes after administration of HCG during 10 days result mainly from modifications of the metabolism of the interstitial cells.

Long-term HCG administration increases the endogenous testosterone content of both seminiferous tubules and interstitial tissue, but it only increases the net production of testosterone in the interstitial tissues, as has also been observed by Van der Vusse *et al.*[39, 43] after a five day treatment with very large doses of HCG (700 I.U./day).

The increase in testosterone production in the interstitial tissue is accompanied by a considerable depression of the normally very active 7α-hydroxylation processes. The high quantities of testosterone and its reduced metabolization through 7α-hydroxylation can explain the increased transformation of [4-^{14}C]-testosterone into androstenedione, which was observed in the metabolization experiments with both whole testis and interstitial tissue, on the basis of a mass action of the larger amounts of unmetabolized testosterone.

Our experiments do not permit us to conclude a specific activation of interstitial 5α-reductase activity as a cause for the increased formation of 5α-androstanediol in the incubations of whole testes after long-term HCG administration. It is possible that tubular 5α-reduction contributes more to this increase through availability of larger amounts of testosterone under these conditions.

REFERENCES

1. Hall P.: Endocrinology of the testis. In *The Testis* (Edited by A. D. Johnson, W. R. Gones and N. L. Vandemark). Academic Press, New York and London, Vol. II (1970) p. 1, pp. 3–7.

2. Inano H. and Tamaoki B.: Bioconversion of steroids in immature rat testes *in vitro*. *Endocrinology* **79** (1966) 579–590.
3. Nayfeh S. N., Barefoot S. W. and Baggett B.: Metabolism of progesterone by rat testicular homogenates. II. Changes with age. *Endocrinology* **78** (1966) 1041–1048.
4. Ficher M. and Steinberger E.: Conversion of progesterone to androsterone by testicular tissue at different stages of maturation. *Steroids* **12** (1968) 491–506.
5. Ficher M. and Steinberger E.: *In vitro* progesterone metabolism by rat testicular tissue at different stages of development. *Acta endocr., Copenh.* **68** (1971) 285–292.
6. Yamada M., Yasue S. and Matsumoto K.: Formation of 5α-reduced products from testosterone *in vitro* by germ cells from immature rats. *Acta endocr., Copenh.* **71** (1972) 393–408.
7. Yoshizaki K., Matsumoto K. and Samuels L. T.: Localization of Δ^4-5α-reductase in immature rat testes. *Endocrinology* **102** (1978) 918–928.
8. Inano H., Tsuno K. and Tamaoki B.: Identification of 7α-hydroxylated androgens as the metabolites of androstenedione by testicular microsomal fraction of rats. *Biochemistry* **9** (1970) 2253–2259.
9. Inano H. and Tamaoki B.: Steroid 7α-hydroxylase of rat testes. *Biochemistry* **10** (1971) 1503–1509.
10. Eechaute W., Lacroix E. and Leusen I.: The conversion of testosterone to 7α-hydroxy-testosterone by incubated rat testes. *Steroids* **24** (1974) 753–764.
11. Inano H., Inano A. and Tamaoki B.: Studies on enzyme reactions related to steroid biosynthesis. II. Submicrosomal distribution of the enzymes related to androgen production from pregnenolone and of the cytochrome P-450 in testicular gland of rat. *J. steroid Biochem.* **1** (1970) 83–91.
12. Bell J. B. G., Vinson G. P. and Lacy D.: Studies on the structure and function of the mammalian testis. III. *In vitro* steroidogenesis by the seminiferous tubules of rat testis. *Proc. R. Soc., London B* **176** (1971) 433–443.
13. Dufau M. L., De Kretser D. M. and Hudson B.: Steroid metabolism by isolated rat seminiferous tubules in tissue culture. *Endocrinology* **88** (1971) 825–832.
14. Sulimovici S. and Lunenfeld B.: The effect of adenosine 3',5'-cyclic-monophosphoric acid on the 17β-hydroxysteroid dehydrogenase of rat testis. *J. steroid Biochem.* **3** (1972) 781–790.
15. Sulimovici S., Bartoov B. and Lunenfeld B.: Localization of 3β-hydroxysteroid dehydrogenase in the inner membrane subfraction of rat testis mitochondria. *Biochim. biophys. Acta* **321** (1973) 27–40.
16. Van der Vusse G. J., Kalkman M. L. and Van der Molen H. J.: 3β-hydroxysteroid dehydrogenase in rat testis tissue inter- and subcellular localization and inhibition by cyanoketone and nagarse. *Biochim. biophys. Acta* **348** (1974) 404–414.
17. Yamada M., Yasue S. and Matsumoto K.: Formation of C_{21}-17-hydroxysteroids and C_{19}-steroids from 3β-hydroxypregn-5-en-20-one and progesterone *in vitro* by germ cells from immature rat testes. *Endocrinology* **93** (1973) 81–87.
18. Coffey J. C., French F. S. and Nayfeh S. N.: Metabolism of progesterone by rat testicular homogenates. IV. Further studies of testosterone formation in immature testis *in vitro*. *Endocrinology* **89** (1971) 865–872.
19. Folman Y., Sowell J. G. and Eik-Nes K. B.: The presence and formation of 5α-dihydrotestosterone in rat testes *in vivo* and *in vitro*. *Endocrinology* **91** (1972) 702–710.
20. Rivarola M. A., Podesta E. J. and Chemes H. E.: *In vitro* testosterone-^{14}C metabolism by rat seminiferous tubules at different stages of development: formation of 5α-androstandiol at meiosis. *Endocrinology* **91** (1972) 537–542.
21. Matsumoto K. and Yamada M.: 5α-reduction of testosterone *in vitro* by rat seminiferous tubules and whole testes at different stages of development. *Endocrinology* **93** (1973) 253–255.
22. Lacy D.: Steroid metabolism by the interstitium and seminiferous tubules of the testes *in vitro*: studies on the rat during development and in the adult in the absence of the boundary tissue. In *Male fertility and sterility* (Edited by R. F. Mancin and L. Martini). Academic Press, London (1974) pp. 175–205.
23. Lacroix E., Eechaute W. and Leusen I.: Influence of age on the formation of 5α-androstanediol and 7α-hydroxy-testosterone by incubated rat testes. *Steroids* **25** (1975) 649–661.
24. Nayfeh S. N., Coffey J. C., Hansson V. and French F. S.: Maturational changes in testicular steroidogenesis: hormonal regulation of 5α-reductase. *J. steroid Biochem.* **6** (1975) 329–335.
25. Inano H., Suzuki K. Wakabayashi K. and Tamaoki B.: Biological activities of 7α-hydroxylated C_{19}-steroids and changes in rat testicular 7α-hydroxylase activity with gonadal status. *Endocrinology* **92** (1973) 22–30.
26. Lacroix E., Eechaute W. and Leusen I.: Modification of testicular steroid metabolism in the rat during gonadotrophin administration. *Arch. Internat. Physiol. Bioch.* **84** (1976) 911–914.
27. Lacroix E., Eechaute W. and Leusen I.: Modification sous l'influence de gonadotrophines du métabolisme des androgènes par les testicules incubés de rats adultes. *Ann. d'Endocr.* **35** (1974) 683–686.
28. Lacroix E., Eechaute W. and Leusen I.: The influence of gonadotrophin (HCG) treatment on the steroidogenesis by incubated rat testes. *J. steroid Biochem.* **8** (1977) 269–275.
29. Rivarola M. A. and Podesta E. J.: Metabolism of testosterone^{14}C by seminiferous tubules of mature rats: formation of 5α-androstan-3α,17β-diol-^{14}C. *Endocrinology* **90** (1972) 618–623.
30. Folman Y., Ahmad N., Sowell J. G. and Eik-Nes K. B.: Formation *in vitro* of 5α-dihydrotestosterone and other 5α-reduced metabolites of [^{3}H]-testosterone by the seminiferous tubules and interstitial tissue from immature and mature rat testes. *Endocrinology* **92** (1973) 41–47.
31. Perez-Lloret A. and Weisz J.: Metabolism of testosterone and 5α-dihydrotestosterone *in vitro* by the seminiferous tubules of the mature rat. *Endocrinology* **95** (1974) 1306–1316.
32. Sowell J. G., Folman Y. and Eik-Nes K. B.: Androgen metabolism in rat testicular tissue. *Endocrinology* **94** (1974) 346–354.
33. Podesta E. J. and Rivarola M. A.: Concentration of androgens in whole testis, seminiferous tubules and interstitial tissue of rats at different stages of development. *Endocrinology* **95** (1974) 455–461.
34. Christensen A. K. and Mason N. R.: Comparative ability of seminiferous tubules and interstitial tissues of rat testes to synthesize androgens from progesterone-4-^{14}C *in vitro*. *Endocrinology* **76** (1965) 646–656.
35. Wattenberg L. W.: Microscopic histochemical demonstration of steroid-3β-ol dehydrogenase in tissue sections. *J. histochem. Cytochem.* **6** (1958) 225–232.
36. Hall P. F., Irby D. C. and de Kretser D. M.: Conversion of cholesterol to androgens by rat testes: comparison of interstitial cells and seminiferous tubules. *Endocrinology* **84** (1969) 488–496.
37. Richards G. and Neville A. M.: Androgen metabolism in rat interstitial tissue and seminiferous tubules. *Nature* **244** (1971) 359–361.
38. Rommerts F. F. G., van Doorn L. G., Galjaard H.,

Cooke B. A. and Van der Molen H. J.: Dissection of wet tissue and of freeze-dried sections in the investigation of seminiferous tubules and interstitial tissue from rat testis. *J. histochem. Cytochem.* **21** (1973) 572–579.

39. Van der Vusse G. J., Kalkman M. L. and Van der Molen H. J.: Endogenous steroid production in cellular and subcellular fractions of rat testis after prolonged treatment with gonadotropins. *Biochim. biophys. Acta* **380** (1975) 473–485.
40. Lacroix E., Eechaute W. and Leusen I.: Influence des gonadotrophines sur la production et le métabolisme des androgènes par les testicules incubés de jeunes rats. *Ann. d'Endocr.* **35** (1974) 687–690.
41. Cooke B. A., De Jong F. H., Van der Molen H. J. and Rommerts F. F. G.: Endogenous testosterone concentrations in rat testis interstitial tissue and seminiferous tubules during *in vitro* incubation. *Nature New Biol.* **237** (1972) 255–256.
42. Van der Vusse G. J., Kalkman M. L. and Van der Molen H. J.: Endogenous production of steroids by subcellular fractions from total rat testis and from isolated tissue and seminiferous tubules. *Biochim. biophys. Acta* **297** (1973) 179–185.
43. Van der Vusse G. J., Kalkman M. L. and Van der Molen H. J.: Endogenous steroid production in preparations of rat testis after long-term treatment with HCG. *J. steroid Biochem.* **6** (1975) 357–359.
44. Payne A. H., Kawano A. and Jaffe R. B.: Formation of dihydrotestosterone and other 5α-reduced metabolites by isolated seminiferous tubules and suspension of interstitial cells in human testis. *J. clin. Endocr. Metab.* **37** (1973) 448–453.
45. Tsang W. N., Collins, P. M. and Lacy D.: Steroid metabolism by the seminiferous tubules *in vitro* and spermatogenesis in the developing rat. *J. Reprod. Fert.* **34** (1973) 513–517.
46. Dorrington J. H. and Fritz I. B.: Metabolism of testosterone by preparations from the rat testis. *Biochem. biophys. Res. Commun.* **54** (1973) 1425–1431.
47. Tencè M. and Drosdowsky M.: Biosynthesis and metabolism of testosterone by Sertoli cell-enriched seminiferous tubules. *Biochem. biophys. Res. Commun.* **73** (1976) 47–55.
48. Tsang W. N., Lacy D. and Collins P. M.: Leydig cell differentiation, steroid metabolism by the insterstitium *in vitro* and the growth of the accessory sex organs in the rat. *J. Reprod. Fert.* **34** (1973) 351–355.
49. Eik-Nes K. B.: Production and secretion of 5α-reduced testosterone (DHT) by male reproductive organs. *J. steroid Biochem.* **6** (1975) 337–339.
50. Van der Molen H. J., Van der Vusse G. J., Cooke B. A. and De Jong F. H.: Cellular and subcellular compartmentalization of steroid metabolism in the rat testis. *J. Reprod. Fert.* **44** (1975) 351–362.