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ANDROGEN METABOLISM AND CONCENTRATION IN THE SEMINIFEROUS TUBULES AT DIFFERENT STAGES OF DEVELOPMENT

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SUMMARY

Testosterone 5α and 3α reducing activities have been studied in whole testis, isolated seminiferous tubules and interstitial tissue of rats from birth to maturity. It was found that 5α-androstan-3α-17-β-diol was the main metabolite of testosterone in seminiferous tubules and that its formation was maximal at the time of the first meiotic division, in the 20 to 26-day-old rat. These *in vitro* studies were confirmed by *in vivo* determinations of the concentrations of testosterone dihydrotestosterone and 5α-androstan 3α,17β-diol in the tubules through sexual development. Testosterone increased from 39 ng/100 ml proteins at 14 days to 94 at 20 days, and then dropped to 13 at 26 days. There was a gradual increase thereafter to reach again a value of 94 at sexual maturation. The concentration of 5α-androstan-3α, 17β-diol also increased from 21 to 89 in 20-day-old rats, but it remained high in 26-day-old animals only to drop thereafter and remain low at sexual maturation. The concentration of dihydrotestosterone did not show a definite peak in young rats but increased markedly between 60 and 90 days of life. It is postulated that testosterone and 5α-androstan-3α,17β-diol might be the active androgens at the time of meiosis. Metabolism of [^{3}H]-testosterone-^{3}H was also studied in the human testis. Formation of reduced products in seminiferous tubules was also correlated with development of spermatogenesis and found to be higher at the time of the first meiotic division, in adolescent boys.

The germinal epithelium of the mature rat goes through a continuing process of cellular proliferation and differentiation from spermatogonium to spermatozoon including a mitotic and a meiotic cellular division. It is generally accepted that androgens are involved in the stimulation of the germinal epithelium. A particularity of the seminiferous tubules, as opposed to other androgen target organs, is their close proximity to Leydig cells that expose them to large concentrations of testosterone. It seems that the androgen-responsive cells of the seminiferous tubules need more androgen for their normal function than the cells of the prostate, seminal vesicles and other target organs [1], with the possible exception of the epididymis.

Testosterone-reducing activity in rat seminiferous tubules at the time of development of the first meiotic division

Some years ago we became interested in studying whether reduction to testosterone to dihydrotestosterone took place in the seminiferous tubules. For this purpose, we incubated isolated seminiferous tubules and interstitial tissue of mature rats with [^{3}H]-testosterone as precursor [2]. The time course of the percentage distribution of radioactivity in testosterone, dihydrotestosterone, 5α-androstane-3α,17β-diol and androst-4-ene-3,17-dione was followed. In the tubules, as substrate testosterone was utilized, 5α-androstane-3α,17β-diol emerged as the main metabolite with little formation of dihydrotestosterone. On the contrary, in the interstitial tissue fraction, no reduced products were observed and 4-androstene-3,17-dione was the main metabolite formed. Therefore, the tubules showed the 5α-reducing activity described in other androgen target organs, but the main metabolite was not dihydrotestosterone. Since 5α-androstane-3α,17β-diol had been found to be the main metabolite of testosterone in preparations of whole testis of maturing rats by other investigators [3–5], we speculated that what these authors detected could have been, at least in part, the activity of the seminiferous tubules. We then incubated isolated tubules and interstitial tissue of 20-day-old rats [6].

It can be observed in Fig. 1 that the seminiferous tubules of these young rats had a faster rate of utilization of testosterone and of formation of 5α-androstane-3α,17β-diol than mature animals. The interstitial tissue fraction did not show formation of 4-androstene-3,17-dione as had been found in mature rats. We

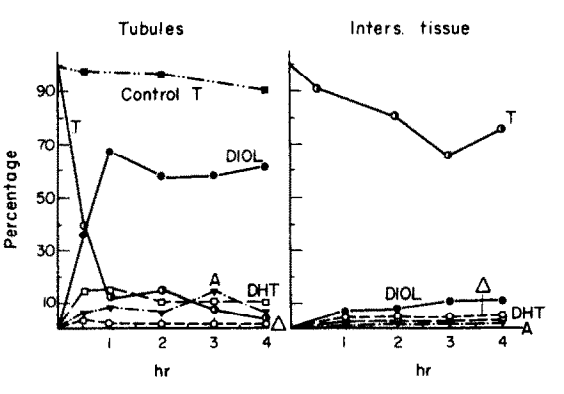

Fig. 1. Time-course of the percentage of total radioactivity in testosterone(T), dihydrotestosterone(DHT), 5α-androstane-3α,17β-diol(DIOL) and 4-androstene-3,17-dione(Δ) after incubation of 20-day-old rat seminiferous tubules and interstitial tissue with [^{14}C]-testosterone. Incubations were carried out at 31°C without addition of cofactors.

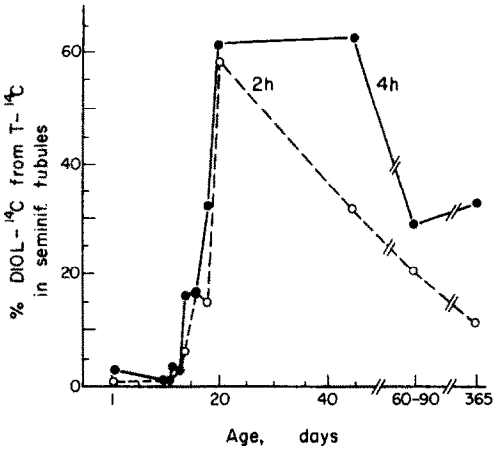

Fig. 2. Percentage radioactivity as 5α-androstane-3α,17β-diol(DIOL) after incubating seminiferous tubules with [^{14}C]-testosterone in rats from birth to maturity. Dotted line, 2 h of incubation; full line, 4 h.

then proceeded to study the formation of 5α-androstane-3α,17β-diol by seminiferous tubules from birth to maturity. Since separation of tubules from surrounding tissue is very difficult during the first two weeks of life, studies were carried out in preparations of whole testis in these very young rats. Separation was performed in rats 14 days old or older. In Fig. 2, the percentage of radioactivity as 5α-androstane-3α,17β-diol in incubations of seminiferous tubules at different ages is plotted. Reducing activity became evident around the 12th day of life and increased rapidly during the following week. As shown by the 2-h-incubation line, the rate of formation was slower in 45-day-old animals and decreased even further in the mature testis to remain low in 1-year-old rats. Table 1 lists the histological finding of the incubated testis in these developing rats. After the 10th day of life, seminiferous tubules of maturing rats start showing a very active process of cellular differentiation and multiplication. The first changes indicating the onset of meiosis were seen between the 10th and 12th day of life in the nuclei of resting primary spermatocytes. From days 14 to 20, there was a progressive development of meiosis. The first zigotene and pachytene spermatocytes were observed on day 16. By day 20, meiosis was already well developed except for the absence of diplotene spermatocytes. Meiosis was completed in the 26-day-old rat. These changes coincided with a gradual and progressive increase in the formation of 5α-androstane-3α,17β-diol by the tubules. Full spermatogenesis was reached by day 60, not shown in the Table, at a time when formation of 5α-androstane-3α,17β-diol had decreased. From these studies, we proposed that 5α-androstane-3α,17β-diol might be involved in the stimulation of the first meiotic division.

Testosterone-reducing activity in human seminiferous tubules at the time of development of the first meiotic division

We then became interested in knowing whether this testosterone-reducing activity of the seminiferous tubules could be shown in the human testis. Maturation of spermatogonia up to the stage of spermatozoa takes around 7 weeks in the rat and 9 weeks in the human. Obviously, if the rat is going to have spermatozoa by age 60 days, it has to start this process of development soon after birth. In the human, a long period of inactivity of the germinal epithelium is present during the years of infancy and childhood when only spermatogonia are seen in the seminiferous tubules. The process of maturation of germ cells begins sometime during adolescence. Therefore, from the standpoint of initiation of spermatogenesis, the equivalent model of the 15- to 26-day-old rat in the human corresponds to the adolescent boy. With this idea in mind, metabolism of testosterone by seminiferous tubules was studied in prepubertal, pubertal and postpubertal human subjects. Biopsy material was obtained at surgery in patients operated upon because of epididymitis or cryptorchidism [9].

Our findings in these testes with different degrees of tubular maturation are shown in Fig. 3. Patient

Table 1. Testicular histology of rats in which the metabolism of testosterone was studied

Age of rats (d)	%[^{14}C]-DIOL at 4 h	Testicular histology
1	2·4	Gonocytes and pre-Sertoli cells in all cords
10	0·8	Degenerating gonocytes, Types A, In and B spermatogonia. A few type R
11	0·3	spermatocytes
12	3·2	
13	2·8	Slight increase in Type R spermatocytes, a few type L spermatocytes
14	15·7	Marked increase in Type L spermatocytes
16	16·9	Further increase in type L spermatocytes. A few Type Z and P spermatocytes
18	34·4	Types L, Z and P spermatocytes in 90% of tubules. No type D spermatocytes
20	61·4	Very good quantitative and qualitative development of meiosis except for absence of Type D spermatocytes
45	62·5	Almost complete maturation of spermatogenesis
365	32·6	Full spermatogenesis. No regressive changes

Primary spermatocytes: R, resting; L, leptotene; Z, zigotene; P, pachytene; D, diplotene.
Cellular types have been classified according to Leblond and Clermont (7) and Clermont and Perey (8).
DIOL = 5α-androstane-3α,17β-diol.

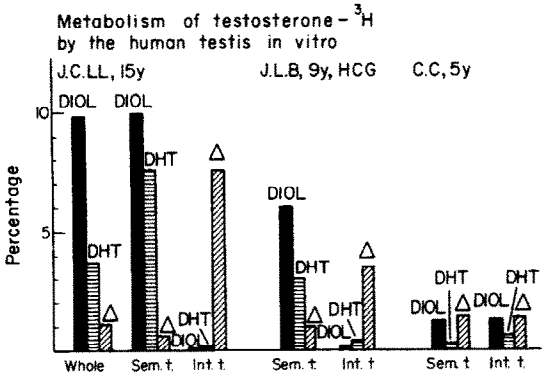

Fig. 3. Percentage distribution of radioactivity among several androgens after incubating testicular tissue with [^{3}H]-testosterone in 3 subjects with different degrees of tubular maturation. Abbreviations are the same as in Fig. 1.

J.Ll. had tuberculous epididymitis with apparently normal testicular tissue showing a maturation up to the stage of spermatids. 5α-androstane-3α,17β-diol and dihydrotestosterone were the main metabolites of testosterone in preparations of whole testis and isolated tubules while 4-androstene-3,17-dione was found in the interstitial tissue. The scrotal testis of the 9-year-old patient J.B. with unilateral cryptorchidism was also studied. HCG administration resulted in partial stimulation of the seminiferous tubules up to the stage of zigotenic spermatocytes. 5α-androstane-3α,17β-diol and dihydrotestosterone could also be detected in the seminiferous tubules. On the other hand, the immature scrotal testis of patient C.C. with unilateral cryptorchidism showed little enzymatic activity.

Two additional subjects are shown in Fig. 4. Patient F.M. had an epididymitis with an unaffected testes. He had partial maturation of the seminiferous tubules showing completion of meiosis but few spermatids. The reducing activity found in the whole testis preparation was again localized in the seminiferous tubules while the interstitial tissue formed 4-androstene-3,17-dione. On the right, patient R.V., who also had an epididymitis, showed some alterations in the microscopic examination of the testis with decreased number of spermatoza. Reducing activity was poor.

Main microscopic findings in the testes of all human subjects studied is summarized in Table 2. They have been arranged according to the degree of maturation of the germinal epithelium. Signs of initiation of meiosis were observed from patient S.P. down. Incomplete maturation was seen in the adult patient R.V., possibly related to his disease. In Fig. 5, percentage conversion to dihydrotestosterone and 5α-androstane-3α,17β-diol in the whole group is plotted. Patients were arranged in order according to histological signs of maturation as seen in the previous Table. It can be observed that as the prepubertal seminiferous tubules matured, there was a progressive increase in the reduced metabolites with a peak in the boys who had just completed their first meiotic division. A decrease was found in the adult patient. The pattern is similar to what we had found in the developing rat testis.

Concentration of testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol in rat whole testis, seminiferous tubules and interstitial tissue during sexual development

As mentioned earlier, the high concentrations of testosterone and other androgens in the testis facilitates their determination. For this reason, we studied the contents of testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol in whole testis, interstitial tissue and seminiferous tubules of rats at different stages of sexual development in order to see if our *in vitro* studies did correlate with this *in vivo*

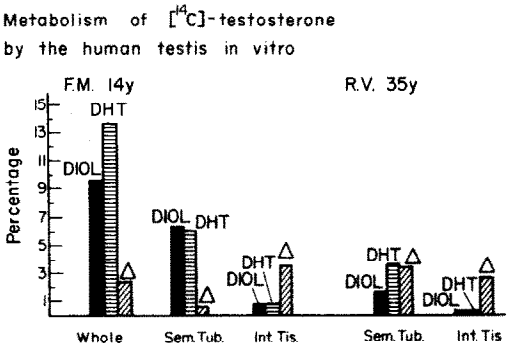

Fig. 4. Percentage distribution of radioactivity among several androgens after incubating testicular tissue with [^{3}H]-testosterone in one adolescent boy and in one adult subject. Abbreviations are the same as in Fig. 1.

Table 2. Testicular histology in patients in whom metabolism of testosterone was studied

Patients	age		ϕ	L	Z	P	D	T	Zo	S
O.C.	9	HCG	60	+	−	−	−	−	−	Imm
C.C.	5	—	43	−	−	−	−	−	−	Imm
S.P.	12	HCG	49	+	+	−	−	−	−	Imm
J.R.	14	—	81	+	+	−	−	−	−	Imm
J.B.	9	HCG	45	++	+	−	−	−	−	Imm
F.M.	14	—	—	+++	++	++	+	+	−	Mat
J.Ll.	15	—	110	++++	++++	++++	+++	++	−	Mat
R.V.	35	—	129	++++	++++	++++	++++	+++	++	Mat

ϕ Mean tubular diameter in μ. L, Z, P, D: leptotenic, zygotenic, pachytenic and diplotenic spermatocytes. T: spermatids. Zo: spermatozoa. S: Sertolli cells. Imm, Mat: immature and mature Sertolli cells.

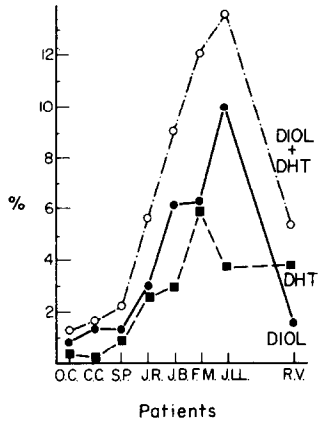

Fig. 5. Percentage conversion of testosterone(T) to dihydrotestosterone(DHT), 5α-androstane-3α,17β-diol(DIOL) and DHT + DIOL by human seminiferous tubules. Developing subjects have been arranged according to the degree of maturation of the germinal epithelium, the adult subject being plotted at the end.

approach. Androgens were determined by a competitive binding technique after purification by thin-layer chromatography [10]. Values were confirmed in a selected group of samples by gas–liquid chromatography.

In Fig. 6 the contents of testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol in whole testis at different ages are plotted. Values are given in nanograms per testis. It can be observed that testosterone rises from less than 1 ng at age 10 days to 10 ng in 20-day-old animals to drop abruptly 6 days later, in spite of the fact that the testis continues to grow. After this, there is a gradual elevation of testosterone up to 60 to 70 ng per testis in mature rats. The contents of 5α-androstane-3α,17β-diol also shows the early peak observed in testosterone but 5α-androstane-3α,17β-diol is still high in 26-day-old rats when testosterone had fallen down. Afterwards, 5α-androstane-3α,17β-diol contents decrease and remain low at sexual maturation. Dihydrotestosterone contents do not show the early peak seen in the other two androgens, but rises in mature rats. In the interstitial tissue, a testosterone peak was also detected at age 20 days. The increment of testosterone during the final period of maturation was similar to that found in whole testis. Since the interstitial tissue represents approximately 10–15% of total testicular weight, it can be easily calculated that the concentration of testosterone per mg of tissue should be much higher in this tissue than in whole testis or seminiferous tubules. Dihydrotestosterone and 5α-androstane-3α,17β-diol could not be detected in the interstitial tissue with the sensitivity of our method.

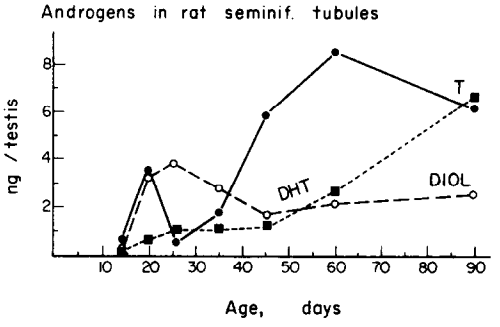

Fig. 7. Contents of testosterone(T), dihydrotestosterone(DHT) and 5α-androstane-3α,17β-diol(DIOL) in seminiferous tubules of rats from the 14th day of life up to sexual maturation (ng/testis).

The contents of the three androgens in the seminiferous tubules are shown in Fig. 7. There was a peak of testosterone in 20-day-old rats identical to that found in whole testis and interstitial tissue. From age 26 days up to sexual maturation, there was a progressive increase in testosterone with a decline of unknown significance from 60 to 90 days.

There was no early peak for dihydrotestosterone, but after the age of 50 days, at the time of maturation of the first spermatozoa, dihydrotestosterone increased markedly to equal the testosterone value in 90-day-old animals. 5α-androstane-3α,17β-diol increased along with testosterone from 10 to 20 days, but fell after the 26th day of life, to remain relatively low at sexual maturation. Therefore, at age 26 days, when meiosis is completed, 5α-androstane-3α,17β-diol

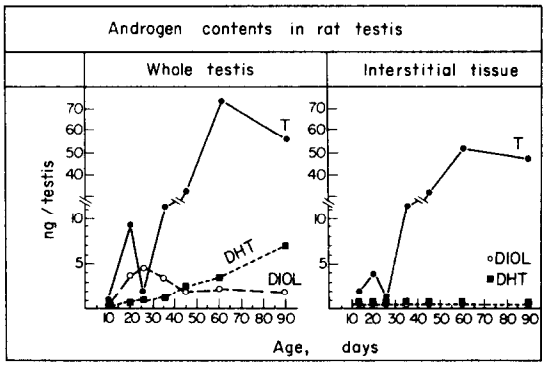

Fig. 6. Contents of testosterone(T), dihydrotestosterone(DHT) and 5α-androstane-3α,17β-diol(DIOL) in whole rat testis and interstitial tissue from 10 days of age to sexual maturation (ng/testis).

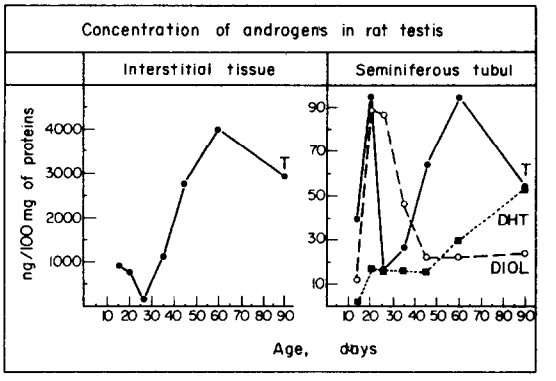

Fig. 8. Concentration of testosterone(T), dihydrotestosterone(DHT) and 5α-androstane-3α,17β-diol in rat interstitial tissue and seminiferous tubules during development and at maturation (ng/100 mg protein).

represented by far the androgen present in highest amounts in the seminiferous tubules.

These marked changes in androgen contents of the testes can be better visualized when these values are expressed as concentrations per tissue mass. Protein contents have been chosen as a parameter of tissue mass. In Fig. 8, previous data are given in ng per 100 mg protein. In the interstitial tissue, testosterone reaches values between 3000 and 4000 ng per 100 mg protein, probably higher than in any other biological tissue. In the seminiferous tubules, it can be seen that the peak of testosterone and 5α-androstane-3α,17β-diol in young rats is of the same magnitude as the testosterone peak in 60-day-old animals.

CONCLUSIONS

The main conclusions drawn from *in vitro* studies were confirmed by *in vivo* determinations, *i.e.* seminiferous tubules of developing rats have the enzymatic activity necessary to form 5α-androstane-3α,17β-diol, and furthermore, this androgen is quantitatively the most important androgen present in the tubules at the time of development of the first meiotic division in the rat. Once meiosis is completed 5α-androstane-3α,17β-diol decreases. Testosterone/5α-androstane-3α,17β-diol ratio is close to 1 in the seminiferous tubules of the 20-day-old rat while it is very high in the interstitial tissue. These different ratios show clearly that the presence of 5α-androstane-3α,17β-diol in the tubules is not due to a contamination of interstitial tissue.

This supports the theory that 5α-androstane-3α,17β-diol is made in the tubules. Otherwise, it would have to be postulated that there is a preferential transport of 5α-androstane-3α,17β-diol from the interstitium into the tubules.

It is interesting that dihydrotestosterone concentration did not increase during the first meiotic division, but did later on, suggesting that this androgen might stimulate the mature stages of spermatogenesis. In summary, the early peak in the concentration of testosterone and particularly 5α-androstane-3α,17β-diol in the seminiferous tubules suggests that these androgens may play a role in the stimulation of the first meiotic division, *i.e.* initiation of spermatogenesis. Testosterone and dihydrotestosterone could be the active androgens necessary for maintaining subsequent meiotic divisions in sexually mature animals.

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