AN ANALYSIS OF THE METABOLITES OF PROGESTERONE PRODUCED BY ISOLATED SERTOLI CELLS AT THE ONSET OF GAMETOGENESIS

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ABSTRACT

Sertoli cells isolated from 17 day old rats were maintained in culture and incubated with [^{14}C]-progesterone for 20 h. The cells and media were extracted with ether/chloroform and the extracts chromatographed two-dimensionally on TLC and the radioactive metabolites visualized by autoradiography. Nine of the metabolites (constituting about 88% of total metabolite radioactivity) were identified by relative mobilities of the compounds and their derivatives in TLC and GC systems and by recrystallizations with authentic steroids as the following: 20α-hydroxypregn-4-en-3-one, 3α-hydroxy-5α-pregnan-20-one, 5α-pregnane-3α,20α-diol, 17β-hydroxy-5α-androstan-3-one, 5α-pregnane-3,20-dione, 17-hydroxypregn-4-ene-3,20-dione, testosterone, 5α-androstane-3α,17β-diol and androst-4-ene-3,17-dione. Over 71% of the metabolite radioactivity was due to 20α-hydroxypregn-4-en-3-one, the major metabolite. 5α-reduced pregnanes constituted about 12% and C_{19} steroids comprised about 2.9% of the radioactivity of the metabolites. Calculation of relative steroidogenic enzyme activities from initial reaction rates suggested the following activities in μunits/mg Sertoli cell protein: 20α-hydroxysteroid oxidoreductase (20α-HSO; 7.71), 5α-reductase (4.77), 3α-HSO (3.57), 17α-hydroxylase (0.93), 17β-HSO (0.34) and C_{17}-C_{20} lyase (0.34). The relatively high rate of steroidogenic enzyme activities in the Sertoli cells of young rats may indicate that Sertoli cells are less dependent on Leydig cell steroidogenesis than has been assumed. Since nearly all the metabolites of progesterone and testosterone are now identified, it is possible to construct a picture of Sertoli cell steroidogenic activity.

INTRODUCTION

Previous studies have demonstrated that the rat testis is capable of a high rate of progesterone metabolism (1,2). Although the interstitial tissue has been considered to be the principal steroidogenic tissue in the testis (3), numerous reports have indicated that the seminiferous tubules of rats are also capable of *in vitro* progesterone metabolism (4-6). Yamada *et al* (5) found that tubules and "nongerm" tubular cells from 30 day old Wistar rats converted progesterone to

5α-pregnane-3,20-dione, 17-hydroxypregn-4-ene-3,20-dione (17-HP), androstenedione (androst-5-ene-3,17-dione) and a trace of testosterone (17β-hydroxyandrost-4-en-3-one). And recently Tcholakian and Steinberger (7) demonstrated that Sertoli cell-enriched cultures from 80 day old rats metabolize progesterone to testosterone, androstenedione, 17-HP, 20α-hydroxypregn-4-en-3-one (20α-DHP) and 17β-hydroxy-5α-androstan-3-one (DHT). We have shown previously that is-lated Sertoli cells (in the absence of Leydig cells and viable germ cells) metabolize androstenedione to testosterone, DHT and 5α-androstane-3α,17β-diol (8), that in cells from young rats (10 to 20 days old) the conversions are stimulated by FSH (8,9) and that the conversion rate is higher between 10 and 20 days than after 20 days of age (8). Recently it was shown that in culture the major metabolite produced from [^{14}C]-progesterone by isolated Sertoli cells from 17 day old rats is 20α-DHP (10). However, Sertoli cells from young rats produce a number of other steroids from progesterone and the present report presents evidence of the identity of nearly 90% of the metabolites formed. The gonadotropin influences and age dependent changes in the progesterone metabolites identified in the present investigation have been indicated in preliminary reports (11,12).

MATERIALS AND METHODS

Animals. Sprague-Dawley strain rats from our own colony (original breeders purchased from Bio Breeding Laboratories of Canada, Ltd., Ottawa) were maintained on a 14-h light, 10-h dark photoperiod, at 24°C and fed Purina Rat Chow and water ad libitum. Seventeen day old males were employed in the study and the rats came from two separate populations. One group had been irradiated in utero on the 18th day of gestation (160 rads of gamma irradiation per pregnant rat) and these contained Sertoli cell-enriched testes (13). The other group was not irradiated.

Chemicals. Inorganic salts and organic solvents were of analytical or spectral grade. Solvents used for extraction were glass distilled prior

to use. The [4-^{14}C]-progesterone (56.95 μCi/μmole) and [1,2,6,7-^{3}H]-progesterone (105 μCi/nmole) were purchased from New England Nuclear and were purified by thin layer chromatography (TLC) prior to use.

Histochemistry and Cytology. In each experiment, several culture dishes were employed for the determination of Sertoli cell numbers and possible contamination with Leydig, peritubular or germ cells. Sertoli cells were identified cytologically (14) after staining with Delafield's hematoxylin and numbers per culture dish were estimated by counts of 20 separate areas produced by a superimposed grid. To test for the presence of Leydig or peritubular cells, cultures were examined for histochemical evidence of 5-ane-3β-hydroxysteroid dehydrogenase activity (15). Cultures were incubated at 37°C in a medium composed of 4 ml 0.1 M Na-K phosphate buffer (pH 8.0), 0.2 ml methanol with 0.138 μmol 3β-hydroxy-5β-androstan-17-one, 0.25 ml Na-K phosphate buffer with 0.612 μmol nitro blue tetrazolium (Sigma), and 0.5 ml distilled water with 6.825 μmol NAD (Sigma). The incubation medium for the blanks lacked the steroid substrate.

Cell Preparation and Incubation. At 17 days of age the rats were weighed and then sacrificed by cervical dislocation. Testes were removed aseptically, washed, finely minced and the Sertoli cells were isolated by sequential enzymatic disaggregation (14) and cultured as previously described (14). Cultures were estimated to comprise 95% to over 98% Sertoli cells, with no Leydig and peritubular cell contamination as determined by the total lack of histochemical 5-ane-3β-hydroxysteroid oxidoreductase activity (15). The contaminating cells consisted of necrotic germ cells within the Sertoli cells (14).

Isolated Sertoli cells were seeded in 35 x 10 mm Corning plastic petri dishes. Each dish contained approximately 1.5 x 10^6 cells. The cells were cultured for 72 h as previously described. After 72 h the medium was replaced with fresh medium containing [4-^{14}C]-progesterone (0.65 μCi/11.56 nmoles/dish) and cells were incubated at 32°C for an additional 1, 3, 6, 20, or 48 hours. Reactions were terminated by scraping the monolayer of cells from each dish and transferring cells and medium to glass tubes containing 2 drops of 1 N HCl.

Steroid Extraction and Identification. For extraction of steroids, the samples were brought up to pH 9-10 with 1 N NaOH. About 8000 dpm [1,2,6,7-^{3}H]-progesterone was added to each sample to serve as recovery tracer. The samples were treated as previously described (10). Each sample was extracted three times with 4 volumes of ether/chloroform (4:1), concentrated under nitrogen and spotted on the lower right hand corner of a 20 x 20 cm silica gel GF thin layer plate (Fisher Redi/Plate, 0.25 mm). Each plate was run 2 times in System I (chloroform/either, 10:3), then turned 90° and run 2 times in System II (hexane/ethyl acetate, 5:2). The plates were then apposed to Kodak Medical X-Ray film (X OMAT R Film) for a 7-day exposure. The R_f values of the radioactive spots were determined, then the spots were scraped from the plates and the gels extracted with ether/chloroform (4:1). The R_f values of the spots were compared to those of 101 authentic steroids chromatographed in the same systems.

To help establish the identities of the progesterone metabolites, procedures similar to those described previously were employed (10). A Hewlett Packard 5830A gas chromatograph equipped with a 150 cm (4 mm I.D.) column packed with 3% OV-210 on Gas Chrom Q 80/100 mesh was employed (oven temp. 240°C, injection port 260°C, FID 260°C) to obtain the retention times (R_T) of authentic steroids which had R_f values similar to those of the metabolite under investigation. Then an aliquot of the metabolite was injected and samples were collected by means of a glass stream splitting device with ratio of approximately 1:9. The material emerging at the splitter was collected in 24 cm snugly fitted glass tubes. The radioactivity of the collected samples was determined with a Beckman LS-255 liquid scintillation counter in a toluene-PPO-POPOP system. Tentative identity of each metabolite was confirmed as outlined under Results by acyl and silyl derivative formation, reduction or oxidation, and when possible by crystallization with authentic unlabelled steroid to constant specific activity.

Formation of Derivatives.

Acetylation. Authentic hydroxy-steroids (1 mg) and unknown samples were dissolved, each in 200 µl pyridine in a glass tube with teflon-lined screw cap. Then 140 µl acetic anhydride was added to each and the mixture stored overnight in the dark and at room temperature. At termination the fluid was evaporated under dry N_2 at 50°C. The dry acetate was dissolved in the appropriate organic solvent, the radioactivity quantitated and the derivative further processed for identification by TLC, GC and/or crystallization.

Silylation. Authentic hydroxy-steroids (1 mg) and unknown samples were dissolved, each in 0.2 ml of either of the following mixtures: bis (trimethylsilyl) acetamide/pyridine (1:10) or bis (trimethylsilyl) acetamide/trimethylchlorosilane/pyridine (5:1:50). The mixture was stored overnight in the dark, at room temperature, and subsequently processed as for acetylation (above).

Reductions. To reduce ketone groups at positions 3, 17, and 20, steroids were dissolved in methanol, the solution was cooled to -20°C for about 10 min and then a few crystals of sodium borohydride were added. The mixture was kept at 4°C for 1 hr and the reaction terminated by the addition of 1.0 N HCl (0.1 ml/ml of mixture). After addition of distilled water (1 ml) the steroid was extracted 3 times with 2 volumes of ether or methylene dichloride. The extract was washed with alkali, acid, water, then dried over sodium sulphate and evaporated under dry N_2 at 45°C.

Oxidations. Steroid was dissolved in 0.2 ml of acetic acid and then 0.15 ml of 2% (w/v) chromium trioxide in water was added. The tube was capped and kept in the dark for 10-120 min. To terminate the reaction, 5-10 volumes of water were added. The steroids were extracted with ether or methylene dichloride. The extract was washed with 0.1 vol of sodium bicarbonate (50% saturated), then with water (0.1 vol), dried with sodium sulphate and evaporated under N_2 at 45°C.

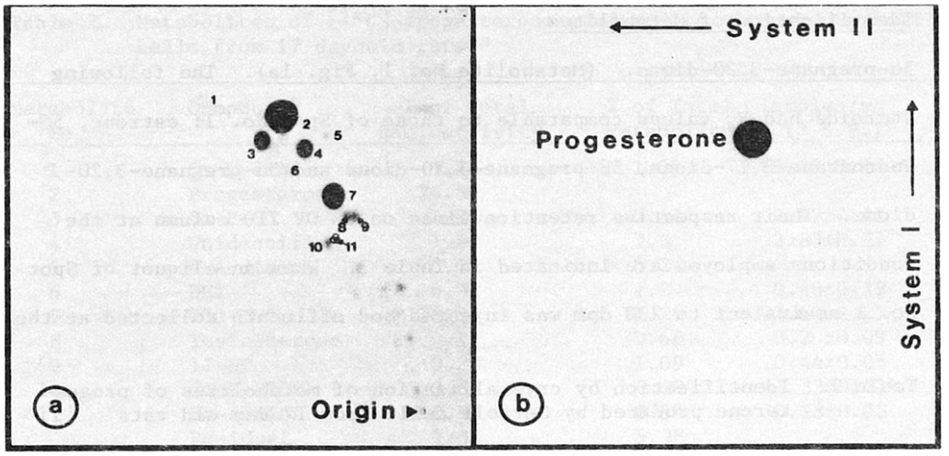

FIGURE 1. Autoradiographs of TLC plates showing the results of incubating [^{14}C]-progesterone for 20 h at 32°C with either (a) culture medium containing 1.5 x 10^6 Sertoli cells from 17 day old rats, or (b) culture medium without any cells. The extracts were spotted on the lower right hand corner and the plates were run 2X in System I (chloroform/ether, 10:3) and then 2X in System II (hexane/ethyl acetate, 5:2) in the directions indicated. Kodak X OMAT R Film was apposed to the plates and exposed for 7 days. The number of each spot is referred to as the metabolite No. in the text. The position of spot No. 11 was determined from non-radioactive standards and is indicated by the three small circles

RESULTS

In Vitro Metabolism of Progesterone

Figure 1a shows that Sertoli cells from 17 day old rats in vitro converted [^{14}C]-progesterone to a number of metabolites. Spot No. 2 (Fig 1a) was identified as the unmetabolized [^{14}C]-progesterone; it had the same R_f values in both TLC systems as [^{14}C]-progesterone incubated without cells (Fig. 1b) and as unlabelled progesterone. Moreover, the radioactivity of Spot No. 2 emerged at the same time as the progesterone peak when applied to an OV 210 column in the gas chromatograph. Crystallization with authentic unlabelled progesterone showed constant specific activity after the first crystallization (Table 1). About 75% of all the recovered radioactivity was due to the unmetabolized [^{14}C]-progesterone (Table 2).

Identification of Metabolites

5α-pregnane-3,20-dione. (Metabolite No. 1, Fig. 1a). The following steroids had R_f values comparable to those of Spot No. 1: estrone, 5α-androstane-3,17-dione, 5β-pregnane-3,20-dione and 5α-pregnane-3,20-dione. Their respective retention times on an OV 210 column at the conditions employed are indicated in Table 3. When an aliquot of Spot No. 1 equivalent to 230 dpm was injected and effluents collected at the

Table 1. Identification by crystallization of metabolites of progesterone produced by Sertoli cells from 17 day old rats

Metabolite No.	Compound*	Theoretical specific activity[a] (dpm/mg)	Specific activity of crystals (dpm/mg) 1st	2nd	3rd	4th
1	5α-P-dione	123	101	119	111	120
2	Progesterone	945	928	933	968	950
3	5α-P-3α[b]	812	840	827	798	827
5	4-A-dione[c]	381	346	369	385	380
6	DHT	186	196	193	171	
7	20α-DHP[d]	192	191	185	192	
8	Testosterone		140	120	150	130
9	17-HP		102	97	100	98
10	5α-P-DIOL[e]	308	355	312	296	
11	5-A-DIOL		194	119	114	

To part of the isolated radioactive fraction approx. 20 mg of the appropriate reference compound was added. Results are given in dpm/mg. The solvents employed were acetone/pentane and methanol/water.

[a]Total radioactivity of the fraction/mg of reference steroid added.

[b]Metabolite No. 3 oxidized and then recrystallized with 5α-pregnane-3,20-dione.

[c]The recrystallizations for androstenedione were done with metabolite from Sertoli cells of 65 day old rats.

[d]Results from Ref (10).

[e]Metabolite No. 10 oxidized and then recrystallized with 5α-pregnane-3,20-dione.

*Abbreviations used: 5α-P-dione: 5α-pregnane-3,20-dione; 5α-P-3α: 3α-hydroxy-5α-pregnan-20-one; 4-A-dione: androst-4-ene-3,17-dione; DHT: 17β-hydroxy-5α-androstan-3-one; 20α-DHP: 20α-hydroxypregn-4-en-3-one; 17-HP: 17-hydroxypregn-4-ene-3,20-dione; 5α-P-DIOL: 5α-pregnane-3α-20α-diol; 5α-A-DIOL: 5α-androstane-3α,17β-diol.

Table 2. Metabolites of [^{14}C]-progesterone produced in vitro by Sertoli cells from 17 day old rats.[a]

Metabolite No.	Compound[b]	% of Total Radioactivity	% of Total Metabolites	nmoles/mg (± S.E.)[c]
1	5α-P-dione	0.28	1.09	0.42±0.14
2	Progesterone	74.39		
3	5α-P-3α	1.95	7.61	2.97±0.11
4	Unidentified	1.87	7.3	2.83±0.31
5	4-A-dione	< 0.05	< 0.2	
6	DHT	0.31	1.2	0.48±0.19
7	20α-DHP	18.25	71.26	27.63±1.21
8	Testosterone	0.17	0.66	0.2 ±0.09
9	17-HP	0.28	1.09	0.44±0.05
10	5α-P-DIOL	0.87	3.39	1.33±0.21
11	5α-A-DIOL	0.15	0.59	0.18±0.05
	Residual	1.38	5.38	

a[^{14}C]-progesterone was incubated in 5 culture dishes, each containing approx 1.5 x 10^6 Sertoli cells from 17 day old rats. The radioactive areas from the TLC plates (Fig. 1) were eluted and quantitated by scintillation spectrometry after corrections for losses. The data represent results from two separate experiments.

[b]Abbreviations used, see footnote to Table 1.

[c]nmoles steroid produced in 20 hours per mg Sertoli cell protein.

times encompassing the peaks of the 4 steroids, 226 dpm were collected in the area of 5α-pregnane-3,20-dione (Table 3). Another aliquot (3000 dpm) of Spot No. 1 was crystallized with 23.9 mg authentic 5α-pregnane-3-20-dione. After the 4th recrystallization, the specific activity of the crystals varied by less than 5% of the initial calculated specific activity (Table 1) and it was concluded that Spot No. 1 (Fig. 1a) is essentially pure 5α-pregnane-3,20-dione.

3α-hydroxy-5α-pregnan-20-one (Metabolite No. 3, Fig. 1a). The following steroids had R_f values comparable to Spot No. 3 in TLC systems I and II: 17β-hydroxy-5α-androstan-3-one (DHT), 3α-hydroxy-5α-pregnan-20-one, 3β-hydroxy-5β-pregnan-20-one, and 20α-hydroxy-5α-pregnan-3-one; their respective GC retention times on the OV 210 column were 7.7, 7.5, 7.6 and 11.6 minutes. All the radioactivity of aliquots of Spot No. 3

Table 3. GC retention times of steroids with R_f values comparable to metabolite No. 1 and the result of splitting off the metabolite with these steroids.[a]

Steroid	Retention Time (min)	Times Split (min)	Radioactivity Captured (dpm)
Estrone	7.1	6.8-8.8	0
5α-androstane-3,17-dione	12.3	11.0-13.5	0
5β-pregnane-3,20-dione	14.3	13.5-14.8	0
5α-pregnane-3,20-dione	15.6	14.9-16.9	226

[a]Aliquots of the known standard steroids were injected along with 230 dpm of metabolite No. 1. Samples were removed from the main stream by means of a splitting device (10) at the times indicated.

was trapped between 7 and 9 minutes on the GC splitter, thus eliminating 20α-hydroxy-5α-pregnan-3-one as the possible metabolite. Another aliquot of Spot No. 3 was crystallized with authentic unlabelled DHT and no radioactivity occurred in the crystals. In order to determine whether either 3α-hydroxy-5α-pregnan-20-one or 3β-hydroxy-5β-pregnan-20-one might be the metabolite, a sample of each and also an aliquot of Spot No. 3 were oxidized and then applied to the GC. The R_T of 5β-pregnane-3,20-dione was 14.3 min and R_T of 5α-pregnane-3,20-dione was 15.6 min. All the radioactivity of the oxidized Spot No. 3 emerged between 15 and 16.5 min. Because 3α-hydroxy-5α-pregnan-20-one was only available in minute quantities, an aliquot of oxidized Spot No. 3 was crystallized with authentic unlabelled 5α-pregnane-3,20-dione. The specific activities of crystals from 4 recrystallizations (Table 1) varied less than 5% from the calculated initial values and it was concluded that metabolite No. 3 is 3α-hydroxy-5α-pregnan-20-one.

Metabolite No. 4

Metabolite No. 4 (Fig. 1a) remains unidentified. Of the 101 steroid standards in our collection, none had R_f values relative to

progesterone (R_{fp}) which were identical to those of No. 4 when run in Systems I and II. The following steroids had R_{fp} values which were approximately in the same range as those of No. 4: 3β-hydroxy-5α-pregnan-20-one, 17β-hydroxy-5α-androstan-3-one (DHT) and estradiol-17β. The radioactivity of eluates of No. 4, applied to gas chromatography (OV 210), emerged at approximately the same time as estradiol-17β (90% of radioactivity applied, emerged between 3-5 min; estradiol-17β R_T = 4.9 min; DHT R_T = 7.7 min; 3β-hydroxy-5α-pregnan-20-one R_T = 8.6). Several attempts were made to crystallize aliquots of No. 4 with unlabelled authentic estradiol-17β or DHT. In each case the radioactivity remained in the mother liquor and it was concluded that No. 4 was not any of the steroids examined. Aliquots of No. 4 were oxidized with Cr_2O_3, then chromatographed two-dimensionally in Systems I and II along with authentic unlabelled progesterone and subjected to autoradiography. The radioactivity of the oxidized No. 4 was coincident with the progesterone spot. An eluate of this radioactive area was applied to gas chromatography; the radioactivity emerged approximately at the same time as progesterone. Another aliquot of the oxidized No. 4 was added to authentic unlabelled progesterone and recrystallized from acetone/n-pentane. The specific activity of crystals and mother liquor remained the same through several recrystallizations (in dpm/mg the first and second crystals and mother liquors were respectively 84, 85, 89, 95). Thus, although its exact identity is unknown, No. 4 metabolite is concluded to be a pregn-4-ene compound with hydroxyl group(s) which can be oxidized to progesterone.

Androst-4-ene-3,17-dione (metabolite No. 5)

The identity of metabolite No. 5 (Fig. 1a) was established to be

androstenedione on the basis of several TLC and GC systems and recrystallizations with authentic androstenedione (Table 1).

17β-hydroxy-5α-androstan-3-one (DHT; metabolite No. 6) and 5α-androstane-3α,17β-diol (metabolite No. 11).

Metabolites 6 and 11 were identified as DHT and 5α-androstane-3α, 17β-diol by procedures published previously (8). An example of crystallization data is reported in Table 1.

20α-hydroxypregn-4-en-3-one (metabolite No. 7; 20α-DHP).

The identity of metabolite No. 7 (Fig. 1a) produced from progesterone by isolated rat Sertoli cells was previously established (10) to be 20α-hydroxypregn-4-en-3-one.

17β-hydroxy-androst-4-en-3-one (testosterone; metabolite No. 8) and 17-hydroxypregn-4-ene-3,20-dione (17-hydroxyprogesterone; 17-HP; metabolite No. 9).

Metabolites 8 and 9 had R_f values comparable to those of testosterone and 17-hydroxyprogesterone. The region containing Nos. 8 and 9 (Fig. 1a) was eluted and then rerun 6 times in System II, which separated the area into 2 distinct spots. No. 8 migrated more rapidly and was coincident with testosterone; No. 9 ran coincident with 17-hydroxyprogesterone. An aliquot of No. 8 was split from the GC (OV 210) and the radioactivity emerged (10-12.5 min) coincident with the unlabelled testosterone (R_T = 11.4 min). The radioactivity of No. 9 emerged (30-40 min) coincident with 17-hydroxyprogesterone (R_T = 35.5 min). Aliquots of Nos. 8 and 9 were recrystallized with authentic unlabelled testosterone and 17-hydroxyprogesterone respectively; the specific activities of the crystals (Table 1) further confirm that metabolite No. 8 is testosterone and metabolite No. 9 is 17-hydroxyprogesterone.

5α-pregnane-3α,20α-diol (metabolite No. 10)

The following steroids had R_f values similar to those of metabolite No. 10 in TLC Systems I and II: androst-5-ene-3β,17β-diol, 5α-androstane-3α,17β-diol, 5β-pregnane-3α,20β-diol, and 5α-pregnane-3α,20α-diol; the respective GC retention times of these four steroids on the OV 210 column were 3.9, 4.2, 5.4, and 5.8 minutes. All the radioactivity of aliquots of No. 6 emerged between 5 and 7 minutes on the GC splitter, thus essentially eliminating androstenediol or androstanediol as the possible metabolite. Another aliquot of No. 10 was oxidized and the radioactivity trapped on the GC splitter; 94% of the radioactivity emerged with 5α-pregnane-3,20-dione (R_T = 15.6 min) and less than 2% was detected with 5β-pregnane-3,20-dione (R_T = 14.3 min). An aliquot of oxidized No. 10 (7,630 dpm) was added to 24.77 mg of authentic unlabelled 5α-pregnane-3,20-dione and recrystallized from acetone/n-pentane. The specific activity of the crystals resulting from the 2nd and 3rd recrystallizations, varied by less than 5% of the initial calculated specific activity (Table 1) and it was concluded that No. 10 is 5α-pregnane-3α,20α-diol.

Relative steroidogenic enzyme activities in Sertoli cells

The relative amounts (nmoles/mg Sertoli cell protein) of each identified metabolite produced from progesterone were calculated and are reported in Table 2. The predominant metabolite was 20α-hydroxypregn-4-en-3-one which constituted over 18% of the total radioactivity recovered from the incubations. 3α-Hydroxy-5α-pregnan-20-one (metabolite No. 3) comprised nearly 2% of the total radioactivity and the other C_{21} and the C_{19} metabolites constituted lesser amounts (Table 2).

In order to determine if rates of formation of the metabolites vary with time, Sertoli cells from 17 day old rats were incubated with

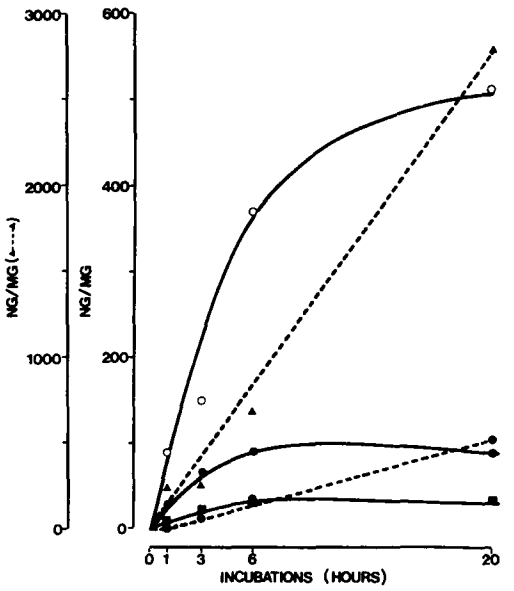

FIGURE 2. Relative rates of conversion of [^{14}C]-progesterone to 20α-DHP (▲--▲), 3α-hydroxy-5α-pregnan-20-one (○—○), 5α-pregnane-3,20 dione (●—●),5α-pregnane-3α,20α-diol (●--●), and testosterone (■—■). Note the separate scale for 20α-DHP.

[^{14}C]-progesterone for 1, 3, 6, 20 or 48 hours and the metabolites were quantitated for the various times. Figure 2 shows the changes, with time, in amounts of metabolites and indicates that each reaction rate examined was linear for at least the first 3 hours and some rates were linear for up to 6 or 20 hours. The rates, for each reaction, during the initial linear parts of the curves were determined and the sum of the metabolic products resulting from a particular enzyme reaction were tabulated. Table 4 shows the calculated activity of specific enzymes in μunits of enzyme activity/mg of Sertoli cell protein. From the values in Table 4 it is evident that the highest steroidogenic enzyme activity, in vitro, in Sertoli cells from 17 day old rats is the 20α-hydroxysteroid oxidoreductase (7.715 μunits/mg). The 5α-reductase and 3α-hydroxysteroid oxidoreductase activities are also considerable (4.77 and 3.57 μunits/mg, respectively) and the 17α-hydroxylase, C_{17}-C_{20} lyase and the 17β-hydroxysteroid oxidoreductase activities are less than 1/20th of the 20α-HSO activity (0.09, 0.34 and 0.34 μunits/mg, respectively).

Table 4. Relative steroidogenic enzyme activity in Sertoli cells from 17 day old rats.

Enzyme[a]	ng/mg/hr[b]	μunits/mg[c]
3α-HSO	67.3	3.57
17β-HSO	6.4	0.34
20α-HSO	145.6	7.71
5α-reductase	90.0	4.77
17α-hydroxylase	17.6	0.93
C_{17}-C_{20} lyase	6.4	0.34

[a] Enzyme activity was determined by summing the identified metabolites of progesterone which require the particular enzyme reaction for their formation:
3α-HSO activity is based on the sum of 5α-pregnane-3α,20α-diol and 3α-hydroxy-5α-pregnan-20-one found during 6 h of incubation
17β-HSO activity is based on the sum of testosterone and DHT formed during 6 h of incubation.
20α-HSO activity is based on the sum of 20α-hydroxypregn-4-en-3-one and 5α-pregnane-3α,20α-diol formed during the first 6 h of incubation with Sertoli cells.
5α-reductase activity is based on the sum of the 5α-reduced metabolites formed from progesterone during 3 or 6 h of incubation.
17α-hydroxylase activity is based on the sum of 17-hydroxypregn-4-ene-3,20-dione and the C_{19} metabolites formed from progesterone during 6 h of incubation.
C_{17}-C_{20} lyase activity is based on the sum of the C_{19} metabolites formed from progesterone during 6 h of incubation.

[b] ng/mg/hr = ng of steroids produced (via the enzyme listed) in 1 h per mg Sertoli cell protein.

[c] μunits/mg = μunits of enzyme activity/mg Sertoli cell protein.
1 μunit of enzyme activity = 1 pmole of products/min.

DISCUSSION

Several groups of investigators have reported the presence of steroidogenic enzyme activities in seminiferous tubules (16). These steroidogenic activities within the tubules now are largely attributable to the Sertoli cells. Studies with isolated Sertoli cells have shown that they possess 5α-reductase, 17β-hydroxysteroid oxidoreductase, 20α-hydroxysteroid oxidoreductase, 17α-hydroxylase and C_{17}-C_{20} lyase (7-10; 17; Table 4). Some reports have suggested the presence of Δ^5-3β-hydroxysteroid oxidoreductase in seminiferous tubules of Sertoli cells (17). However, we have been unable to detect any such activity in

isolated Sertoli cells, either histochemically or biochemically. [^{3}H]-3β-Hydroxy-5-pregnen-20-one was not converted to [^{3}H]-progesterone or any other steroid with a 3-keto group. Conversely, none of the identified progesterone metabolites had a 3β-OH group and those remaining unidentified could not be matched chromatographically with known 3β-OH steroids.

The present results extend the list of steroids capable of being produced by Sertoli cells from young rats. It is now known that Sertoli cells produce androstenedione, DHT and 5α-androstane-3α,17β-diol from testosterone (9), and 5α-pregnane-3,20-dione (Table 2), 20α-DHP (10), 5α-pregnane-3α,20α-diol (Table 2), 17-HP (7; Table 2), testosterone (7; Table 2), androstenedione and DHT (Table 2) from progesterone and pregn-5-ene-3β,20α-diol from pregnenolone (10).

Recently Tcholakian and Steinberger (7), using cells from 80 day old rats and incubating for 3 h, were able to account for 8.45% of the [^{3}H]-progesterone metabolism. In their study 3.45% of the progesterone metabolites were identified as testosterone, androstenedione, 17 - hydroxypregn-4-ene-3,20-dione and DHT and about 59% of the progesterone metabolism remained unidentified (7). In our study with cells from 17 day old rats, we found that after 20 h of incubation, over 25% (or nearly 12 nmoles) of the progesterone was metabolized and we have identified nearly 90% of the metabolite fraction. For cells from 80 day old rats, Tcholakian and Steinberger (7) list DHT as the major identified metabolite of progesterone followed by 17-HP and then 20α-DHP. They (7) suggest, moreover, that 5α-reductase is the primary active steroid metabolic enzyme in Sertoli cells from 80 day old rats. In our present study with Sertoli cells from 17 day old rats, the amount of 20α-DHP produced from progesterone in 20 h was 65 x the amount of

17-HP (Table 2) and the most active steroid metabolic enzyme is 20α-hydroxysteroid oxidoreductase (Table 4). An age-dependent shift in progesterone metabolism is suggested. We have previously demonstrated age-dependent changes in C_{19} steroid metabolism (8) and will be reporting on age-dependent changes in progesterone metabolism by Sertoli cells in a forthcoming publication (18).

The possible biological functions of the various C_{21} and C_{19} steroid metabolites have never been fully explored. However, several observations suggest that they are more than waste products or merely metabolic intermediates in the synthesis of testosterone or DHT. (a) The rates of production of some of the "non-testosterone" steroids by Sertoli cells change markedly with age, being highest between 10 and 20 days and showing the greatest stimulation by FSH at the time of onset of gametogenesis (8, 12). (b) Several reports indicate that compounds such as 20α-DHP or 5α-pregnane-3,20-dione may inhibit C_{17}-C_{20} lyase (19, 20) or 17α-hydroxylase (21), and 3α-hydroxy-5α-pregnan-20-one can trigger LH release from the pituitary (22) while 20α-DHP may inhibit LH release (23). (c) "Non-testosterone" steroids have been shown to be able to maintain aspects of spermatogenesis (24). On the other hand, marked reduction of testicular and plasma testosterone due to pharmacological treatments need not impair spermatogenesis (25) while spermatogenic arrest due to cryptorchidism is accompanied by greatly elevated testicular levels of testosterone and FSH (26). Estradiol-17β has been reported, on the basis of RIA measurements, to be produced by rat Sertoli cells (27) and it is suggested to have a regulatory role in the male. The rates of conversion of progesterone to the steroids reported in Table 2 indicate that at 17 days Sertoli cells produce various

steroids at concentrations that are from 3X to 162X the concentration of testosterone; moreover, the concentrations of progesterone metabolites amount to from 300X to 19,400X the reported concentration of estradiol-17β (27). As the rate of formation of C_{21} and C_{19} metabolites (Table 2) is particularly high at the onset of gametogenesis (12, 18), it may be important to examine the metabolites for possible biological effects. And since Sertoli cells from young rats are able to synthesize steroids de novo from acetate (28) it may be that the biologically active steroids within the tubules are less dependent upon the result of Leydig cell activity than has hitherto been presumed.

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30. Trivial Names and Abbreviations:
androstenedione: androst-4-ene-3,17-dione; DHT: 17β-hydroxy-5α androstan-3-one; 17-HP: 17-hydroxyprogesterone, 17-hydroxy-pregn-4-ene-3,20-dione: 20α-DHP: 20α-hydroxypregn-4-en-3-one; pregnenolone: 3β-hydroxy-5-pregnen-20-one; progesterone: pregn-5-ene-3,20-dione; HSO: hydroxysteroid oxidoreductase.