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Testicular Steroidogenesis in the Mature and Immature Baboon *Papio anubis*

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The following studies were undertaken to compare testicular steroidogenesis in the mature and immature baboon. Testicular fragments (50 mg) were incubated for 3 hr with [7-^{3}H]pregnenolone, or with [7-^{3}H]progesterone. The mature testis formed more testosterone (4.6%), androstenedione (1.6%), and progesterone (28.5%) from pregnenolone than did the immature testis (0.6, 0.5, and 26.1%). The immature testis formed more 17α-hydroxyprogesterone (34.7%) and 20α-dihydroprogesterone (23.2%) from pregnenolone than did the mature testis. Similar conversions were obtained in progesterone incubates. 5α-Androstanediol was identified only in mature incubates. These results suggest that the mature baboon testis has greater C_{17}-C_{20} lyase, 17β-hydroxysteroid dehydrogenase, and 5α-reductase activities than the immature testis, while the immature testis has greater 20α-reductase activity.

Although there is considerable information available regarding testicular steroidogenesis in mammalian species, primarily in rodents (Steinberger and Ficher, 1968, 1969; Coffey *et al.*, 1971; Bardin and Peterson, 1967; Nayfeh *et al.*, 1966), little is known about this process in subhuman primates. However, we have recently investigated steroidogenesis in testes of marmosets, which are New World primates of the family Callithricidae. In *Saguinus oedipus*, pregnenolone and progesterone substrates are converted primarily to 17α-hydroxyprogesterone, with a significant accumulation of testosterone (Preslock and Steinberger, 1976, 1977a). In the common marmoset *Callithrix jacchus*, progesterone is the predominant metabolite identified in testicular incubates with radiolabeled pregnenolone (Preslock and Steinberger, 1977b).

The baboon *Papio anubis* has been extensively used as a model for studies in reproductive biology (Goldzieher and Axelrod, 1969; Kulkarni *et al.*, 1970; Ishihara *et al.*, 1975). However, there have been no studies reported which describe testicular steroidogenesis in this primate species. The following studies were therefore conducted to investigate the synthesis of androgens by the testis of the baboon.

MATERIALS AND METHODS

Baboons. A total of two immature and one mature *Papio anubis* were utilized in these studies. The immature testes, which were not descended, were removed and each testis was bisected into approximately equal 50-mg fragments, for a total of eight fragments. One-half of each immature testis was incubated with radiolabeled pregnenolone, and one-half was incubated with radiolabeled progesterone. The left descended testis from the mature baboon was cut into eight equal fragments weighing approximately 50 mg. Four fragments were incubated with radiolabeled pregnenolone, and four were incubated with radiolabeled progesterone.

Materials. Nanograde solvents and Silicar TLC-7Gf silica gel were from Mallinckrodt Chemical Works, St. Louis, Mo., nonradioactive steroid carriers were purchased from Steraloids, Rawling, N.Y., while radioactive substrates (^{3}H) and tracers (^{14}C) were

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from Amersham Searle, Inc., Arlington Heights, Ill.; cofactors for incubations were obtained from Sigma Chemical Co., St. Louis, Mo.; chromatography paper (No. 1) was from Whatmann Paper Co. Steroids were checked for purity, while paper and silica gel were washed with methanol.

Incubation procedures. Testes were removed from one mature and two immature *Papio anubis* and placed into ice-cold 0.25 *M* Tris–sucrose buffer, pH 7.4. The testes were weighed, decapsulated, and cut into fragments weighing approximately 50 mg/fragment. The fragments were teased apart and placed into incubation flasks containing Krebs–Ringer bicarbonate, pH 7.4, fortified with NADH, an NADPH-generating system, glucose, glucose-6-phosphate dehydrogenase, and lactic dehydrogenase. They were incubated for 3 hr with [7-^{3}H]pregnenolone (10 μCi; 24 Ci/mmol) or with [7-^{3}H]progesterone (10 μCi; 21 Ci/mmol). A total of four fragments each from immature and mature testes was incubated with radiolabeled pregnenolone, and four each were incubated with progesterone for a total of 16 separate incubations. The incubations were in a Dubnoff metabolic shaking incubator at 37° under an atmosphere of 95% O_2/5% CO_2. After completion of the incubations the reactions were terminated with 1 *N* HCl (0.5 ml), and the incubates were frozen.

Extraction of metabolites. The incubates were thawed at room temperature, and the fragments were homogenized in Krebs–Ringer bicarbonate buffer, pH 7.4. The homogenized fragments were pooled with their respective incubation media, whereupon radiolabeled tracers (^{14}C) and unlabeled carriers were added to each pool. The pools were extracted 10 times with cold diethyl ether:chloroform, 4:1, and the solvents were evaporated with nitrogen. Upon concentration of the residues, 5 ml of methanol was added to each tube, and aliquots were removed for estimates of recovery. The remaining solvent was evaporated under nitrogen, and the residues were utilized in paper chromatography.

Chromatographic procedures. Residues were applied to 2.5 × 50-cm Whatmann No. 1 paper strips which had been impregnated with formamide as the stationary phase. The strips were chromatographed in two separate solvent systems, with hexane as the initial mobile phase and a subsequent separation with a hexane:benzene mobile phase. Radioactive peaks were located with a Packard Model 385 recording ratemeter. The peaks were eluted with 80 ml of methanol, and the steroids were isolated by thin-layer chromatography in selected solvent systems. These solvent systems consisted of benzene:ethyl acetate (80:20, 60:40), benzene:methanol (98:2, 95:5), and chloroform:acetone (90:10, 80:20). Testosterone, 20α-dihydroprogesterone, 5α-androstanediol, and dehydroepiandrosterone were acetylated and separated from other metabolites by thin-layer chromatography. Aliquots for recovery determinations were obtained prior to each thin-layer separation and prior to recrystallization of each metabolite.

Crystallization procedures. Following isolation by chromatography, metabolites were identified by crystallization to constant specific activities and ^{3}H/^{14}C ratios through three successive solvent combinations (acetone:hexane, acetone:cyclohexane, acetone:hexane). The conversion of precursors to metabolites was calculated by determining the total ^{3}H-disintegrations per minute for each metabolite from crystallization data, correcting the total ^{3}H-disintegrations per minute for procedural losses and dividing the corrected total ^{3}H-disintegrations per minute for each metabolite by the ^{3}H-disintegrations per minute of the total incubate. Conversions were expressed as percentages of the ^{3}H-disintegrations per minute of the total incubate. Although 20α-dihydroprogesterone and 5α-androstanediol were not crystallized, their tentative identities were established by acetylation and comparison of relative mobilities with those of authentic standards run in four separate thin-layer chromatographic systems. Percentage conversion of each substrate into these metabolites was estimated from the recovery data.

RESULTS

Pregnenolone incubations. The testes of mature *Papio anubis* converted radiolabeled pregnenolone primarily to progesterone, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, testosterone, androstenedione, and 5α-androstanediol. The major metabolite formed was progesterone (28.5%) with substantial conversion of the substrate to both 17α-hydroxyprogesterone (18.5%) and 20α-dihydroprogesterone (14.7%). Testosterone was 4.6% of the radioactivity, while androstenedione was 1.6%. The mature testis also formed some 5α-androstanediol (1.7%) and dehydroepiandrosterone (0.23%) from radiolabeled pregnenolone.

The immature baboon testis converted pregnenolone substrate into metabolites similar to those of the mature testis. However, 17α-hydroxyprogesterone (34.7%) was the major metabolite formed, while progesterone and 20α-dihydroprogesterone were, respectively, 26.1 and 23.2% of the total radioactivity. Testosterone and an-

drostenedione were 0.6 and 1.6% of the radioactivity, while dehydroepiandrosterone was 0.2%. There was no 5α-androstanediol identified in the immature incubates.

Figure 1 demonstrates the percentage conversion of pregnenolone substrate into each metabolite, while Table 1 lists the recrystallization data for these conversions.

Progesterone incubations. Testes from the mature baboon converted progesterone substrate primarily into 17α-hydroxyprogesterone (26.9%) and 20α-dihydroprogesterone (22.1%). Progesterone was also metabolized to testosterone (4.8%), androstenedione (0.7%), and to 5α-androstanediol (5.5%). The nonmetabolized progesterone substrate was 27.4% of the total radioactivity.

17α-Hydroxyprogesterone and 20α-dihydroprogesterone were the major metabolites formed from incubation of immature testes with progesterone substrate, these metabolites representing 34.3 and 22.7% of the respective radioactivity. There was substantially less conversion of the progesterone substrate into testosterone (0.2%) and androstenedione (0.6%). As reported above for incubations of immature testis with pregnenolone, there was no 5α-

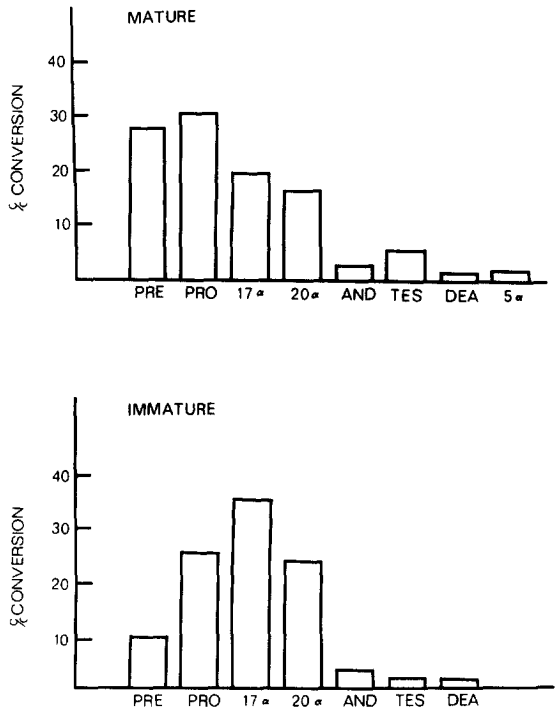

FIG. 1. Percentage (%) conversion of radiolabeled [^{3}H]pregnenolone to metabolites at 3 hr of incubation by 50 mg of mature and immature baboon testes. The following abbreviations are used: PRE, pregnenolone substrate; PRO, progesterone; 17α,17α-hydroxyprogesterone; 20α,20α-dihydroprogesterone; AND, androstenedione; TES, testosterone as testosterone acetate; DEA, dehydroepiandrosterone as dehydroepiandrosterone acetate; 5α, 5α-androstanediol as 5α-androstanediol acetate.

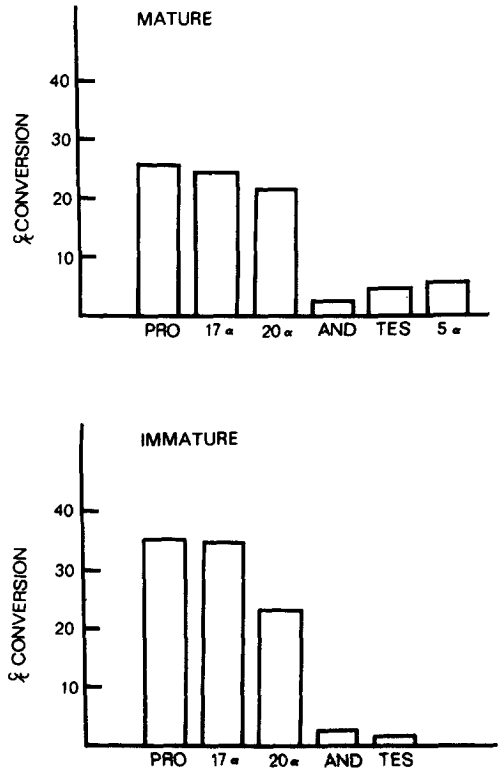

FIG. 2. Percentage (%) conversion of radiolabeled [^{3}H]progesterone to metabolites at 3 hr of incubation by 50 mg of mature and immature baboon testes. The following abbreviations are used: PRO, progesterone substrate; 17α, 17α-hydroxyprogesterone; 20α, 20α-dihydroprogesterone; AND, androstenedione; TES, testosterone as testosterone acetate; 5α, 5α-androstanediol as 5α-androstanediol acetate.

TABLE 1
CRYSTALLIZATION DATA FOR INCUBATION OF [7-^{3}H] PREGNENOLONE WITH TESTICULAR FRAGMENTS FROM MATURE AND IMMATURE BABOONS

Metabolite[a]	^{3}H (dpm/mg)[b]	^{14}C (dpm/mg)[b]	^{3}H/^{14}C
Mature testis			
Preg[c]	83.7 ± 3.2	1.5 ± 0.1	53.3 ± 2.6
Prog	135.4 ± 4.7	1.5 ± 0.09	85.4 ± 3.5
17α-OH Prog	77.6 ± 2.6	1.1 ± 0.09	66.8 ± 2.9
Andros	1.6 ± 0.1	0.8 ± 0.01	1.9 ± 0.1
Testost	1.4 ± 0.1	1.3 ± 0.1	0.1 ± 0.08
DEA	0.8 ± 0.02	1.2 ± 0.09	0.6 ± 0.01
Immature testis			
Preg[c]	33.6 ± 2.3	1.2 ± 0.08	27.5 ± 2.1
Prog	82.4 ± 3.0	0.9 ± 0.09	85.7 ± 3.7
17α-OH Prog	120.6 ± 4.1	0.8 ± 0.06	136.7 ± 4.3
Andros	4.7 ± 0.3	0.7 ± 0.04	6.2 ± 0.4
Testost	2.4 ± 0.1	1.2 ± 0.09	1.9 ± 0.1
DEA	0.7 ± 0.02	0.9 ± 0.08	0.7 ± 0.06

[a] The following abbreviations are used: Preg, pregnenolone; Prog, progesterone; 17α-OH Prog, 17α-hydroxyprogesterone; Andros, androstenedione; Testost, testosterone as testosterone acetate; DEA, dehydroepiandrosterone as dehydroepiandrosterone acetate.

[b] Data ($\bar{X}$ ± SD) represented as mean disintegrations per minute (× 10^3) of three successive crystallizations of quadruplicate samples.

[c] Nonmetabolized pregnenolone substrate.

androstanediol formed from progesterone by the immature baboon testis. Nonmetabolized substrate was 34.5% of the total radioactivity in the immature incubations.

Figure 2 demonstrates the percentage conversion of progesterone substrate into each metabolite, and Table 2 lists the recrystallization data for these conversions.

DISCUSSION

The results of these experiments demonstrate that the mature and immature baboon testes convert pregnenolone and progesterone to similar metabolites. 17α-Hydroxyprogesterone and 20α-dihydroprogesterone were the principal metabolites formed from these substrates, with substantially lower conversion to testosterone and androstenedione. However, there are some important differences when comparing conversions between the mature and the immature testes. The mature testis forms approximately 10-fold more testosterone from both pregnenolone and progesterone substrates than does the immature testis. The mature testis also forms 5α-androstanediol from both pregnenolone and progesterone, while the immature testis forms none. However, the immature testis forms more 17α-hydroxyprogesterone and 20α-dihydroprogesterone from these substrates.

It is apparent from these studies that 5α-reductase is active in the mature baboon testis, but is inactive in the immature testis. The increased 5α-reductase actity is responsible for the formation of 5α-reduced metabolites, primarily 5α-androstanediol, from testosterone. This contrasts with reports in the rat where 5α-reductase resulted in elevated formation of androsterone from progesterone in the immature testis (Steinberger and Ficher, 1968, 1969). The formation of 5α-androstanediol in the mature baboon testis is correlated with increased

TABLE 2
CRYSTALLIZATION DATA FOR INCUBATION OF [7-^{3}H] PROGESTERONE WITH TESTICULAR FRAGMENTS FROM MATURE AND IMMATURE BABOONS

Metabolite[a]	^{3}H (dpm/mg)[b]	^{14}C (dpm/mg)[b]	^{3}H/^{14}C
Mature testis			
Prog[c]	237.7 ± 6.8	1.5 ± 0.1	154.9 ± 5.0
17α-OH Prog	64.5 ± 3.1	1.1 ± 0.08	60.0 ± 3.2
Andros	4.1 ± 0.4	1.1 ± 0.09	4.3 ± 0.2
Testost	0.6 ± 0.03	1.3 ± 0.1	0.5 ± 0.01
Immature testis			
Prog[c]	158.1 ± 4.2	1.2 ± 0.1	131.7 ± 4.1
17α-OH Prog	113.2 ± 3.0	0.8 ± 0.04	150.4 ± 3.8
Andros	3.2 ± 0.2	0.9 ± 0.03	3.5 ± 0.2
Testost	0.6 ± 0.01	1.4 ± 0.1	0.4 ± 0.01

[a] The following abbreviations are used: Prog, progesterone; 17α-OH Prog, 17α-hydroxyprogesterone; Andros, androstenedione; Testost, testosterone as testosterone acetate.

[b] Data ($\bar{X}$ ± SD) represented as mean disintegrations per minute (×10^3) of three successive crystallizations of quadruplicate samples.

[c] Nonmetabolized progesterone substrate.

formation of testosterone, suggesting increased 5α-reductase activity as a consequence of testosterone production. However, whether the immature testis has the capability to metabolize testosterone to 5α-reduced androgens remains to be determined.

These results suggest that the testis of the baboon *Papio anubis* converts pregnenolone to testosterone primarily via the delta-4 pathway. A substantial portion of the pregnenolone substrate was converted to progesterone and 17α-hydroxyprogesterone, metabolites indicative of a predominant delta-4 pathway. However, a small portion of the radioactivity was identified as dehydroepiandrosterone (< 1%), suggesting a minor metabolism of pregnenolone to testosterone via the delta-5 pathway. It is possible, however, that the rate of conversion of pregnenolone to testosterone through dehydroepiandrosterone is very rapid, with a consequent low accumulation of the intermediate dehydroepiandrosterone in the incubates. It is also possible that situation similaa to that described earlier in the mouse testis (Ellis and Berliner, 1965) exists in the baboon testis, whereby there is an early conversion of pregnenolone to testosterone via the delta-5 pathway, with a subsequent slower formation of testosterone from progesterone through the delta-4 pathway.

Previous work in our laboratory has demonstrated a predominant delta-4 pathway in the testis of the marmoset *Saguinus oedipus* (Preslock and Steinberger, 1976, 1977a). This pathway involved conversion of pregnenolone to progesterone, 17α-hydroxyprogesterone, androstenedione, and then to testosterone. Intermediates indicative of a delta-5 pathway were not detected in marmosets. In rhesus monkey testis, Sharma *et al.*, (1967) reported a predominant delta-4 pathway, while Hoschoian and Brownie (1967) reported the delta-5 pathway as predominant. The results of the present studies with baboons, and former studies with marmosets (Preslock and Steinberger, 1976, 1977a) and rhesus monkeys (Sharma *et al.*, 1967), suggest that several subhuman primate testes may convert pregnenolone to testosterone via a predominant delta-4 pathway.

It is interesting that in both the marmoset (Preslock and Steinberger, 1976, 1977a) and

the baboon testis there is substantial accumulation of 17α-hydroxyprogesterone from both pregnenolone and progesterone substrates. This contrasts to other species such as the rat (Barry *et al.*, 1952; Steinberger and Ficher, 1968, 1969; Slaunwhite and Burgett, 1965), the mouse (Ellis and Berliner, 1965), the rabbit (Rosner *et al.*, 1964; Taylor and Scratcher, 1967), and the dog (Eik-Nes and Kekre, 1963), where testosterone is the predominant metabolite formed from these precursors.

It is perhaps significant that substantial portions of pregnenolone and progesterone substrates were converted into 20α-dihydroprogesterone by both the mature and immature baboon testis. Steinberger *et al.* (1970, 1973) earlier reported high levels of this metabolite in incubates of human testicular fragments from normal, azoospermic, and Klinefelter subjects incubated with radiolabeled progesterone. It was recently reported that a significant conversion of progesterone into 20α-dihydroprogesterone occurs in the testis of human males, with the highest conversion occurring in the prepubertal testis (Kjessler and Å:son, 1976; Å:son *et al.*, 1976). As such, there appear to be two major pathways for metabolism of progesterone in testes primate species: One pathway is via 17α-hydroxylase and formation of 17α-hydroxyprogesterone, while the second pathway is via 20α-reductase with formation of 20α-dihydroprogesterone.

Results from the present studies suggest that the activity of C_{17}-C_{20} lyase is higher in the mature than in the immature baboon testis. 17α-Hydroxyprogesterone accumulated in incubates of immature testes but was substantially lower in incubates of mature testes, suggesting defective conversion of 17α-hydroxyprogesterone to androstenedione by C_{17}-C_{20} lyase, particularly in the immature testis. The decreased formation of 17α-hydroxyprogesterone in the mature testis was correlated with increased testosterone formation. As such, C_{17}-C_{20} lyase may be the rate-determining step in the baboon testis and thereby regulate the synthesis of androgens from pregnenolone and progesterone precursors.

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