Comp. Biochem. Physiol., 1969, *Vol.* 31, *pp.* 379 *to* 390. *Pergamon Press. Printed in Great Britain*

STEROID METABOLISM *IN VITRO* BY THE ADRENAL CORTEX FROM MALE AND FEMALE MONKEYS, *MACCA MULATTA**

DINESH C. SHARMA and J. LESTER GABRILOVE

Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48104; University of California, Berkeley, California; and The Mount Sinai Hospital and Medical School, New York, N.Y. 10029

(*Received* 27 *March* 1969)

Abstract—1. Biosynthesis of steroid hormones from radioactive progesterone, 17α-hydroxyprogesterone, androst-4-ene-3,17-dione and testosterone by the adrenals of male and female Rhesus monkeys was studied.

2. On a comparative basis the adrenals from the female monkeys yielded a higher ratio of estrogens to androst-4-ene-3,17-dione and/or testosterone among the biosynthetic products.

3. Probably due to the common embryologic origin, the sex chromatin constitution (*XX*, or *XY*) reflected is not only in gonadal but also in adreno-cortical enzyme activities.

4. Following gonadectomy, decreased sex difference in the biosynthetic activities of the adrenals was observed.

INTRODUCTION

THE ADRENAL cortex is capable of secreting not only the corticoids but the androgens and estrogens also. Although ordinarily this contribution is modest as compared to the gonads (Baggett *et al.*, 1956, 1959), under certain circumstances the production of sex hormones by the adrenal cortex may be of great significance (Soffer *et al.*, 1961). It has been suggested that the common biosynthetic pathway to androgens and estrogens found in the gonads and the adrenal cortex reflects their common embryologic origin in the urogenital ridge (Gabrilove, 1961, 1964; Sellman & Dougherty, 1967). Earlier we have reported on the biosynthesis of testosterone by monkey testes (Sharma *et al.*, 1967a). Present studies were carried out to investigate the biosynthesis of corticoids by monkey adrenals and to ascertain whether the biosynthetic capabilities of the adrenal cortex with respect to sex hormone production also reflect the sex chromatin genetic constitution, and, if so, whether these capabilities might be modified following gonadectomy.

MATERIALS AND METHODS

Progesterone-7-α-^{3}H (SA 32·2 mc/mg), 17α-hydroxyprogesterone-4-^{14}C (SA 108 μc/mg), androst-4-ene-3,17-dione (SA 158 μc/mg) and testosterone-4^{14}C (SA 75 μc/mg) were

* This investigation was supported in part by a grant-in-aid from the National Institutes of Health (HD 02764-01), and a Fellowship (1-FO3-AM 41138-01) from the National Institute of Arthritis and Metabolic Diseases.

purchased from New England Nuclear Corp. and Nuclear Chicago Corp. and were further purified by paper chromatography. NADP "98 per cent" pure as the sodium salt, glucose-6-phosphate "99 per cent" pure also as the sodium salt, Tris and maleic anhydride were obtained from Sigma Chemical Company. Glucose-6-phosphate dehydrogenase was obtained from Calbiochem, Inc. The nonradioactive steroids and other chemicals were also obtained commercially. Analytical grade solvents were redistilled before use.

Experimental subjects

Four healthy adult male (weight 12lb), and four adult female (weight about 10lb) Rhesus monkeys (*Macca mulatta*) were acquired from Woodward Asiatic Animal Imports Corp., Burlingame, California, and housed in individual cages in their large primate colony under the supervision of staff veterinary doctors. After 4 weeks, the adrenal glands of two male and two female experimental subjects were removed and immediately placed in ice for *in vitro* studies commenced within 1 hr. The remaining two male and two female monkeys were transported to the Veterinary School, University of California, Davis for surgery. The males were castrated and the females were ovariectomized under 8 mg of Sernylan injected intravenously. Post-operatively the subjects were maintained at the hospital for recovery for 2 weeks after which they were housed again at the primate colony (Woodward Asiatic Corp). for about 2 months. At the end of this period, the adrenals from these animals were also studied *in vitro* as in the case of the control subjects.

Incubation procedure

The adrenal tissue was homogenized with twenty times its weight/volume of ice cold Tris-maleate buffer 0·05 M, pH 7·4, in an all-glass homogenizer. The homogenate was centrifuged at 3000 *g* for 20 min to remove the unbroken cells, connective tissue and the nuclei. The supernate was incubated with radioactive substrates at 37°C for 60 min in air in a Dubnoff metabolic shaker. In addition to the substrate the incubation mixture contained NADP 3 μmoles, glucose 6-phosphate 20 μmoles, glucose-6-phosphate dehydrogenase 3 Kornberg Units, $MgCl_2$ 15 μmoles, Tris-maelate buffer (pH 7·4) 150 μmoles, in a total volume of 3 ml. Additional details on individual incubations are given under Results below.

Extraction, preliminary purification and separation of metabolites in neutral and phenolic fractions

Following incubation, the radioactive metabolites were extracted from the incubation mixture with methylene chloride; details of the extraction procedure have been given earlier (Sharma *et al.*, 1965a). The extracted steroids were dissolved in 10 ml of methylene chloride, a suitable aliquot was taken to determine the recovery of radioactivity, and the remainder was chromatographed in a 5-g silica gel column (1 × 24 cm) to remove the non-polar lipids. The column was first eluted with 20–25 ml of methylene chloride–methanol (97 : 3) and then with 25 ml of methylene chloride–methanol (85 : 15); the steroids were eluted in the latter fraction and the recovery of radioactivity was 97–99 per cent. The radioactive metabolites in the eluate were partitioned into the neutral and phenolic fractions by the method also described in detail earlier (Sharma *et al.*, 1965b).

Separation of the metabolites in the neutral fraction

To the neutral fraction nonradioactive steroid carriers viz. 17α-OH-progesterone, 16α-OH-progesterone, 11-deoxycorticosterone, corticosterone, 11-deoxycortisol, cortisol, androst-4-ene-3,17-dione and testosterone, 150 μg each were added, and the mixture was subject to partition chromatography on a 35 g Celite (No. 545) column, using the mobile phase 2,2,4-trimethylpentane and the stationary phase 80% methanol–20% H_2O (Engel *et al.*, 1961). An exponential gradient of 1,2-dichloroethane was started after 110 fractions of 1·6 ml each had been collected; a total of 600 fractions were collected.

The fractions were evaporated under nitrogen and the residue was dissolved in 3 ml of 95% aqueous ethanol. An aliquot (0·05 ml) of alternate fractions was taken to determine

the radioactivity by liquid scintillation counting and the absorbance of the remainder was determined in a spectrophotometer at 240 mμ to locate the steroid (Δ^4-3-ketones) carriers. Fractions corresponding to the various radioactive peaks along with washings of the tubes, in which the fractions were collected, were pooled and evaporated in a flash evaporator. The radioactive material was dissolved in a known volume of methanol, an appropriate aliquot was taken for radioactivity determination and the remaining portion was further purified by successive paper chromatography. The various systems used are mentioned in Table 1 and Fig. 1; recovery of the radioactivity was calculated after each step. Details of locating radioactive zones and authentic standards on paper chromatograms and determination of the radioactivity in eluates of the zones have been reported earlier (Sharma *et al.*, 1965b). Final characterization of the metabolites listed in Table 1 was done by isotopic dilution analysis (Sharma *et al.*, 1965b, 1967a); unconverted substrates mentioned in Tables 2 and 4 were not subjected to the isotopic dilution analysis.

Separation of estrogens in the phenolic fraction

Since the total radioactivity in the phenolic fraction from various samples was about 100,000 dis/min or less, instead of a column chromatographic separation of metabolites (Sharma *et al.*, 1965a) successive paper chromatographic separation (Fig. 1) was employed. The phenolic fraction (estrogens) was chromatographed first in Bush B_3 system along with

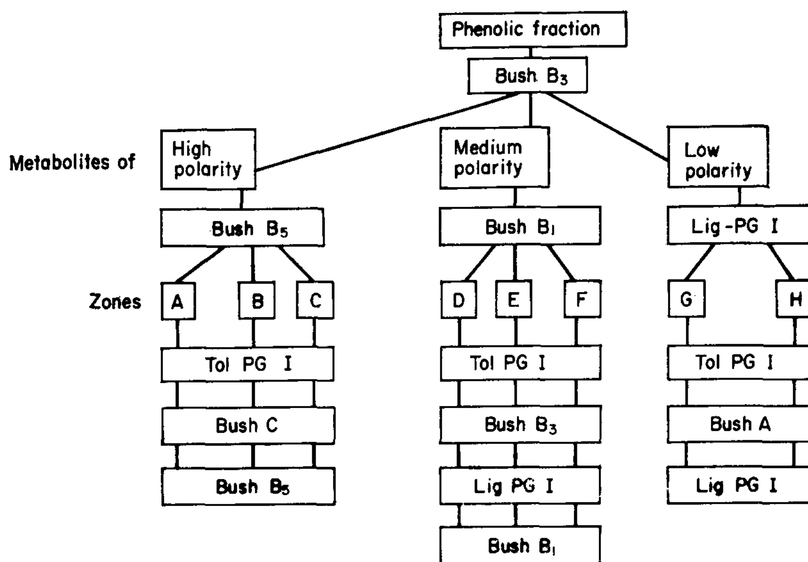

FIG. 1. Separation of the radioactive metabolites in the "phenolic fraction". Zone A = estriol; B = 16-ketoestradiol: C = 16α-hydroxyestrone; D = estradiol-17β; E = estradiol-17α; F = not characterized; G = estrone; H = not characterized. These and other authentic standards particularly, 7α-hydroxyestrone, 15α-hydroxyestrone, 2-hydroxyestradiol, 6-ketoestriadiol, 7α-hydroxyestradiol, 11α-hydroxyestradiol, 15α-hydroxyestradiol and epiestriol were chromatographed on parallel strips. The nonradioactive estrogens were located on the chromatograms by colour reaction with a mixture of equal parts of 1% aqueous potassium ferricyanide and 2% aqueous ferric chloride solutions. Each metabolite was chromatographed separately with various standards.

authentic estriol, estradiol-17β and estrone. The chromatogram was divided into three portions, viz. (i) material of high polarity (comparable to estriol), (ii) material of intermediate polarity (comparable to estradiol-17β), (iii) and material of low polarity (comparable to estrone). Highly polar fraction (i) was rechromatographed in Bush B_5, the intermediate fraction (ii) was rechromatographed in Bush B_1 and the least polar fraction (iii) was chromatographed in ligroin–propylene glycol, 50 : 50 propylene glycol–methanol impregnation. Besides estriol, estradiol-17β, and estrone, several other authentic phenolic steroids of comparable polarity were chromatographed as reference standards. As a result of these and other successive paper chromatographies (Fig. 1) the phenolic metabolites could be divided into eight different zones A through H in decreasing order of polarity. Of these eight phenolic steroids estriol (zone A), estradiol-17β (zone D) and estrone (zone G) were positively characterized by isotopic dilution analysis (Sharma *et al.*, 1965a, b). Identification of the other steroids, based on R_f values in four chromatography systems is tentative. It appears that the steroids in zone B and zone C were 16-ketoestradiol and 16α-hydroxyestrone respectively; the steroid from zone E had the mobility of estradiol-17α. Most of the radioactivity in the strippings was from the material more polar than estriol.

RESULTS

Studies on normal male and female monkeys

*Progesterone-*7α-^{3}H *as the substrate.* Aliquots (35 mg) from each of the four samples of homogenized adrenals (two samples from two normal male and two samples from two normal female monkeys) were incubated in duplicate (eight incubations) with about 11·9 μc (20 μg) of progesterone-7α-^{3}H in presence of a TPNH generating system. Following incubation the radioactive metabolites were extracted from the incubation mixture and partitioned into the neutral and the phenolic fraction. The radioactive metabolites in the neutral fraction along with the nonradioactive carrier steroids (150 μg each) added to this fraction were first separated by column chromatography on a Celite column as described above; a pattern for the separation of various metabolites has been given earlier (Sharma *et al.*, 1965b, 1967b). Further purification and characterization of the various metabolites was done by paper chromatography as listed in Table 1 and by isotopic dilution analysis.

The results of these incubations are summarized in Table 2. About 40 per cent of the substrate was metabolized. Besides 11-deoxycorticosterone, corticosterone and cortisol, 16α-OH progesterone was also a significant product. Despite the use of a NADPH generating system, biosynthesis of testosterone was less than 1 per cent although formation of androst-4-ene-3,17-dione was about 3 per cent. Formation of the phenolic steroids (estrogens) was quite low; distribution of radioactivity in the phenolic fraction into various estrogens is given in Table 3.

*17α-Hydroxyprogesterone-*4-^{14}C *as the substrate.* The amount of the tissue and conditions for the incubations of the adrenal tissue from normal male and female monkeys were the same as in case of the substrate progesterone-7α-^{3}H; however, the amount of the substrate used for each incubation was about 1·77 μc (16 μg). The results of these incubations are also summarized in Table 2. The formation of 11-deoxycortisol and cortisol was much higher than in the case of the substrate progesterone-7α-^{3}H. However, neither the formation of the androgens viz.

TABLE 1—SUCCESSIVE PAPER CHROMATOGRAPHY OF RADIOACTIVE METABOLITES IN THE NEUTRAL FRACTION* FOLLOWING THEIR SEPARATION ON A CELITE COLUMN

Metabolite	Chromatography systems	Running time (hr)
Progesterone	Tol-PG-I	4
	Lig-PG-I	10
	Bush A	To solvent front
17α-OH-Progesterone	Tol-PG-I	10
	Acetylation[2]	
	Tol-PG-I	6
	Bush B_4	To solvent front
16α-OH-Progesterone	Tol-PG-I	14
	Bush B_5	To solvent front
	Tol-PG-II	18
	Cycl + Benz-Form	15
11-Deoxycorticosterone	Tol-PG-I	5
	Bush-B_3	To solvent front
	Tol-PG-II	20
Corticosterone	Tol-PG-I	12
	Bush-B_5	To solvent front
	Tol-PG-II	36
11-Deoxycortisol	Tol-PG-I	12
	Bush-B_5	To solvent front
	Tol-PG-II	36
Cortisol	Bush-B_5	To solvent front
	E_2B	To solvent front
	Tol-PG-II	36
Testosterone	Tol-PG-I	10
	Acetylation	
	Tol-PG-I	6
	Bush-A	To solvent front
	Lig-PG-II	8
Androst-4-ene-3,17-Dione	Tol-PG-I	4
	Bush-A	To solvent front
	Lig-PG-I	10

* Authentic standards were chromatographed on parallel strips at each step. Abbreviations: Tol = toluene; Lig = ligroin, b.p. 39–45°C; PG = propylene glycol; Cycl = cyclohexane; Benz = bensene; Form = formamide repurified by amberlite cation exchange resin IRC-50; MeOH = methanol. (Sharma *et al.*, 1965b, 1967a, b.)

androst-4-ene-3,17-dione and testosterone, nor that of the phenolic steroids was significantly improved; about 62 per cent of the substrate was not metabolized, indicating that its availability was not the limiting factor. The radioactive metabolites in the phenolic fraction are summarized in Table 3.

Androst-4-*ene*-3,17-*dione*-4-^{14}C *and testosterone*-4-^{14}C *as the substrate.* Since in the adrenal cortex a major portion of the C_{21} steroids is chanelled towards the

biosynthesis of corticoids due to a very active C-21 hydroxylating enzyme system, aromatization of androst-4-ene-3,17-dione and testosterone by the male and female adrenals was also studied. The amount of tissue and incubation conditions were similar to those described above in experiments with progesterone-7α-^{3}H; the amount of the substrate androst-4-ene-3,17-dione-4-^{14}C used per incubation was $3{\cdot}84 \times 10^{6}$ dis/min, (10 μg) and that of testosterone-4-^{14}C was $3{\cdot}66 \times 10^{6}$ dis/min (10 μg) in the case of the female adrenals and $4{\cdot}03 \times 10^{6}$ dis/min (10 μg) in the case

TABLE 2—METABOLISM OF PROGESTERONE AND 17α-HYDROXYPROGESTERONE BY ADRENALS FROM NORMAL FEMALE AND MALE MONKEYS*

Substrate	Products	Subjects			
		Females		Males	
		No. 5	No. 6	No. 1	No. 2
		(Percentage of conversion)			
Progesterone-7α-^{3}H	Progesterone (unconverted)	59·2	60·1	60·7	58·0
($26{\cdot}55 \times 10^{6}$ dis/min; 20 μg)	17α-OH-Progesterone	3·2	3·1	2·3	3·4
	16α-OH-Progesterone	5·3	5·1	6·9	6·6
	11-Deoxycorticosterone	10·6	9·8	10·2	10·5
	Corticosterone	2·3	2·5	2·6	2·8
	11-Deoxycortisol	3·2	2·9	2·3	2·6
	Cortisol	4·8	4·5	4·8	4·7
	Androst-4-ene-3,17-dione	3·0	2·9	3·9	4·0
	Tetsosterone	0·3	0·5	0·9	1·0
	Phenolic steroids	0·5	0·4	0·4	0·4
17α-OH-Progesterone-4^{14}C	17α-OH-Progesterone (unconverted)	63·9	61·7	62·2	63·5
($3{\cdot}93 \times 10^{6}$ dis/min; 16 μg)	11-Deoxycortisol	18·1	17·5	14·4	16·4
	Cortisol	7·2	6·9	7·4	7·6
	Androst-4-ene-3,17-dione	3·6	3·3	4·1	4·3
	Testosterone	0·6	0·7	1·0	1·2
	Phenolic steroids	0·5	0·4	0·2	0·3

Distribution of the phenolic fraction into different estrogens is given in Table 3.
Average weight of adrenals from a male monkey was 780 mg and from a female monkey was 870 mg.

* Female and male monkey adrenals, 35 mg, were incubated with radioactive substrates as described under Materials and Methods.

of the male adrenals. The radioactive metabolites extracted from the incubation mixtures were separated into the neutral and phenolic fractions; only the phenolic fraction was further investigated. From the results summarized in Table 3 it is apparent that the aromatization of testosterone by the female adrenal was far greater than by the male adrenal although despite the presence of excess substrate

TABLE 3—BIOSYNTHESIS OF ESTROGENS BY MONKEY ADRENALS*

Substrate (Dis/min × 10^{-6})	Animal no. and sex	Phenolic fraction (Percentage of substrate)	Products (phenolic metabolites) Zone A	Zone B	Zone C	Zone D	Zone E	Zone F	Zone G	Zone H	Strippings
			(Percentage of phenolic fraction (100))								
Progesterone-7α-^{3}H	No. 6 F	0·43	3	6	17	14	26	—	9	15	9
(20 μg)	No. 5 F	0·49	5	7	12	16	21	—	11	17	12
26·55	No. 1 M	0·37	2	8	14	13	23	—	10	17	11
	No. 2 M	0·41	2	9	17	12	20	—	12	19	8
17α-OH-Prog.-4-^{14}C	No. 6 F	0·36	4	4	17	10	31	—	12	18	9
(16 μg)	No. 5 F	0·47	5	6	13	14	26	—	15	11	7
3·93	No. 1 M	0·18	2	7	16	7	29	15	9	14	12
	No. 2 M	0·27	4	8	10	9	27	10	11	12	8
Androst-4-ene-3,17-dione-	No. 6 F	0·39	18	13	12	8	7	10	10	—	15
4-^{14}C (20 μg)	No. 5 F	0·48	14	11	15	10	8	14	16	—	10
3·58	No. 1 M	0·31	7	19	—	8	15	—	4	22	13
	No. 2 M	0·29	11	15	—	9	12	—	11	19	16
Testosterone-4-^{14}C (20 μg)	No. 6 F	1·21	4	7	6	12	15	—	32	—	13
	No. 5 F	1·13	6	9	8	16	12	—	29	—	16
3·37	No. 1 M	0·45	3	9	15	8	12	—	42	—	8
	No. 2 M	0·49	4	11	12	14	10	—	33	—	11

Zone A = estriol; B = 16 ketoestradiol; C = 16α-hydroxyestrone; D = estradiol-17β; E = estradiol-17α; F = not characterized; G = estrone; H = not characterized.

* Incubation conditions and other metabolites are given in Table 2.

the total conversion was low. The difference in the metabolism of androst-4-ene-3, 17-dione, a normal secretory product of the adrenal cortex, by the male and female adrenal was not that marked.

Studies on castrated monkeys

Twelve weeks following surgery, the adrenals from the two orchidectomized males and the two ovariectomized females were obtained for *in vitro* studies. The experimental design for these studies was similar to the one described for control animals; progesterone-7α-^{3}H and 17α-hydroxyprogesterone-4-^{14}C were employed as the substrate again and the tissue to substrate ratio was maintained the same. Table 4 summarizes the data on the conversion of these substrates into the major metabolites. Since the biosynthesis of phenolic steroids was not appreciably different from the experiments with adrenals from control animals, further separation of these radioactive metabolites into various estrogen fractions was not attempted.

TABLE 4—METABOLISM OF PROGESTERONE AND 17α-HYDROXYPROGESTERONE BY ADRENALS FROM MONKEYS 12 WEEKS FOLLOWING GONADECTOMY*

		Subjects			
		Females		Males	
Substrate	Products	No. 7 (Percentage of conversion)	No. 8	No. 3	No. 4
Progesterone-7α-^{3}H	Progesterone (unconverted)	56·1	58·6	59·7	58·2
(28·97 × 10^{6} dis/min; 10 μg)	17α-OH-Progesterone	5·9	5·1	4·1	5·0
	16α-OH-Progesterone	9·0	9·6	3·8	4·2
	11-Deoxycorticosterone	7·1	6·2	9·9	9·4
	Corticosterone	5·0	5·3	4·5	4·7
	11-Deoxycortisol	2·5	2·1	2·0	2·4
	Cortisol	5·2	4·9	3·9	4·3
	Androst-4-ene-3,17-dione	3·8	4·0	4·1	4·4
	Testosterone	0·3	0·2	0·4	0·6
	Phenolic steroids	0·5	0·5	0·5	0·4
17α-OH-Progesterone-4-^{14}C	17α-OH-Progesterone (unconverted)	65·2	61·4	67·3	66·8
(1·04 × 10^{6} dis/min; 8 μg)	11-Deoxycortisol	15·7	14·4	11·9	10·6
	Cortisol	8·1	8·4	6·6	7·7
	Androst-4-ene-3,17-dione	3·7	3·2	3·0	4·1
	Testosterone	0·3	0·4	0·6	0·8
	Phenolic steroids	0·5	0·5	0·3	0·4

Average weight of adrenals from the orchidectomized monkey was 600 mg and from the ovariectomized monkey was 450 mg.

* Female and male monkey adrenals, 16 mg, were incubated with radioactive substrates as described under Materials and Methods.

In these experiments also more than 50 per cent of the substrate remained unmetabolized assuring that its availability was not the limiting factor in the formation of androgens and estrogens. Nevertheless about 4–5 per cent of the substrate was converted to sex hormones, mainly to androst-4-ene-3,17-dione although a NADPH generating system was used to supply the cofactor. Besides C_{21} hydroxylated products, 16α-hydroxyprogesterone was a major metabolite of the substrate progesterone; it was formed in much greater amount by the adrenal from the female as compared to the male adrenal. Among the C_{21} hydroxylated products, both 11-deoxycorticosterone and corticosterone were formed in larger quantity than their 17α-hydroxylated analogs.

As expected the major metabolites of 17α-hydroxyprogesterone were 11-deoxycortisol and cortisol. The amount of androst-4-ene-3,17-dione and testosterone formed was not much different as compared to the experiments with the substrate progesterone. The formation of phenolic steroids was again very low and comparable to the values obtained in experiments with normal monkeys.

DISCUSSION

Results on experiments with normal male and female monkeys (Table 2) as well as the castrated subjects (Table 4) indicate the presence of a very active C-21 hydroxylating system when either progesterone or 17α-hydroxyprogesterone was the substrate. Both 11-deoxycorticosterone and 11-deoxycortisol were recovered in far greater amounts than corticosterone and cortisol indicating that 11β-hydroxylation was the limiting step in the formation of these corticoids from the substrates progesterone and 17α-OH-progesterone.

Formation of 16α-hydroxyprogesterone by hyperplastic human adrenal tissue (Villee *et al.*, 1962; Weliky & Engel, 1963), feminizing human adrenal tumor, feminizing testes (Sharma *et al.*, 1965b) and interstitial cell tumor of the testes (Sharma *et al.*, 1967b) has been reported. Conversion of progesterone to 16α-hydroxyprogesterone by normal human testes (Sharma *et al.*, 1967b) and normal monkey testes (Sharma *et al.*, 1967a) has also been observed although the conversion is much lower. In present studies this metabolite was formed in significant amounts by the adrenals from normal male and female monkeys and its formation by the adrenal from ovariectomized female monkey was far greater than by the adrenal of the orchidectomized male. Neither a physiological role nor an explanation to an increase in the formation of 16α-hydroxyprogesterone in abnormal adrenal and testis has so far been reported, although it has been suggested (Fukushima *et al.*, 1961) that 16α-hydroxylated compounds may have an inhibitory effect on Δ^5-3 ketosteroid isomerase.

When 17α-hydroxyprogesterone was the substrate (Tables 2 and 4) only 40 per cent of the substrate was metabolized and the formation of C_{19} and C_{18} products, viz. androgens and estrogens was not higher than in the case of the substrate progesterone indicating that the limitation is at the C_{17}–C_{20} demolase step. However, this enzyme is present in the adrenal cortex as evidenced by the present studies as well as the fact that androst-4-ene-3,17-dione is a normal secretory

product of the adrenal cortex. A limitation of this biosynthetic step probably explains the very minor role of the adrenal cortex as a sex hormone producing endocrine organ under normal circumstances. The formation of testosterone was minimal although this could be due to the presence of NADPH and not NAD which favors 17α-hydroxysteroid dehydrogenase activity. A decreased activity of this enzyme system as a controlling factor in virilization has been reported in certain disorders (Dominguez, 1961; Sharma *et al.*, 1967b).

The presence of C_{17-20} desmolase and aromatizing enzyme system in the adrenal cortex is probably a consequence of the common embryologic origin and a close morphologic relationship between the gonads and the adrenal cortex (Witschi, 1951; Arey, 1954), however, these enzymes are far less active in the adrenal cortex as compared to the gonads. Although the conversion of progesterone and 17α-OH-progesterone was mainly to the C_{21} hydroxylated compounds and only a limited

TABLE 5—CONVERSION OF STEROID SUBSTRATES INTO ESTROGENS BY THE ADRENALS FROM FEMALE AND MALE RHESUS MONKEYS*

Substrate		Ratio of products × 10			
		Females		Males	
		No. 5	No. 6	No. 1	No. 2
Progesterone-7α-^{3}H	Estrogens / Androgens	1·5	1·2	0·8	0·8
17α-OH-Progesterone-4-^{14}C	Estrogens / Androgens	1·2	1·0	0·4	0·5
Androst-4-ene-3,17-dione-4-^{14}C	Estrogens / Androst-4-ene-3,17-dione	3·9	4·8	3·1	2·9
Testosterone-4-^{14}C	Estrogens / Testosterone	12·1	11·3	4·5	4·9
		Females		Males	
	After gonadectomy	No. 7	No. 8	No. 3	No. 4
Progesterone-7α-^{3}H	Estrogens / Androgens	1·2	1·2	1·1	0·8
17α-OH-Progesterone-4-^{14}C	Estrogens / Androgens	1·2	1·4	0·9	0·8

* Ratios for the substrates progesterone and 17α-OH-progesterone have been calculated from the data given in Tables 2 and 3; ratios for the substrates androst-4-ene-3,17-dione and testosterone have been calculated from the data given in Table 3. The values for "androgens" in this Table are the sums of androst-4-ene-3,17-dione and testosterone figures listed in Table 2.

amount of androgens (androst-4-ene-3,17-dione + testosterone) and estrogens was formed (Table 2), on a relative basis the adrenal from the female monkey indicated a higher activity of the aromatizing enzyme system (Table 3). This was particularly apparent when the substrate was testosterone; major phenolic metabolites of this steroid was estradiol-17β and estrone.

Since the development of the gonadal anlage into the ovary or the testis is genetically determined, it is also expected that the XX genetic sex constituton would favor the conversion of androgen into estrogen and the XY chromosomal complement would minimize the aromatization of the androgens into estrogens. Results of the present studies indicate that a similar effect of the genetic constitution is observable in the minor sex hormone producing role of the adrenal cortex.

The experiment with castrated monkeys was carried out in an attempt to determine whether castration and the consequent increased elaboration of gonadotropins might exert a secondary regulating effect on the conversion of androgen to estrogen in the adrenal cortex. Following castration, the sex difference in the ratio of estrogen/androst-4-ene-3,17-dione in the end-products decreased when progesterone or 17α-OH-progesterone was employed as the substrate (Table 5). Further studies employing varying periods following castration will be necessary, however, to delineate the temporal course of this phenomenon. It may be necessary to note that only a small number of gonadectomized and normal male and female monkeys were available for investigation due to various limitations and a statistical evaluation of the data is not possible in the context of the present experimental design.

REFERENCES

AREY L. B. (1954) *Developmental Anatomy*, 6th edition. W. B. Saunders, Philadelphia.

BAGGETT B., ENGEL L. L., SAVARD K. & DORFMAN R. I. (1956) The conversion of testosterone-3-^{14}C to ^{14}C estradiol-17β by human ovarian tissue. *J. biol. Chem.* **221**, 931–994.

BAGGETT B., ENGEL L. L., BALDERAS L., LANMAN G., SAVARD K. & DORFMAN R. I. (1959) Conversion of ^{14}C-testosterone to ^{14}C-estrogenic steroids by endocrine tissues. *Endocrinology* **64**, 600–608.

BURTON R. B., ZAFFARONI A. & KEUTMANN E. H. (1951) Paper chromatography of steroids —II. Corticosteroids and related compounds. *J. biol. Chem.* **188**, 763–771.

BUSH I. E. (1952) Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. *Biochem. J.* **50**, 370–378.

DOMINGUEZ O. V. (1961) Biosynthesis of steroids by testicular tumours complicating congenital adrenocortical hyperplasia. *J. clin. Endocr. Metabol.* **21**, 663–674.

EBERLEIN W. R. & BONGIOVANNI A. M. (1955) New solvent systems for resolution of corticosteroids by paper chromatography. *Archs Biochem. Biophys.* **59**, 90–96.

ENGEL L. L., CAMERON C. B., STOFFYN A., ALEXANDER J. A., KLEIN O. & TROFIMOW N. D. (1961) The estimation of urinary metabolites of administered estradiol-17β-16-^{14}C. *Analyt. Biochem.* **2**, 114–125.

FUKUSHIMA D. K., SMULOWITZ M. & WILLIAMS K. I. H. (1961) Δ^5-Pregnene-3α,16α,20α-triol and related steroids in adrenocortical carcinoma. *J. biol. Chem.* **236**, 3147–3150.

GABRILOVE J. L. (1961) A biologic concept of adrenocortical function. *Acta Endoc.* (*Kobenhavn*) **36**, 281–286.

GABRILOVE J. L. (1964) Diseases associated with some enzymic defects in the gonads and adrenal cortex. *J. Mount Sinai Hosp., N.Y.* **31**, 449–455.

GABRILOVE J. L., SHARMA D. C., WOTIZ H. H. & DORFMAN R. I. (1965) Feminizing adreno-cortical tumours in the male. *Medicine, Baltimore* **44**, 37–39.

SELLMAN A. H. & DOUGHERTY C. M. (1967) The development of the female genital tract, *Ann. N.Y. Acad. Sci.* **142**, 576–585.

SHARMA D. C., DORFMAN R. I. & SOUTHREN A. L. (1965a) Steroid biosynthesis *in vitro* by feminizing testes. *Endocrinology* **76**, 966–978.

SHARMA D. C., RAHEJA M. C., DORFMAN R. I. & GABRILOVE J. L. (1965b) Studies on a feminizing human adrenal tumour. *J. biol. Chem.* **240**, 1045–1053.

SHARMA D. C., JOSHI S. G. & DORFMAN R. I. (1967a) Biosynthesis of testosterone by monkey-testes *in vitro*. *Endocrinology* **80**, 499–504.

SHARMA D. C., RACZ E. A., DORFMAN R. I. & SCHOEN E. J. (1967b) A comparative study of the biosynthesis of testosterone by human testes and a virilizing interstitial cell tumour. *Acta Endocr.* (*Kobenhavn*) **56**, 726–736.

SOFFER L. J., DORFMAN R. I. & GABRILOVE J. L. (1961) *The Human Adrenal Gland.* Lea & Febiger, Philadelphia.

VILLEE D. B., DIMOLINE A., ENGEL L. L., VILLEE C. A. & RAKER J. (1962) Formation of 16α-hydroxyprogesterone in hyperplastic adrenal tissue. *J. clin. Endocr. Metabol.* **22**, 726–734.

WELIKY I. & ENGEL L. L. (1963) Metabolism of progesterone-4-^{14}C and pregnenolone-7α-^{3}H by human adrenal tissue. *J. biol. Chem.* **238**, 1302–1307.

WITSCHI E. (1951) Embryogenesis of the adrenal and the reproductive glands. *Recent Progr. horm. Res.* **6**, 1–27.

Key Word Index—Monkeys; *Macca mulatta*; adrenal cortex; steroids; corticoids; androgens; estrogens; sex difference; gonadectomy; biosynthesis of hormones.