activité. Afin de vérifier si une partie au moins de l'activité était liée à la molécule des entraîneurs, nous avons appliqué la radiochromatographie fonctionnelle (cf. MATHIS et OURISSON[5]). (La bande No. 5, correspondant à la fois à la 11-déhydrocorticostérone et à la 11-désoxy-17α-hydroxycorticostérone, a été divisée en 2 moitiés, la 11-déhydrocorticostérone recherchée dans l'une et la 11-désoxy-17α-hydroxycorticostérone, dans l'autre.) Après 3 transformations, plus aucune activité n'accompagnait les dérivés de l'aldostérone, de la cortisone et de la 11-désoxy-17α-hydroxycorticostérone. Par contre, elle resta liée aux produits de transformation de l'hydrocortisone, de la corticostérone, de la 11-déhydrocorticostérone et de la 11-désoxycorticostérone. (L'hydrocortisone a été acétylée, l'acétate hydrolysé et l'hydrocortisone régénérée oxydée par $NaBiO_3$; la corticostérone a été acétylée, l'acétate oxydé par CrO_3 et l'acétate de déhydrocorticostérone obtenu hydrolysé.) La Figure 2 montre les radiochromatogrammes se rapportant, le 1er à la 11-désoxycorticostérone régénérée à partir de l'acétate, le 2e au produit de réduction par $NaBH_4$, le Δ^5-prégnène-3,20,21-triol, le 3e à la 11-déhydrocorticostérone régénérée à partir de l'acétate et le 4e au produit de réduction par $NaBH_4$, le Δ^5-prégnène-11-one-3,20,21-triol.

Conclusion. Nous pouvons donc affirmer la présence d'hydrocortisone, de corticostérone, de 11-déhydrocorticostérone et de 11-désoxycorticostérone radioactives dans l'extrait chromatographié et conclure à la sécrétion de ces 4 hormones par la surrénale embryonnaire de Veau cultivée in vitro.

Summary. Pieces of foetal bovine adrenals were cultured in vitro in the presence of ^{14}C-1 sodium acetate. The following radioactive corticosteroids were sought: aldosterone, cortisone, hydrocortisone, corticosterone, 11-dehydrocorticosterone, 11-deoxycorticosterone and 11-deoxy-17α-hydroxycorticosterone. Four of them have been identified by thin-layer radiochromatography and derivative formation, i.e. hydrocortisone, corticosterone, 11-dehydrocorticosterone and 11-deoxycorticosterone.

J. CHOURAQUI et J.-P. WENIGER

Laboratoire de Zoologie et d'Embryologie expérimentale de la Faculté des Sciences, 67 Strasbourg et Centre de Recherches nucléaires, Applications biologiques, 67 Strasbourg-Cronenbourg (France), 18 octobre 1967.

[5] C. MATHIS et G. OURISSON, J. Chromat. *12*, 94 (1963).

In vivo Perfusion of the Human Ovary with 4-^{14}C-Dehydroepiandrosterone and 7α-^{3}H- Dehydroepiandrosterone ^{35}S-Sulfate. Biosynthesis of Steroid Hormones in Human Gonads, V

Only recently, the in vivo perfusion of human testes with suitable precursors revealed the direct transformation of sulfoconjugated dehydroepiandrosterone (DHEA) to sulfoconjugated androgens and estrogens[1-3]. On the other hand, the contribution of sulfoconjugated DHEA to the biogenesis of free androgens and estrogens remained insignificant. The present communication deals with similar investigations on the biosynthesis of C_{19}- and C_{18}-steroids by the human ovary.

In a 37-year-old female patient undergoing ovariectomy due to breast cancer, the left ovary was perfused via the Ramus tubarius-Vena ovarica with radiochemically pure substrate, consisting of 91.5 μg 4-^{14}C-DHEA with 10,130,000 cpm ^{14}C and 75.3 μg Na-7α-^{3}H-DHEA ^{35}S-sulfate with 8,104,000 cpm ^{3}H and 536,000 cpm ^{35}S (^{3}H/^{35}S = r = 15.1). Ovarian vein blood was collected for 40 min, yielding 212.0 ml of heparinized plasma. After exhaustive extraction of free steroids with 3 × 2 Vol chloroform the pre-extracted plasma was treated with 2 × 10 Vol acetone-ethanol (1:1 v/v). For isolation of the various conjugate fractions in the filtrate column chromatography on DEAE-Sephadex A-50 and subsequent thin-layer chromatography of resulting steroid sulfatides and sulfates on silica gel G in chloroform-methanol-ammonia (15:5:0.2 v/v) proved to be efficient[4]. While $^{2}/_{3}$ of the combined sulfoconjugates were subjected to solvolysis in ethyl acetate/sulfuric acid[5], individual sulfoconjugates in the remaining aliquot were separated by thin-layer chromatography in chloroform-methanol-ammonia (10:10:0.2 v/v) and by paper chromatography on M&N paper 2214 FF in hexane-isopropyl ether-*t*-butanol-water-ammonia (2:5:3:9:1 v/v)[6]. Several of the isolated steroid sulfates were submitted to additional purification by consecutive chromatography in the foregoing 2 systems, aliquots being solvolysed in order to establish the ^{14}C-content of the steroid moiety and to characterize the liberated compound. From the different fractions of free and liberated steroids estrogens were removed by routine procedures[7], separated by repeated thin-layer chromatography[8] and quantitated by gas-liquid chromatography of the acetates[9]. Neutral steroids were resolved by repeated thin-layer chromatography in different solvent systems[8] and assayed by the 2,4-dinitrophenylhydrazine method[10] or the OERTEL–EIK-NES reaction[11]. Radioactive zones on chromatograms were localized in a Berthold TL Scanner Mod. 2720, while ^{14}C-, ^{3}H- and ^{35}S-activity in aliquots of all fractions were

[1] P. KNAPSTEIN, F. WENDLBERGER, P. MENZEL and G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *348*, 990 (1967).
[2] G. W. OERTEL, F. WENDLBERGER, P. MENZEL, L. TREIBER and P. KNAPSTEIN, Europ. J. Steroids, in press (1968).
[3] P. KNAPSTEIN, F. WENDLBERGER, P. MENZEL, L. TREIBER and G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *348*, 1066 (1967).
[4] G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *336*, 236 (1964).
[5] S. BURSTEIN and S. LIEBERMAN, J. biol. Chem. *233*, 331 (1958).
[6] E. E. BAULIEU, C. CORPECHOT and R. EMILIOZZI, Steroids *2*, 429 (1963).
[7] L. L. ENGEL and I. T. NATHANSON, J. biol. Chem. *185*, 255 (1950).
[8] G. W. OERTEL, P. KNAPSTEIN and L. TREIBER, Hoppe-Seyler's Z. physiol. Chem. *345*, 221 (1966).
[9] G. W. OERTEL and L. TREIBER, Europ. J. Biochem., in press (1968).
[10] L. TREIBER and G. W. OERTEL, Z. klin. Chem. Biochem. *5*, 83 (1967).
[11] G. W. OERTEL and K. B. EIK-NES, Analyt. Chem. *31*, 98 (1959).

Table I. Free C_{19}- and C_{18}-steroids in ovarian vein plasma

Steroid	cpm $^3H/^{14}C$	r	% ^3H	% ^{14}C	µg	SA ^3H / SA ^{14}C [a]	SA ^3H / SA ^{14}C [b]
Dehydroepiandrosterone	620,000 2,516,000	0.25	84.01	85.06	32.0	19,400 78,600	18,700 75,900
Androstenediol	14,300 42,200	0.34	1.93	1.43	1.6	8,930 26,400	8,340 24,700
Androstenetriol	16,700 72,800	0.23	2,27	2.53	2.0	8,360 36,400	8,820 38,100
16α-Hydroxy-dehydro-epiandrosterone	8,680 40,600	0.21	1.18	1.37	1.1	7,890 36,900	
16-Keto-androstenediol	4,340 19,600	0.22	0.59	0.66	0.6	7,230 32,700	
Androstenedione	27,900 136,000	0.21	3.79	4.60	3.1	8,990 43,900	8,520 40,400
Testosterone	10,900 29,600	0.37	1.47	1.00	0.7	15,500 42,300	15,900 43,800
Androsterone	3,820 9,060	0.42	0.52	0.31	1.2	3,180 7,550	
Etiocholanolone	4,900 12,600	0.39	0.66	0.43	1.1	4,450 11,500	
Androstandione / Etiocholandione	5,260 18,700	0.28	0.71	0.64	0.6	8,770 31,200	
Estrone	3,240 4,620	0.70	0.44	0.16	0.76	4,260 6,080	4,520 6,200
Estradiol	4,620 736	6.28	0.63	0.02	0.96	4,810 767	4,440 764
Estriol	2,980 4,440	0.67	0.40	0.15	0.55	5,420 8,070	5,510 8,300
X_1	10,440 30,600	0.34	1.41	1.03			

[a] After third chromatography, [b] after fifth chromatography. SA, specific activity.

determined in a Packard Tricarb Spectrometer Mod. 3010 by simultaneous counting[12]. For further characterization of free and sulfoconjugated steroids, purification to constant specific activity and isotope ratios, eventually after reverse isotope dilution with standard material, seemed adequate.

Besides 7,832,000 cpm ^{14}C or 77.2% of original ^{14}C-activity the fraction of free steroids also contained 1,786,000 cpm ^3H or 22.0% of infused ^3H-activity, thus hinting at a pronounced sulfatase activity in ovarian tissue[13]. The composition of the free fraction and the specific activity of isolated individual steroids are shown in Table I. From substantial ^3H-activity in free androstenedione, testosterone, as well as estrone, estradiol and estriol, it becomes quite evident that a considerable portion of free androgens and estrogens in ovarian vein blood has been formed from sulfoconjugated DHEA. At the same time the lower specific activity of metabolites indicates a dilution by endogenous compounds.

The fraction of sulfoconjugates, comprised of 60.2% steroid sulfatides and 39.8% steroid sulfates, yielded 4,822,000 cpm ^3H or 59.5% of injected ^2H-activity and 358,000 cpm $^{35}S + ^{14}C$. After subtraction of ^{14}C the isotope ratio $^3H/^{35}S$ was found to be 21.0 instead of 15.1, reflecting a 28.2% loss of ^{35}S in steroid sulfoconjugates. On the basis of this loss of ^{35}S and the appearance of ^{14}C in the fraction of sulfoconjugates, it could be calculated that the fraction of combined steroid sulfatides and sulfates in ovarian vein blood was composed of approximately 69.4% ^3H- and ^{35}S-labelled, 27.1% only ^3H- and 3.5% only ^{14}C-labelled sulfoconjugates. No labelled steroid glucuronosides were detected in ovarian vein blood. The presence of ^{14}C-labelled as well as merely ^3H-labelled sulfoconjugated C_{19}- and C_{18}-steroids in ovarian effluent clearly proves an appreciable sulfokinase activity of ovarian tissue, part of the substrate being formed through hydrolysis of sulfoconjugates. In addition to

[12] K. J. OKITA, J. J. KABARA, F. RICHARDSON and G. V. LEROY, Nucleonics *15*, 110 (1957).

[13] E. C. SANDBERG and R. C. JENKINS, Biochim. biophys. Acta *113*, 190 (1966).

Table II. Sulfoconjugated C_{19}- and C_{18}-steroids in ovarian vein plasma

Steroid	cpm $^3H/^{35}S$ [a]	r	cpm $^3H/^{35}S$ [b]	r	cpm $^3H/^{14}C$ [c]	% ^3H	% ^{14}C	μg	SA ^3H / SA ^{14}C
Dehydroepiandrosterone	398,000	22.6	350,000	21.2	2,059,000	87.46	85.52	93.5	22,000
	17,600		16,500		45,600				488
Androstenediol	6,340	20.4	5,750	20.7	16,200	0.69	1.14	2.4	6,750
	310		278		610				254
Androstenetriol	18,200	19.3	15,400	20.8	60,000	2.55	2.48	2.8	21,400
	945		742		1,320				471
16α-Hydroxy-dehydro-epiandrosterone					95,900	3.80	3.90	4.2	22,800
					2,080				496
16-Keto-androstenediol					62,600	2.66	3.23	2.9	21,600
					1,720				593
Androstenedione	2,610	22.3	2,040	21.5	5,420	0.23	0.36	0.9	6,020
	117		95		150				167
Testosterone	2,530	21.1	2,120	21.9	16,000	0.68	0.73	1.1	14,500
	120		97		390				355
Androsterone					8,560	0.36	0.84	17.4	491
					448				26
Etiocholanolone					9,690	0.41	0.53	5.8	1,670
					282				49
Androstandione / Etiocholandione					2,500	0.11	0.15	1.7	1,470
					78				46
Estrone	2,460	20.3	2,050	22.3	4,920	0.20	0.23	0.89	5,530
	121		92		120				135
Estradiol	2,520	21.0	2,140	22.3	3,660	0.16	0.28	0.61	6,000
	120		96		150				246
Estriol					3,470	0.15	0.20	0.54	6,420
					106				196
X_1					7,230	0.31	0.50		
					268				

[a] Calculated after third and [b] after fourth chromatography of sulfoconjugates and solvolysis of an aliquot. [c] After solvolysis of $^2/_3$ of sulfoconjugates. SA, specific activity.

DHEA as the predominant steroid in the fraction of sulfoconjugates (Table II), also double-labelled androstenedione, testosterone, estrone and estradiol sulfate were isolated and properly identified. Such data supports previous findings concerning the direct transformation of sulfoconjugated DHEA under in vivo conditions[8]. Again, the apparent decrease in the specific activity of numerous metabolites in the fraction of sulfoconjugates is caused by varying dilution with non-labelled endogenous compounds. These may be contributed from different pools within the ovary, from the general plasma pool (as is probable in the case of androsterone and etiocholanolone), or perhaps by biosynthetic pathways not involving sulfoconjugated DHEA.

Although definite conclusions as to the biosynthesis of individual steroids by specific routes appear to be difficult in view of rather high substrate concentrations and sometimes limited radioactivity, the direct transformation of sulfoconjugated DHEA and its role as precursor of free androgens and estrogens in ovarian vein blood seems of particular interest[14].

Zusammenfassung. Nach in-vivo-Perfusion eines menschlichen Ovars mit 4-^{14}C-DHEA und 7α-^{3}H-DHEA-^{35}S-sulfat lagen rund 15% der freien C_{19}- und C_{18}-steroide im Ovarialvenenblut als ^{3}H- und 85% als ^{14}C-markierte Verbindungen vor. Die Fraktion der sulfokonjugierten Steroide, bestehend aus 60% Steroidsulfatiden und 40% Steroidsulfaten, enthielt etwa 69% doppeltmarkierte, 27% ^{3}H- und 4% ^{14}C-markierte Sulfokonjugate. Neben dem vorzugsweise direkten Metabolismus des sulfokonjugierten DHEA liess sich auch ein indirekter Stoffwechsel zu Androgenen und Östrogenen nachweisen.

G. W. Oertel, L. Treiber,
D. Wenzel, P. Knapstein,
F. Wendlberger and P. Menzel

Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik, 65 Mainz and Chirurgische Universitätsklinik, Universität des Saarlandes, 665 Homburg (Germany), 6 December 1967.

[14] This investigation was supported by the Deutsche Forschungsgemeinschaft, 532 Bad Godesberg (Germany).