J. steroid Biochem. Vol. 21, No. 3, pp. 347–349, 1984
Printed in Great Britain

0022-4731/84 $3.00 + 0.00
Pergamon Press Ltd

SHORT COMMUNICATION

CONVERSION OF TESTOSTERONE AND PROGESTERONE TO OESTRONE BY THE OVARY OF THE RAT EMBRYO IN ORGAN CULTURE

J.-P. WENIGER*, J. CHOURAQUI and A. ZEIS
Laboratoire de Zoologie et d'Embryologie expérimentale, Université Louis-Pasteur,
12 rue de l'Université, 67000 Strasbourg, France

(*Received* 28 *November* 1983)

Summary—Ovaries from 19- and 20-day old rat embryos were cultured in the presence of [^{3}H]testosterone and [^{3}H]progesterone respectively, and the conversion of these precursors into [^{3}H]oestrone and [^{3}H]oestradiol was studied. By adding tracer amounts of [^{14}C]oestrone and [^{14}C]oestradiol to the culture media at the beginning of the analysis and crystallizing oestrone and oestradiol to constant specific activity, a conversion percentage could be calculated.

There was no oestradiol formation either from progesterone or from testosterone. Oestrone was formed in measurable amounts from both precursors. It is concluded that in the ovary of the 19–20-day old rat embryo 17β-hydroxysteroid dehydrogenase favors the formation of oestrone.

INTRODUCTION

Until 1975 it was thought that the ovary of the mammalian embryo did not form oestrogens, since neither oestrone nor oestradiol could be identified after incubation of human foetal ovaries, bovine embryonic ovaries or rat embryonic ovaries with [^{14}C]acetate, [^{14}C]pregnenolone or [^{14}C]testosterone [1, 2, 3]. Authors did not worry about this negative finding, since it was known that, contrary to the testis, the ovary did not play any significant role in normal sex differentiation [4].

However, in 1977, Milewich *et al.*[5], using [^{3}H]testosterone and [^{3}H]androstenedione of high specific activity as precursors, demonstrated the formation of radioactive oestradiol and oestrone by the ovary of the rabbit embryo. Shortly thereafter, Mauléon *et al.*[6], using radioimmunoassay, measured large amounts of oestradiol in the culture medium of sheep embryonic ovaries. Since then, these early results have been confirmed in the guinea-pig embryonic ovary [7], the human foetal ovary [8] and the bovine embryonic ovary [9], either by radioimmunoassay or by the radiochemical methods.

Whenever both oestrogens were measured [5, 8], oestradiol was formed in a significantly higher amount than oestrone. Our goal was to study the formation of oestrone and oestradiol by the ovary of the rat embryo. It turned out that oestrone was most formed, oestradiol being barely detectable.

MATERIALS AND METHODS

Ovaries were removed from 19- and 20-day old embryos of the Wistar strain. They were halved and cultured for 24 h at 37°C on the agar containing medium of Wolff and Haffen[10] in the presence of [^{3}H]testosterone or [^{3}H]progesterone.

[1,2,6,7-^{3}H]testosterone came from the Centre d'études nucléaires (Gif-sur-Yvette) and [1,2,6,7-^{3}H]progesterone from the Radiochemical Centre. Their radiochemical purity, checked by thin-layer chromatography, was $\geqslant$95% and their sp. act. 83 and 85 Ci/mmol respectively. They were dissolved in a 1:3 (v/v) mixture of propylene glycol-1,2 and Tyrode's balanced salt solution. The radioactive concentration of the testosterone and progesterone solutions used was respectively 0.16 and 0.40 μCi/μl, which was equivalent to a substance concentration of 0.6–1.5 ng/μl or to a molar concentration of 2–5 μM. 6.25 μl of the testosterone solution or 4.5 μl of the progesterone solution were dropped on the culture medium after explanting the ovaries.

At the end of the 24 h culture period, 100 μg of each carrier oestrone and oestradiol were added to the media, as well as tracer amounts of [4-^{14}C]oestrone (New England Nuclear, Boston) and [4-^{14}C]oestradiol (The Radiochemical Centre, Amersham) for the determination of the losses occurring during the analysis. Since the semi-solid culture media are not suitable for oestrogen extraction after only mechanical dissociation [11], they were subjected to acid hydrolysis (15% HCl) for 1 h under reflux in order to obtain a homogeneous phase. Undoubtedly, this procedure destroys part of the oestrogens, but, on the other hand, it may loosen their binding to proteins or other molecules.

The hydrolysate was brought to neutrality and extracted with diethyl ether. The ether extract was washed and evaporated to dryness. The residue was dissolved in a 1:1 (v/v) benzene–petroleum ether mixture, from which the oestrogens were extracted with 0.4 N Na OH and subjected to methylation [12]. Oestrone methyl ether and oestradiol methyl ether were separated from each other by thin-layer chromatography on silica gel in chloroform–diethyl ether, 9:1 (v/v). They were viewed in u.v., light, scraped off and eluted. Oestrone methyl ether was further purified by reducing it to oestradiol methyl ether, which was chromatographed in cyclohexane–ethyl acetate, 7:3 (v/v). This same system was used for the rechromatography of the original oestradiol methyl ether.

Fifteen mg of non-radioactive oestradiol methyl ether were added to each eluate and repeated crystallizations were carried out in either methanol–dichloromethane–petroleum ether or acetone–chloroform–petroleum ether. Specific activity determinations relative to both ^{3}H and ^{14}C were made on 1 mg samples of the successive crystals and mother

*To whom correspondence should be addressed.

liquors. The channels of the liquid scintillation spectrometer were windowed so that no ^3H passed into the ^{14}C channel. Twelve percent of the ^{14}C passed into the ^3H channel and were subtracted from the radioactivity therein. Under these conditions, ^3H was counted with an efficiency of 40% and ^{14}C with an efficiency of 65%. The background values were respectively 12 and 15 cpm. The constancy of the specific activity of three successive crops of crystals was taken as proof that the radioactive molecule was identical with the carrier oestradiol methyl ether.

The error of weighings was $\leq 1\%$. Counting time was long enough to afford an error $\leq 3\%$. Under these conditions, the total error in specific activity determination was $\leq 5\%$. Specific activity was considered constant whenever three successive crystallizations yielded values which were within $\pm 5\%$ of the average [13]. The constant specific activity value relative to ^{14}C allowed the calculation of the recovery percentage, that relative to ^3H the calculation of the conversion percentage.

Since hydrogen atoms in the positions 1β and 2β are lost during aromatization, it should be added that no ^3H was in these positions in the case of [1,2,6,7-^{3}H]progesterone and that 7% of ^3H were in these positions in the case of [1,2,6,7-^{3}H]testosterone. The supplementary loss in this latter case was not taken into account in the calculation of the conversion percentage. For full information, the distribution of the label was the following:

	1α	1β	2α	2β	6α	6β	7α	7β
In testosterone (%)	29	3.5	12.5	3.5	12.5	3.5	32	3.5
In progesterone (%)	29	0	21.2	0	21.2	0	28.6	0

RESULTS

The results of a control experiment indicated that the losses of ^3H- and ^{14}C-labelled oestrone and oestradiol occurring during the analysis were identical (Table 1). So, the utilization of ^{14}C-labelled tracers to determine the losses of [^{3}H]oestrogens formed was valid.

In neither incubation experiments could oestradiol be brought to constant specific activity. So, its identification has failed. It seems that the ovary of the 19–20-day old rat embryo lacks the capacity to convert progesterone or testosterone into oestradiol. But the conversion of both precursors into oestrone was easily demonstrable (Table 1).

DISCUSSION

When the capacity of the ovary of the 19-day old rabbit embryo to convert [^{3}H]testosterone or [^{3}H]androstenedione into oestrogens was studied, it was found that both precursors yielded much more oestradiol than oestrone [5]. The same result was obtained with the ovary of the human foetus of 13 cm CR length [8].

In the present study, oestradiol formation by the ovary of the 19–20-day old rat embryo could not be demonstrated, either from progesterone or from testosterone. But both precursors yielded measurable amounts of oestrone. So, it can be concluded that in the ovary of the 19–20-day old rat embryo 17β-hydroxysteroid dehydrogenase favors the formation of oestrone.

Further studies should be aimed at elucidating whether this feature is peculiar to the rat embryo or to the specific stage under investigation. The effects of the gonadotrophins could also be studied.

From a more general standpoint, the physiological mean-

Table 1. Recrystallization of oestradiol methyl ether

	Control experiment		Experiment 1		Experiment 2	
Precursor:			Fifty 19-day old ovaries 6.25 μl = 858,000 cpm [^{3}H]T		Thirty-six 20-day old ovaries 4.5 μl = 1,492,000 cpm [^{3}H]Pro	
OESTRONE						
Tracer:	14,800 cpm [4-^{14}C]E_1 10,700 cpm [6,7-^{3}H]E_1		18,400 cpm [4-^{14}C]E_1		18,400 cpm [4-^{14}C]E_1	
	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H
Crystals 1	**290**	**219**	**374**	**820**	**225**	**276**
Mother liquors 1	315	245	401	910	254	338
Crystals 2	**290**	**214**	**374**	**786**	**244**	**254**
Mother liquors 2	306	252	389	828	248	282
Crystals 3	**301**	**208**	**351**	**764**	**234**	**267**
Mother liquors 3	309	**214**	383	813	236	279
Crystals 4			**357**	**776**	**238**	**250**
Mother liquors 4			**374**	832	246	**264**
Constant sp. act.	294	214	366	775	235	259
% Constancy	2.50%	2.69%	4.10%	1.46%	4.36%	3.19%
Total activity (×15)	4410	3210	5490	11,625	3525	3885
% Recovery	30%	30%	30%		19%	
% Conversion				4.5%		1.4%
OESTRADIOL						
Tracer:	11,750 cpm [4-^{14}C]E_2 33,500 cpm [6,7-^{3}H]E_2		15,300 cpm [4-^{14}C]E_2		15,300 cpm [4-^{14}C]E_2	
	^{14}H	^3H	^{14}C	^3H	^{14}C	^3H
Crystals 1	**252**	**717**	**290**	16.2	**175**	7.9
Mother liquors 1	**249**	803	305	32	197	60
Crystals 2	**240**	**746**	**276**	18.5	**171**	2.9
Mother liquors 2	**257**	**732**	**282**	19.4	192	15
Crystals 3	**248**	**729**	**282**	15.0	**174**	5.7
Mother liquors 3	**250**	**764**	**288**	13.5	177	4.4
Constant sp. act.	249	738	284		173	
% Constancy	3.74%	3.58%	2.68%		1.35%	
Total activity (×15)	3735	11,070	4260		2595	
% Recovery	32%	33%	28%		17%	
% Conversion						

Constant specific activity values, i.e. values which are within $\pm 5\%$ of the average, have been scored.

Specific activity (sp. act.) of crystals and mother liquors, expressed in cpm/mg, recovery percentage of [^{14}C]labelled tracers and conversion rate of [^{3}H]testosterone (T) and [^{3}H]progesterone (Pro) into oestrone (E_1) and oestradiol (E_2).

ing of oestrogen secretion by the ovary of the mammalian embryo was not clear from previous work. George *et al.*[14, 15] had suggested that any effect of ovarian oestrogen would probably be exerted within the ovary itself. This could be verified by using aromatase inhibitors.

REFERENCES

1. Jungmann R. A. and Schweppe J. S.: Biosynthesis of sterols and steroids from acetate-^{14}C by human fetal ovaries. *J. clin. Endocr. Metab.* **28** (1968) 1599–1604.
2. Weniger J.-P., Chouraqui J. and Zeis A.: Sur l'activité sécrétoire de l'ovaire embryonnaire de mammifère. *Ann. Endocr.* **33** (1972) 243–250.
3. Schindler A. E. and Friedrich E.: Steroid metabolism of pregnenolone-4-^{14}C by human foetal ovaries. *Endokrinologie* **65** (1975) 72–79.
4. Jost A.: Recherches sur la différenciation sexuelle de l'embryon de Lapin. III. Rôle des gonades foetales dans la différenciation sexuelle somatique. *Arch. Anat. micr. Morph. exp.* **36** (1946–47) 271–315.
5. Milewich L., George F. W. and Wilson J. D.: Estrogen formation by the ovary of the rabbit embryo. *Endocrinology* **100** (1977) 187–196.
6. Mauléon P., Bézard J. and Terqui M.: Very early and transient 17β-estradiol secretion by fetal sheep ovary. *In vitro* study. *Ann. Biol. anim. Biochim. Biophys.* **17** (1977) 399–401.
7. Sholl S. A. and Goy R. W.: Androgen and estrogen synthesis in the fetal guinea pig gonad. *Biol. Reprod.* **18** (1978) 160–169.
8. George F. W. and Wilson J. D.: Conversion of androgen to estrogen by the human fetal ovary. *J. clin. Endocr. Metab.* **47** (1978) 550–555.
9. Shemesh M.: Estradiol-17β biosynthesis by the early bovine fetal ovary during the active and refractory phases. *Biol. Reprod.* **23** (1980) 577–582.
10. Wolff E. and Haffen K.: Sur le développement et la différenciation sexuelle des gonades embryonnaires d'Oiseau en culture *in vitro*. *J. exp. Zool.* **119** (1952) 381–403.
11. Akram H. and Weniger J.-P.: Effet de l'hypophysectomie sur la sécrétion d'oestrogènes par les gonades de l'embryon de Canard femelle. *Gen. comp. Endocr.* **21** (1973) 543–546.
12. Brown J. B.: A chemical method for the determination of oestriol, oestrone and oestradiol in human urine. *Biochem. J.* **60** (1955) 185–193.
13. Axelrod L. R., Matthijssen C., Goldzieher J. W. and Pulliam J. E.: Definitive identification of microquantities of radioactive steroids by recrystallization to constant specific activity. *Acta endocr., Copenh* (Suppl.) **99** (1965).
14. George F. W., Milewich L. and Wilson J. D.: Oestrogen content of the embryonic rabbit ovary. *Nature* **274** (1978) 172–173.
15. George F. W. and Wilson J. D.: Regulation of the onset of gonadal function in the fetal rabbit. In *Testicular Development, Structure and Function* (Edited by A. Steinberger and E. Steinberger). Raven Press, New York (1980) pp. 33–40.