CL appear 20α-HSD negative as happens in the non-operated control animals. After hysterectomy the CL behave in exactly the opposite way; i.e. when hysterectomy is performed before the 11th day of pregnancy, the pregnancy CL appear as usual, 20α-HSD negative; when it is performed after the 13th day, an intense enzymatic activity can be detected in the pregnancy CL 3 days after the hysterectomy. (Table II.)

(2) Pseudopregnancy. As shown in Table III, the pseudopregnancy CL, i.e. the ones due to the last ovulation, are, as previously shown[6] 20α-HSD negative at least until the 9th day of pseudopregnancy. This enzymatic activity is present only in the involuting CL, i.e. those of previous generations. In hypophysectomized pseudopregnant rats, the pseudopregnancy CL show 20α-HSD activity 3 days after the operation.

Discussion. The above-mentioned results show that the hypophysal incretion inhibits in pseudopregnancy and in early pregnancy (i.e. in the period of pre-implantation, implantation and placenta formation) the onset of the 20α-HSD activity in CL. As a consequence of the absence of this enzyme, present only in the involuting CL, the concentration of the progesterone (P) in the blood increases and the concentration of the 20α-hydroxy-progesterone (20α-OH-P) decreases[1,9].

When hypophysal incretion is lacking and 20α-HSD appears in all the CL the particular equilibrium between P and 20α-OH-P, which is necessary to allow pregnancy or pseudopregnancy, is altered and both the situations are interrupted. When the placenta is already formed, the control of the 20α-HSD activity in the CL goes from the pituitary to the placenta itself. In late pregnancy, in fact, hypophysectomy does not cause the onset of the 20α-HSD activity in the CL, which is instead brought about by hysterectomy, as shown by the present data, or by placental dislocation[1]. In the second half of pregnancy the placenta is the organ responsible for the proper P/20α-OH-P ratio, by maintaining 20α-HSD negative the pregnancy CL.

It should be noted that the placental incretion, while inhibiting the onset of the 20α-HSD activity when it is lacking, is not able to abolish it when present. In fact in pregnant ergocornine-treated rats in which pregnancy is maintained by progesterone administration, the presence of placenta and foetuses does not abolish the 20α-HSD activity induced in all the CL by ergocornine[7,10].

Table II. Effect of hysterectomy on corpora lutea of pregnant rats

Day of operation	Day of sacrifice	No. of animals	No. of animals with 20α-HSD negative CL
1	4	5	5
1	6	3	3
1	8	4	4
8	11	5	5
11	14	5	4
13	16	5	2
15	18	6	0

Table III. Effect of hypophysectomy on corpora lutea of pseudopregnant rats

Day of operation	Day of sacrifice	No. of animals	No. of animals with 20α-HSD negative CL
–	5	4	4
–	7	4	4
–	9	5	5
2	6	6	0
4	7	5	0

Riassunto. Sono stati studiati con metodi enzimoistochimici alcuni meccanismi che controllano la comparsa della 20α-idrossi-steroide-deidrogenasi (20α-HSD) in ratte gravide o pseudogravide dopo ipofisectomia o isterectomia. I risultati dimostrano che nel primo periodo della gravidanza e in pseudogravidanza, l'ipofisi è necessaria per mantenere i corpi lutei privi di attività 20α-HSD.

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The Formation of Androgens in Human Foetal Liver in vitro

The source of testosterone in the human organism is not only the tissue of steroidogenic endocrine glands, but also the periphery, as can be deduced from the differences in the secretion and production rates of testosterone[1]. An important role in the formation of testosterone in the periphery is played by the liver, and an appreciable proportion – about 40% – of the hepatic production of testosterone comes from 3β-hydroxy-5-androsten-17-one[2–5].

The reports of a virilizing hepatoma in a young boy[6] and of the presence of several of the enzymes involved in steroidogenesis in foetal liver[7], imply that the liver is an endocrine tissue. Slaunwhite et al.[7] found small amounts of oestradiol and oestriol isolated from the incubation of human foetal liver with 3β-hydroxy-5-androsten-17-one, which demonstrate that foetal liver possesses an aromatizing enzyme system and indirectly signifies the presence of a 3β-hydroxy-Δ^5-steroid dehydrogenase system. Goldman et al.[8] demonstrated histochemically the activity of 3β-hydroxy-Δ^5-steroid dehydrogenase in hepatic cells; the activity increased roughly in proportion to foetal age. This paper reports a study showing directly the in vitro conversion of 3β-hydroxy-5-androsten-17-one to 5-androstene-3β,17β-diol, 4-androstene-3,17-dione and testosterone in human foetal liver.

Human foetal liver tissue was obtained from 9 foetuses of both sexes removed by legal interruptions of pregnancy from healthy women. 50 mg of homogenized liver was incubated in 3 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 20 m*M* glucose/l, each with 1.05 μCi [4-^{14}C]-3β-hydroxy-5-androsten-17-one (specific activity 27.5 mCi/m*M*, in 0.1 ml ethanol). NAD^+ (1.5 mg) and NADH (1.5 mg) were added and the mixture was incubated at 37 °C in air atmosphere for 90 min. After the incubation, 10 μg each of testosterone, 4-androstene-3,17-

dione and 5-androstene-3β,17β-diol were added and the mixture was extracted with ethyl acetate and chloroform. The dry residue was chromatographed on a thin layer of silica gel in benzene-methanol 6:1, the zones corresponding in mobility to authentic steroids were eluted and rechromatographed on paper in Bush A system (petroleum ether–methanol–water 5:4:1). The radioactivity of the steroids was measured by liquid scintillation spectrometry [9].

The identity of [4-^{14}C]-testosterone and [4-^{14}C]-4-androstene-3,17-dione formed was confirmed as described elsewhere [9]. The chromatographic mobility of a further radioactive metabolite was identical with that of authentic 5-androstene-3β,17β-diol in 4 systems (thin layer of silica gel; cyclohexane-ethyl acetate 1:1, Rf 0.52, benzene-methanol 6:1, Rf 0.21; paper Whatman No. 1: Bush A, Rf 0.12, and tetrachloromethane on triethylene glycol impregnated paper, Rf 0.15). In all metabolites under investigation, the identity was confirmed by crystallization to constant specific activity (see Table I).

Table I. Successive recrystallization of [4-^{14}C]-testosterone, [4-^{14}C]-4-androstene-3,17-dione and [4-^{14}C]-5-androstene-3β,17β-diol obtained after chromatography of foetal liver extracts after incubation with [4-^{14}C]-3β-hydroxy-5-androsten-17-one

Crystallization No.	[4-^{14}C]-testosterone 12-week-old foetus (dpm/mg. 10^{-3})		[4-^{14}C]-4-androstene-3,17-dione 12-week-old foetus (dpm/mg. 10^{-3})		[4-^{14}C]-5-androstene-3,17-diol 7-week-old foetus (dpm/mg. 10^{-3})	
	Crystalls	Mother liquor	Crystalls	Mother liquor	Crystalls	Mother liquor
0	1.10	–	2.60	–	1.08	–
1	0.79	3.00	2.52	2.72	0.98	2.90
2	0.76	1.85	2.63	2.42	0.83	1.85
3	0.65	0.76	2.34	2.18	0.76	1.34
4	0.76	0.41	2.45	1.93	0.77	0.96

As a carrier 10 mg of authentic steroid was added.

Table II. The formation of [4-^{14}C]-testosterone, [4-^{14}C]-4-androstene-3,17-dione and [4-^{14}C]-5-androstene-3β,17β-diol from [4-^{14}C]-3β-hydroxy-5-androsten-17-one ([4-^{14}C]-DHA) in human foetal liver during the 1st half of pregnancy

Foetal age	Total activity extracted	[4-^{14}C)-DHA recovered		[4-^{14}C]-testosterone		[4-^{14}C]-4-androstene-3,17-dione		[4-^{14}C]-5-androstene-3β,17β-diol	
Weeks	dpm	dpm	%[a]	dpm	%[a]	dpm	%[a]	dpm	%[a]
7	1,941,900	100,098	5.16	661	0.03	5,083	0.26	12,100	0.62
9–10	1,709,307	170,416	9.97	4,949	0.29	19,016	1.11	2,772	0.16
10–11	1,690,158	99,473	8.82	950	0.08	3,690	0.33	24,436	2.17
11	1,921,420	122,990	6.40	4,246	0.22	7,916	0.41	22,807	1.19
12	1,809,850	82,269	4.55	10,875	0.60	28,713	1.59	–	–
12	1,426,460	39,476	2.77	1,081	0.08	1,855	0.13	3,312	0.23
14	1,770,750	219,304	12.39	2,244	1.27	9,781	0.55	82,648	4.67
16	1,382,500	23,656	1.71	820	0.06	1,160	0.08	3,310	0.27
20	1,630,040	37,330	2.28	10,563	0.65	15,942	0.98	5,330	0.33
Mean values	1,698,043	99,446	6.00	2,143	0.24	10,351	0.60	19,589	1.20

[a] Calculated from total activity extracted.

The average total radioactivity extracted amounted to 72% of the incubated activity; it is less than in experiments with other foetal organs [9], evidently due to the conjugation and hydroxylation of the steroids in the liver. The highly polar metabolites escape extraction. 3β- hydroxy-5-androsten-17-one recovered amounted to only 6% of the radioactivity extracted, indicating that the metabolic activity of foetal liver exceeds the metabolic rates in other foetal organs [9]. Quantitatively the most important metabolites were high polar substances, products of effective hydroxylations in the foetal liver, which were not examined further.

From the $C_{19}O_2$ metabolites of 3β-hydroxy-5-androsten-17-one in foetal liver, 5-androstene-3β,17β-diol was the most abundant, followed by 4-androstene-3,17-dione and testosterone (Table II). It is not excluded that a part of the metabolites escaped determination due to conjugate formation. Similarly, as in previous investigations [9,10], no significant correlation between the rate of androgen formation and the foetal age was observed, in contrast to the activity of 3β-hydroxy-Δ^5-steroid dehydrogenase as described by GOLDMAN et al.[8].

Zusammenfassung. Nach Inkubation des homogenisierten Lebergewebes menschlicher, 7–20 Wochen alter, Foeten mit 3β-Hydroxy-5-androsten-17-on wird die Bildung von Testosteron, 4-Androsten-3,17-dion und 5-Androsten-3β,17β-diol nachgewiesen.

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