

CHOLESTEROL BIOSYNTHESIS IN HUMAN FETAL LIVER AND ADRENAL⁽¹⁾

Morris L. Givner and Robert B. Jaffe

Department of Biochemistry, Ayerst Research Laboratories, Montreal, Canada and Steroid Research Unit, Reproductive Endocrinology Program, Department of Obstetrics and Gynecology, University of Michigan Medical Center, Ann Arbor, Michigan 48104.

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ABSTRACT

Hepatic and adrenal cholesterol biosynthesis were investigated in the human fetus in vitro by measuring the incorporation of acetate-2-¹⁴C into cholesterol by slices of liver and adrenals from the same fetus. To differentiate neutral lipids from cholesterol biosynthesis, the radioactivity was measured in the nonsaponifiable neutral lipid fraction and in cholesterol isolated and purified as its crystalline 5,6-dibromo-derivative. Expressed in μ moles acetate incorporated/hr/g tissue into neutral lipids (n.l.) and cholesterol, the values were: fetus I (11 wk. old), liver (84.4), 49.1; adrenals (48.8), 9.3; fetus II (14 wk. old), liver (91.3), 47.3; adrenals (15.6), 5.5, respectively. Liver and adrenal cholesterologenesis was inhibited by a specific inhibitor [trans-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride = AY-9944] of 7-dehydrocholesterol Δ^7 -reductase. This finding provided (indirect) evidence that 7-dehydrocholesterol is an obligatory intermediate in the biosynthesis of cholesterol in both of these organs. The percent incorporation of acetate into cholesterol in the human fetal liver was greater than that in any other tissue previously reported.

We have previously shown that cholesterol, perfused to the midterm (2) and term (3) human placenta in situ can be utilized in the synthesis of neutral steroids. Recently, Hellig et al (4) have demonstrated that circulating cholesterol (primarily of maternal origin) is utilized for most if not all of placental progesterone biosynthesis in pregnancies marked by anencephalic fetuses. However, the de novo synthesis of cholesterol by the placenta is minimal at best (5). In contrast, we and others have shown that the human midterm fetal testis has the capacity for de novo synthesis of free steroids; whereas, the fetal adrenal synthesizes both free and conjugated steroids (6-8). It seemed of interest to report our studies on the relative capacities of the human fetal adrenal and liver to synthesize cholesterol. Studies were also performed to assess indirectly whether 7-dehydrocholesterol is an obligatory intermediate in the biosynthesis of cholesterol in these organs.

MATERIALS AND METHODS

Human male fetal livers and adrenals were obtained following hysterotomy for therapeutic termination of pregnancy. The adrenals and livers were freed of extraneous tissue, blotted, and slices prepared within 15 min. of operation with a Stadie-Riggs microtome. Both adrenal and liver slices were preincubated (30 min.) and incubated (3 hr.) in Krebs-Ringer bicarbonate buffer (with added glucose), based on earlier studies on cholesterologenesis in bovine adrenals (9). Cholesterol was isolated (9) by the addition of 100 mg carrier, brominated (10,11) and the resulting 5,6-dibromocholestan-3 β -ol recrystallized (4 times from ethyl acetate-methanol) to radiochemical purity.

To assess whether 7-dehydrocholesterol is an obligatory intermediate in cholesterol biosynthesis in these organs, identical studies, from the same fetal specimens, were carried out in the presence of trans-1,4-bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY-9944), $1 \times 10^{-4}M$ final concentration.

RESULTS

The extent of cholesterol biosynthesis from acetate in the human fetal adrenal and liver and the almost total inhibition of this conversion by AY-9944 are shown in Table 1 and Figure 1.

TABLE 1

Human Fetal Liver and Adrenal Cholesterogenesis In Vitro

Fetal age (wk)	Organ (mg) ^a	Groups ^b	Total radioactivity, dpm/mg tissue ^d	
			neutral lipids	cholesterol
11	liver (182-210)	controls	21,200 (19.4) ^e	12,300 (11.3) ^e
		AY-9944 ^c	16,900 (15.8)	120 (0.1)
	adrenal (10-15)	controls	12,300 (0.8)	2,300 (0.1)
		AY-9944	11,200 (0.6)	50 (<0.01)
14	liver (28-44)	controls	22,900 (3.8)	11,900 (2.4)
		AY-9944	24,400 (4.2)	100 (0.01)
	adrenal (27-29)	controls	3,900 (0.5)	1,400 (0.2)
		AY-9944	-	40 (<0.01)

^aTissue/incubation flask.

^bDuplicate incubations, each flask contained 10 µc of sodium acetate-2-¹⁴C, specific activity 38 µC/µM, purchased from Radiochemical Centre, Amersham, England.

^cTrans-1,4-bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride, 1x10⁻⁴M (final concentration).

^dAvg. values.

^ePercent conversion of sodium acetate-2-¹⁴C per incubation.

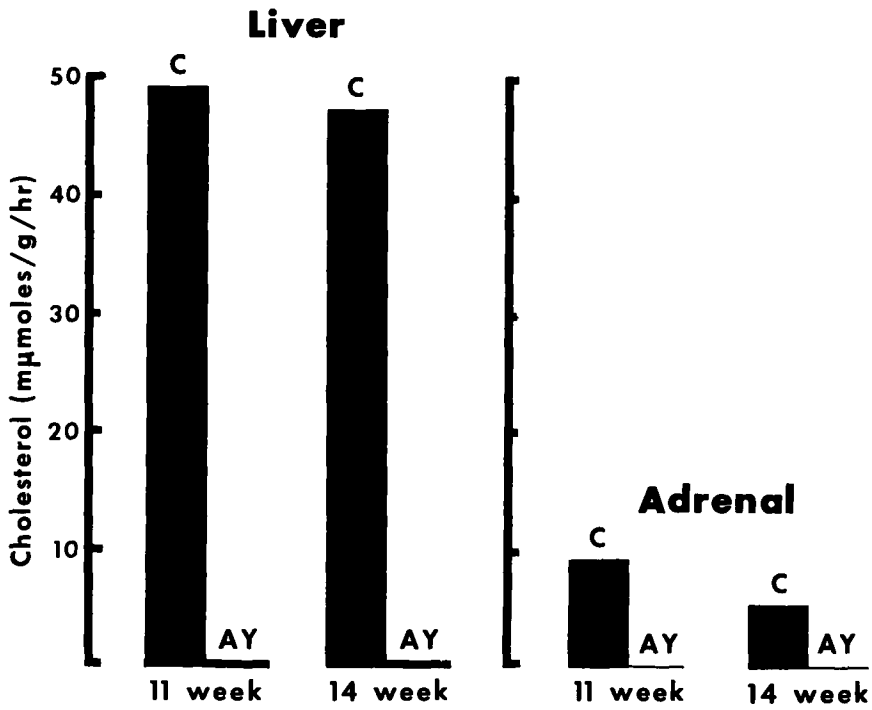


Fig. 1. The effect of AY-9944 on cholesterogenesis in human fetal liver and adrenal slices. C = Controls, AY = AY-9944.

DISCUSSION

This study demonstrates the unequivocal biosynthesis of cholesterol in human fetal liver and adrenal slices. The neutral lipid and cholesterol fractions (dpm/mg tissue, Table 1) are higher in the liver than in the adrenal. In regard to cholesterol biosynthesis, the human fetal liver is 5 to 9 times more active on a per gram tissue basis than the fetal adrenal under our experimental conditions (Table 1, Fig. 1). The magnitude of these differences is even greater when the relative organ weights are considered (e.g. Table 1, 11 wk. fetal liver 985 mg vs 81 mg for the 2 adrenals). Up to 11% of the acetate-2-¹⁴C was converted to cholesterol by the fetal liver slices. In this series of experiments, up to 0.2% conversion was observed with fetal adrenal slices. However, in three other similar studies with fetal adrenal slices (unpublished data), we have observed up to 6% of the substrate acetate-2-¹⁴C incorporated into cholesterol. The percent incorporation of acetate-2-¹⁴C into tissue cholesterol in any given experiment may reflect the amount of tissue and substrate per incubation, the activity of the enzyme systems, and the duration of the incubation. To our knowledge, there have not been reports in which more than 2% of acetate-2-¹⁴C was incorporated into tissue cholesterol. Kritchevsky et al (12) have reported up to 2% acetate-1-¹⁴C was incorporated into cholesterol by rat liver slices.

In fetal adrenal slices, Bloch and Benirschke (8) demonstrated the capacity to biosynthesize steroids from acetate-1-¹⁴C

with cholesterol as the probable intermediate. However, in their study, the cholesterol was isolated by digitonin precipitation, a method that has been shown to be non-specific for cholesterol (10). In vivo, Davis et al (13) administered acetate-1-¹⁴C intravenously to a pregnant woman and isolated cholesterol from the fetal liver and adrenal by digitonin precipitation. Solomon et al (14) perfused a human fetus with cholesterol-7 α -³H and acetate-1-¹⁴C and isolated the dibromo derivative of cholesterol-¹⁴C from the blood, adrenals and liver. In the latter study, the amount of cholesterol-¹⁴C isolated from the adrenal was 11 times less than the quantity found in the liver. Following perfusion of fetuses with ¹⁴C-acetate, Telegdy et al (15) isolated cholesterol-¹⁴C from the adrenals, liver, testes and blood. In these in vivo studies, the evidence that the fetal adrenal had the capacity to biosynthesize cholesterol was inconclusive because of the possibility that the cholesterol-¹⁴C biosynthesized in the liver was transported via the bloodstream to the adrenal.

In both liver and adrenal slices in the present studies (Table 1, Fig. 1), cholesterol biosynthesis was inhibited by AY-9944, a specific inhibitor of 7-dehydrocholesterol Δ^7 -reductase (16,17). This finding indicates that 7-dehydrocholesterol is an obligatory intermediate in the biosynthesis of cholesterol in both of these organs. In the control liver samples, more than 50% of the neutral lipid fraction is cholesterol (Table 1); in the adrenal, this percentage is less. In the presence of AY-9944 in both the liver and

adrenal incubations, <1% of the neutral lipid fraction is cholesterol. It should be noted that the total radioactivity in the neutral lipid fraction was much less affected by AY-9944 than the cholesterol fraction. This latter finding may be explained by previous in vitro (16,17) and in vivo (18-20) studies in the rat that showed that AY-9944 inhibits the conversion of 7-dehydrocholesterol to cholesterol. The 7-dehydrocholesterol accumulates, is present in the neutral lipid fraction, but is separated from cholesterol when the latter is converted to 5,6-dibromocholesterol and recrystallized to radiochemical purity (18).

In the newborn (3,21), the total serum cholesterol (67 mg \pm 4 mg/100 ml) is lower than the maternal serum (222 \pm 9 mg/100 ml). A low level of circulating blood cholesterol in the fetus might explain a high rate of fetal liver cholesterogenesis, i.e., the absence of a negative feedback inhibition by cholesterol. In the adult, dietary cholesterol apparently provides no feedback control over cholesterol synthesis (22).

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