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HDL_2-DEPENDENT BILE ACID SYNTHESIS IN RABBIT HEPATOCYTE CULTURE: EFFECTS OF OXIDIZED CHOLESTEROL DERIVATIVES

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Preparations of cholesterol kept for a long time at room temperature undergo autooxidation, the principal products of which are 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 3,5-cholestadien-7-one, 3β,5α,6β-cholestanetriol, and 25-hydroxycholesterol [13]. Feeding rabbits with cholesterol in this form accelerates the development of high hypercholesterolemia by 1-1.5 months compared with rabbits kept on a diet with purified cholesterol [1]. Massive deposition of lipid inclusions, consisting of cholesterol and its esters, is observed under these circumstances in the hepatocytes [7]. Since excretion of cholesterol is determined mainly by its oxidation into bile acids [12], the writers postulated previously [7] that depression of bile acid synthesis under the influence of oxidized cholesterol derivatives is one of the principal mechanisms of more rapid development of hypercholesterolemia. Support for this hypothesis is given by data obtained on liver microsomes of rats, showing that 7β-hydroxycholesterol and 7-ketocholesterol are competitive inhibitors of cholesterol-7α-hydroxylase [11]. Meanwhile there are no data directly relating to the effect of oxidized sterols on bile acid synthesis in the literature at the present time.

The aim of this investigation was to study the effect of oxidized cholesterol derivatives in bile acid synthesis by cultures of rabbit hepatocytes.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 2.5-3 kg were used. Hepatocytes were isolated by perfusion of the liver with collagenase solution [7]. The cells were cultured in Eagle's minimal medium containing 10% fetal calf serum, 100 μg/ml of kanamycin, 1 mM essential amino acids and L-glutamine, at 38°C in an atmosphere of 95% air and 5% CO_2. Fraction 2 of high-density lipopro

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TABLE 1. Distribution of ^{14}C (in cpm/mg cell protein) in Fractions of Cholesterol and Bile Acids (M ± s.d.)

Parameter		Cholesterol		Bile acids
		cells	medium	
VLDL	0 h	155 631+1215		
	24 h	105 125+9270	35 363+2 313	7 796+113 (5)
HDL_2	0 h	174 274+1427		
	24 h	131 111+1216	11 757+998	26 771+1817 (15)

Legend. Percent of [4-^{14}C]-cholesterol oxidized into bile acids during incubation for 24 h given between parentheses.

TABLE 2. Effect of Hydroxysterols on Synthesis of [4-^{14}C]-Bile Acids (M ± s.d.)

Substance in medium, μg/ml		Bile acids, per cent of control
7β-hydroxycholesterol	1	57+3**
	10	25+9**
3,5-cholestadien-7-one	1	75+15*
	10	41+9**
7α-hydroxycholesterol	1	76+6**
	10	65+10**
25-hydroxycholesterol	1	72+6**
7-ketocholesterol	1	86+5*
	10	79+4*

Legend. *$p < 0.05$, **$p < 0.01$.

teins (HDL_2) was isolated from healthy human blood serum by ultracentrifugation [3], Labeling of HDL_2 and of very low density lipoproteins (VLDL) with [4-^{14}C]-cholesterol was carried out as described previously [14]. The protein concentration was determined by the method in [8]. Bile acids were isolated from the cells and culture medium by differential extraction of lipids and bile acids with a mixture of chloroform and methanol (1:1 by volume), as described previously [6]. Incorporation of the radioactive label into lipids and bile acids was determined by scintillation radiometry. The results were subjected to statistical analysis by Student's t-test. The results in the graph and tables are presented in the form of mean ± standard deviation (n = 3).

EXPERIMENTAL RESULTS

In order to study bile acid synthesis, hepatocytes were preincubated in nutrient medium in the presence of lipoproteins labeled with [4-^{14}C]-cholesterol for 24 h. The cells were then washed to remove unbound label, a fresh portion of medium was added, and the rate of accumulation of bile acids in the medium and cells was determined. It was shown that [4-^{14}C]-cholesterol, supplied to the hepatocyte in the composition of HDL_2 is metabolized three times more rapidly into bile acids than cholesterol of VLDL (Table 1). This confirmed observations of other workers obtained on cultures of hepatocytes of rats and chick embryos [2, 4], showing that the principal substrate for bile acid synthesis is cholesterol of HDL_2. In the subsequent experiments we therefore used HDL_2 labeled with [4-^{14}C]-cholesterol. It follows from the results given in Fig. 1 that rabbit hepatocytes secrete bile acids at a constant rate for 72 h. Meanwhile, intracellular accumulation of bile acids was not observed. Consequently, secretion of bile acids into the culture medium in fact reflects their synthesis. In the study of the effect of oxidized sterols on bile acid synthesis, incorporation of [4-^{14}C]-cholesterol of HDL_2 into the combined bile acids of cells and medium was measured. Addition of the oxidized sterols to the culture medium caused a decrease in the rate of bile acid synthesis (Table 2). Maximal inhibition was produced by 7β-hydroxycholesterol, in agreement with data published previously on competitive inhibition of the limiting enzyme in bile acid synthesis, namely cholesterol-7α-hydroxylase, by this compound [11]. 3,5-cholestadien-7-one, and 7α- and 25-hydroxycholesterol also had a marked inhibitory action. Thus hydroxysterols are factors capable of influencing bile acid synthesis in rabbit hepatocytes in culture.

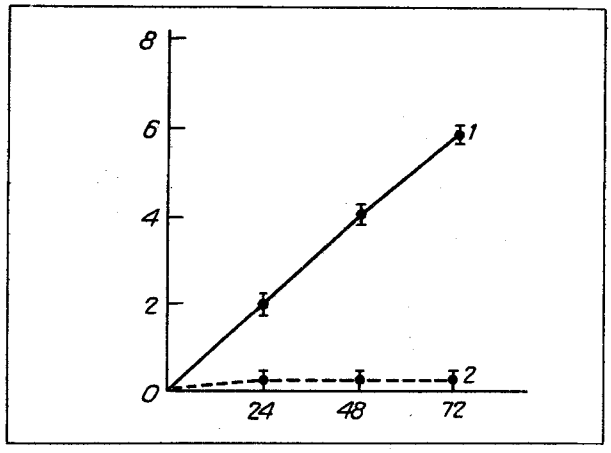

Fig. 1. Accumulation of [^{14}C]-bile acids in culture medium (1) and hepatocytes (2). Abscissa, duration of incubation of cells (in h); ordinate, number (in cpm/mg cell protein) $\times 10^{-4}$

In persons with a low level of bile acid synthesis, the predisposition to the development of hypercholesterolemia and atherosclerosis is enhanced [9]. Regulation of bile formation assumes particular importance against the background of an atherogenic diet. Studies on rabbits [15] have shown that addition of cholesterol to the food is accompanied by an increase in cholesterol-7α-hydroxylase activity, increased secretion of bile acids, and their increased elimination from the body. A disturbance of these processes must lead to accumulation of cholesterol in the body. In turn, the rate of secretion of bile acids determines the excretion of free cholesterol with the bile [5]. There is evidence that the cholesterol of the bile and cholesterol secreted into the blood in the composition of VLDL constitute a separate pool in the hepatocyte, which differs from the pool of cholesterol intended for bile acid synthesis [10]. Consequently, depression of bile acid synthesis should be accompanied by a redistribution of intracellular cholesterol toward an increase in its secretion in the composition of VLDL. This kind of redistribution was observed in hepatocytes isolated from rabbits kept on a diet with cholesterol which had undergone partial autooxidation [7].

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