ROLE OF ESTERIFIED CHOLESTEROL OF THE LIVER IN BILE ACID FORMATION

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The common bile duct of albino rats was exteriorized and the bile collected for 24 h. After removal of the bile for this period the concentration of esterified cholesterol in the liver fell sharply compared with control rats (mock operation). Neither the content of total and free cholesterol in the liver or the content of cholesterol fractions in the blood plasma showed any significant change. It is postulated that cholesterol esters, through conversion into free cholesterol, are utilized in bile acid biosynthesis.

Conversion of cholesterol into bile acids and neutral steroids of the feces is a fundamental process of cholesterol catabolism and excretion in animals and man. For this reason the study of cholesterol catabolism in the liver is of great interest to the elucidation of the cholesterol balance in the body and its regulation. The content of cholesterol in the mammalian liver is about 2-3 mg/g fresh weight and the ratio of free to esterified cholesterol is 4:1 [8, 11]. Bile acids are formed from free cholesterol via hydroxylation in the C-7α position [13, 12]. The sequence of stages of conversion of cholesterol into cholic acid is considered to be as follows [4]: 1) hydroxylation at C-7 and C-12; 2) inversion of the 3β hydroxy group; 3) saturation of the double bond; 4) degradation of the side chain. At the same time, it has been argued on theoretical grounds that the first stage of hydroxylation involves, not free cholesterol, but its ester [7]. In experiments on rats it is possible to exteriorize the common bile duct and thereby to remove the bile acids present in the enterohepatic circulation, thereby stimulating their increased synthesis [5].

The object of this investigation was to study the content of cholesterol fractions of the liver and plasma of rats after removal of bile acids by draining the bile for 24 h.

EXPERIMENTAL

Male albino rats weighing 180-220 g were starved for 16 h and the common bile duct was then exteriorized [5]. Under ether inhalation anesthesia a polyethylene tube about 1 mm in external diameter was inserted into the common bile duct at the point where it leaves the liver, the abdominal wound was sutured, and the rats were placed in individual cages restricting their mobility, where they were given a 5% solution of glucose in 0.9% NaCl to drink. Rats undergoing a mock operation without exteriorization of the bile duct, and kept in similar cages, acted as the control. The outflowing bile was collected for 24 h, after which the rats were sacrificed. Blood was taken into tubes containing heparin and the liver was perfused with 80 ml of a cold 0.9% NaCl solution. Samples of plasma and liver homogenate were extracted for 24 h with a chloroform–methanol (2:1, v/v) mixture in the ratio of tissue : mixture = 1:20. The extracts were filtered, one-fifth of their volume of water was added, and after mixing they were left to stand overnight at 4°C. Aliquot volumes were taken from the bottom chloroform layer to determine cholesterol fractions by the method of Sperry and Webb [16]. Total bile acids in the portions of bile were determined by the method of Kul'berg and Malyarskaya [2].

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TABLE 1. Content of Cholesterol Fractions in Liver and Plasma of Control Rats and Rats Losing Bile for 24 h (mg%)

Object	Index	Control rats (mock operation; n = 7)			Rats after removal of bile for 24 h (n = 7)		
		F	E	T	F	E	T
Liver	M	201	55	256	227	10	237
	$\pm m$	10,04	6,97	±11,70	±12,03	±3,82	±11,55
	%	78,5	21,5	100,0	95,8	4,2	100,0
Plasma	$\pm M$	20,2	31,9	52,1	24,8	24,0	38,8
	m	2,74	4,65	5,63	2,36	2,39	3,68
	%	39,0	61,0	100,0	50,4	49,6	100,0

Legend: F) Free, E) esterified, T) total.

EXPERIMENTAL RESULTS AND DISCUSSION

The mean volume of bile draining from the rats was 10.5 ± 0.68 ml/day, and the content of bile acids excreted in the 24-h portion of bile was 24.12 ± 0.92 mg. During the first hours after the rats recovered from the anesthetic the rate of bile drainage was 0.6-0.9 ml/h, but by the end of the experiment this had fallen to 0.3-0.4 ml/h. The maximal concentration of total bile acids (5-7 mg/ml) was found 2-3 h after exteriorization of the common bile duct, while the lowest concentration (1-2 mg/ml) occurred at the end of the experiment.

The results of determination of the content of cholesterol fractions in the liver and plasma of the control and experimental rats are given in Table 1. The relative content of esterified cholesterol in the liver of the control rats (mock operation) was 21.5%, while in the rats with the exteriorized bile duct it was only 4.2%, a fivefold decrease ($P < 0.001$). The decrease in esterified cholesterol took place despite maintenance of a constant level of the free and total cholesterol of the liver ($P > 0.05$). The content of cholesterol fractions in the blood plasma showed no significant change ($P > 0.05$).

In rats weighing 200 g, with a mean weight of the liver of 8 g, the content of circulating bile acids is 10 mg [12]. In the present experiments the mean loss of esterified cholesterol was 3.6 mg (0.45 mg/g × 8 g), or about 5 mg of bile acids. This total (15 mg) is much less than the daily loss of bile acids (24.12 ± 0.92 mg), indicating that additional quantities of free cholesterol were being utilized to form bile acids.

Experiments with cholesterol-C^{14} have shown that free cholesterol is a precursor of bile acids [6, 12]. Free cholesterol is also utilized in corticosteroid biosynthesis in the adrenal cortex [9], despite the fact that most of the cholesterol is present in esterified form [8, 11]. Esterified cholesterol in the liver and adrenals can thus be regarded as a reserve form of cholesterol, metabolically inert, in a state of equilibrium with free cholesterol which takes part in subsequent conversions. Boyd's hypothesis [7] that esterified cholesterol can be converted directly into bile acids has not yet received experimental verification. In this discussion it is therefore assumed that the free cholesterol of the hepatocytes is the direct precursor of bile acids. Besides phosphatides, cholesterol is another lipid component of the plasma membrane [1,10]. In the writer's opinion most of the free cholesterol in the liver cells, just as in other cells of the body, is located in the membranes. The greater the surface area of the membrane, the higher the free cholesterol content in the cell. The discovery of cholesterol in a mainly nonesterified form in the nuclear, mitochondrial, and, in particular, microsomal fractions of the rat liver [14, 15, 3] can be attributed to its occurrence as an obligatory structural component of the subcellular membranes. The absence of changes in the content of free cholesterol in the liver of rats with an exteriorized bile duct confirms its importance as a structural component of the membranes. The discovery of a small quantity of cholesterol esters in the subcellular fractions in a previous investigation enabled the writer to postulate that cholesterol esters are located in the hyaloplasm and within the subcellular organoids, i.e., in their matrix [3]. A small fraction of free cholesterol in a state of equilibrium with esterified cholesterol must also be present there. The utilization of this fraction of free cholesterol in bile acid biosynthesis led to a marked decrease in the reserve of esterified cholesterol in rats with an exteriorized bile duct. A change in the content of total free cholesterol could not be detected because this fraction is relatively small in magnitude.

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