EFFECT OF FEEDING CHOLESTEROL ON ITS DISTRIBUTION IN SUBCELLULAR STRUCTURES OF THE LIVER IN RATS OF DIFFERENT AGES

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Keeping rats of two age groups (10-12 months and 22-24 months) for 6-8 weeks on a diet including cholesterol and bile caused a marked increase in the level of cholesterol and, in particular, of its esters in the liver (the nuclear fraction and the supernatant) and in the blood plasma. A higher concentration of free cholesterol in the nuclei and of esterified cholesterol in the supernatant was found in the old rats than in the adults. The total cholesterol content in the microsomal fraction was increased very slightly on account of an increase in the content of cholesterol esters. It is postuled that considerable accumulation of cholesterol esters in the liver takes place as a result of an increase in their concentration in the hyaloplasm and in the matrix of the subcellular structures.

The liver plays a special role in cholesterol metabolism and participates actively in the synthesis of cholesterol and its elimination. Data in the literature on age changes in the cholesterol metabolism in the liver are very contradictory and are concerned chiefly with the total cholesterol content in the liver tissue [3, 5, 9]. During the production of experimental hypercholesteremia and atherosclerosis, lipid infiltration of the liver develops as a rule, and it is more marked in old animals [1, 2].

The object of the present investigation was to determine the content of cholesterol (and its fractions) not only in whole liver tissue, but also in subcellular structures of the liver in animals of different ages under normal conditions and with hypercholesteremia.

EXPERIMENTAL METHOD

Experiments were carried out on 24 female albino rats of two age groups: adult (10-12 months) and old (22-24 months). Twelve of these animals (six adult and six old) were kept on the ordinary laboratory diet (control group). The experimental animals (six from each age group) received the following preparations daily for 6-8 days through a gastric tube (calculated per 100 g body weight): 0.25 g cholesterol as a suspension in sunflower oil and 0.25 ml of bile previously concentrated to one-fifth of its original volume. The subcellular fractions were isolated from the rats' liver tissue [4, 7]: nuclei, mitochondria, microsomes, and supernatant. Lipids were extracted (24 h, 20°C) by Folch's method (chloroform–methanol, 2 : 1). The total, free, and esterified cholesterol fractions were estimated in the chloroform layer [10].

EXPERIMENTAL RESULTS

It will be clear from the results in Table 1 that the content of total, free, and esterified cholesterol in the plasma and whole tissue of the liver was not significantly different in the adult and old rats. The exception was some increase in the content of free cholesterol in the liver of the old rats.

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TABLE 1. Content of Cholesterol Fractions in Blood Plasma and Whole Liver of Rats (M±m)

Diet	Group of animals (age in months in parentheses)	Cholesterol in blood plasma (in mg/ml)			Cholesterol in liver (in mg/g)		
		free	esters	total	free	esters	total
Ordinary	1 (10—12)	0,22±0,015	0,37±0,041	0,59±0,040	1,7±0,13	0,4±0,04	2,1±0,15
	2 (22—24)	0,33±0,076	0,33±0,047	0,66±0,056	2,1[a]±0,13	0,4±0,21	2,5±0,12
Cholesterol added	3 (10—12)	0,61[b]±0,07	1,9[d] ±0,213	2,51[b]±0,257	8,4[b] ±0,75	57,3[b]±6,62	65,6[b]±7,01
	4 (22—24)	1,04[c,d]±0,157	2,15[d]±0,360	3,19[d]±0,492	8,6[d]±0,65	69,0[d]±4,38	77,5[d]±4,84

Legend, here and in Table 2: a) difference statistically significant ($P < 0.05$) between groups 1 and 2, b) between groups 1 and 3, c) between groups 3 and 4, d) between groups 2 and 4. In all other cases differences are not significant.

TABLE 2. Content of Free, Esterified, and Total Cholesterol in Subcellular Fractions of Rat Liver (in mg/g protein: M±m)

Diet	Group of animals (age in months in parentheses)	Cholesterol in nucleus			Cholesterol in mitochondria		
		free	esters	total	free	esters	total
Ordinary	1 (10—12)	7,8±0,58	1,2±0,17	9,0±0,67	7,4±0,88	1,9±0,10	9,3±0,82
	2 (22—24)	8,9±0,67	1,4±0,19	10,3±0,90	8,5±0,59	2,1±0,16	10,6±0,66
Cholesterol added	3 (10—12)	75,6[b]±5,96	235,4[b]±39,55	311,0[b]±38,0	21,1[b]±1,32	28,7[b]±3,39	49,8[b]±4,31
	4 (22—24)	146,7[c,d]±18,22	256,4[d]±34,81	403,1[d]±44,7	22,5[d]±2,09	17,2[d]±1,98	39,7[d]±3,00

Continuation

Diet	Group of animals (age in months in parentheses)	Cholesterol in microsomal fraction			Cholesterol in supernatant		
		free	esters	total	free	esters	total
Ordinary	1 (10—12)	57,1±4,53	12,3±1,02	69,4±5,19	4,2±0,34	2,5±0,19	6,7±0,51
	2 (22—24)	58,3±5,8	14,1±1,44	72,4±7,12	4,5±0,57	7,8[a]±0,76	12,3[a]±0,87
Cholesterol added	3 (10—12)	64,8±4,05	30,1[b]±2,97	94,9[b]±4,57	86,6[b]±8,45	137,0[b]±13,55	223,6[b]±8,06
	4 (22—24)	67,9±3,82	34,5 [d]±5,72	102,4[d]±4,34	68,8[d]±4,31	253,2[c,d]±19,28	322,0[c,d]±21,7

In all the experimental animals the cholesterol content in the blood plasma and liver was considerably increased. The increase in the level of free cholesterol in the liver was the same in the adult and the old rats, but that of the cholesterol esters was somewhat greater in the old than in the adult animals. For example, cholesterol feeding led to an increase of 4.9 times in the content of free cholesterol in the liver of the adult rats, but of 143 times in the content of cholesterol esters. The corresponding increases in the old animals were 4.4 and 173 times.

No significant age changes in the content of the cholesterol fractions were found in the nuclei, mitochondria, and microsomes of the liver in the control group of rats (Table 2). However, the content of esterified cholesterol was 3 times higher in the supernatant from the old rats than the corresponding parameters in the adult animals.

During feeding with cholesterol the compound accumulated in large quantities in all subcellular fractions but, in particular, in the nuclei and supernatant. The relative increase in the content of esterified cholesterol was greater than that of free cholesterol. For example, in the liver nuclei of adult rats the content of free cholesterol was increased by 9.7 times and the content of cholesterol esters by 196 times; the corresponding increases in the mitochondria were 2.9 and 15.1 times, and in the supernatant 20.6 and 54.6 times. Similar changes also were observed in the old experimental rats (Table 2). Comparison of the adult and old experimental animals showed a higher content of free cholesterol in the nuclei and of total and esterified cholesterol in the supernatant in the latter. It is an interesting fact that the content of free cholesterol in the microsomal fraction was virtually unchanged in both adult and old experimental rats. The content of total cholesterol, however, was slightly increased on account of the esterified fraction.

The transport and reserve functions of cholesterol esters in the plasma and lymph [6] and in the cell and subcellular organoids and their participation in the free cholesterol metabolism of the cell membranes [4] have been postulated previously. The considerable accumulation of cholesterol esters probably takes place through an increase in their concentration in the hyaloplasm and matrix of the subcellular structures (nucleus, mitochondria, endoplasmic reticulum). The content of free cholesterol located in the structure of the cell membranes evidently does not change significantly. The observed increase in the content of free cholesterol takes place on account of its fraction in equilibrium with cholesterol esters in the hyaloplasm and matrix of the subcellular formations.

Weakening of hormonal regulation by the pituitary and thyroid glands is known to lead to slowing of cholesterol metabolism in old rats [8]. The higher accumulation of cholesterol and, in particular, of its esterified fraction in old rats than in adult animals during alimentary cholesterol loading, observed in the present experiments, confirms the conclusion that cholesterol metabolism is slowed in old age.

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