EFFECT OF ATHEROGENIC DIET ON BLOOD CHOLESTEROL AND BILE SECRETING FUNCTION OF THE LIVER OF RATS OF DIFFERENT AGES

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UDC 612.357.32 +616.13.002.2

After isolation of the common bile duct the quantity of bile secreted during 24 h and the concentration of total bile acids in it were determined in adult (11-12 months) and old (27-29 months) rats. Differences between the adult and old rats as regards bile formation and the concentration of the cholesterol fractions of the liver and blood plasma were not significant if the animals were kept on a standard diet. If kept on an atherogenic diet, the secretion of bile acids was significantly higher both in the adult and in the old rats. Meanwhile the level of total and esterified cholesterol in the blood plasma was higher in the old than in the adult rats.

KEY WORDS: free cholesterol, cholesterol esters, total bile acids, aging, atherogenic diet.

There is as yet no general agreement regarding the changes with age in the blood serum cholesterol concentration in rats or the causes of these changes. For instance, the increase in the cholesterol concentration in rats up to 18 months of age, which some workers [7, 10] have observed, has not been confirmed by other investigations [12, 13]. A high cholesterol diet can cause more severe lipid infiltration of the liver in old than in young animals [1]. A decrease in the formation of bile acids from cholesterol may be one possible cause of changes in the cholesterol concentration in the blood and liver. There is evidence of a decrease in the conversion of cholesterol into bile acids in rats between the ages of 2 and 18 months [13]. However, the character of the changes in bile formation at an older age remains unknown.

The object of this investigation was to study age differences in the cholesterol level in the blood plasma and liver of rats kept on ordinary and atherogenic diets and to compare these findings with the bile secreting function of the liver.

EXPERIMENTAL METHOD

Rats of two age groups were used: adult animals aged 11-12 months (groups 1, 3, 5, and 6) and old rats aged 27-29 months (groups 2 and 4). The rats of groups 1 and 2 were kept on the ordinary animal house diet and the animals of groups 3 and 4 received an atherogenic diet, including cholesterol, concentrated bile, and 6-methylthiouracil, for 5 months. The adult rats of group 5 received a diet with cholesterol and those of group 6 cholesterol together with bile. The conditions of experimental feeding were described previously [2]. The bile-secreting function of the liver was studied in an acute experiment. Under inhalational ether anesthesia, a polyethylene tube was inserted into the common bile duct and fixed [4]. Bile was collected for 24 h, after which the rats were decapitated and the total, free, and esterified cholesterol concentrations were determined in the blood plasma and liver [4]. Bile acids were determined in the bile samples [3].

EXPERIMENTAL RESULTS

Data on the level of the cholesterol fractions in the blood, the volume of bile secreted, and the concentration of bile acids in it are given in Table 1. They show that the relative volume of bile secreted during the 24 h period falls with age in rats kept on a standard diet. Meanwhile, the quantity of bile acids per unit weight of the animal remains substantially unchanged. The cholesterol concentration in the liver and blood plasma of the adult and old rats kept on a standard diet was about the same.

Research Institute of Physiology, Kiev University. Laboratory of Pathological Physiology, Institute of Gerontology, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 155-157, February, 1978. Original article submitted April 18, 1977.

0007-4888/78/8502-0147$07.50

TABLE 1. Secretion of Bile Acids with Bile and Level of Cholesterol Fractions in Blood Plasma and Liver of Rats of Different Ages Kept on Standard and Atherogenic Diets (M ± m)

Diet	Group	Age	Weight of rats, g	Volume of bile, ml/100 g/24 h	Quantity of bile acids, mg/100 g/24 h	Liver cholesterol, mg/g tissue			Plasma cholesterol, mg/100 ml		
						free	esterified	total	free	esterified	total
Standard	1	Adult $n=7$	227 ± 4	4,70 ± 0,35	10,90 ± 0,42	2,11 ± 0,12	0,09 ± 0,03	2,21 ± 0,14	31,0 ± 3,9	22,0 ± 1,9	53,0 ± 3,8
	2	Old $n=8$	364[a] ± 8	3,50[a] ± 0,25	10,10 ± 1,53	2,10 ± 0,13	0,08 ± 0,06	2,10 ± 0,15	38,0 ± 3,07	20,0 ± 2,2	58,0 ± 3,5
Atherogenic	3	Adult $n=6$	207 ± 7	6,30 ± 0,79	29,40[b] ± 5,32	9,90[b] ± 1,23	24,50[b] ± 3,57	3,44[b] ± 4,45	151,0[b] ± 12,9	257,0[b] ± 18,4	409,0[b] ± 27,5
	4	Old $n=6$	338[c] ± 20	4,9[c] ± 0,84	36,3[c] ± 11,08	22,20[c, d] ± 4,78	16,70[c, d] ± 3,33	39,0[c] ± 6,3	189,0[c, d] ± 32,5	324,0[c, d] ± 20,2	516,0[c, d] ± 26,2
Cholesterol	5	Adult $n=7$	262 ± 16	3,83 ± 0,47	21,0[e] ± 2,59	2,38 ± 0,42	1,22[e] ± 0,55	3,61 ± 0,77	21,0 ± 6,7	43,0[e] ± 7,8	63,0 ± 10,2
Cholesterol + bile	6	Adult $n=5$	265 ± 18	4,68 ± 1,12	20,3[f] ± 4,41	11,0[f] ± 3,93	13,4[f] ± 1,83	24,40[f] ± 5,74	61,0[f] ± 7,0	190,0[f] ± 21,0	251,0[f] ± 26,0

Legend. Difference significant ($P < 0.05$) between groups: a) 1 and 2; b) 1 and 3; c) 2 and 4; d) 3 and 4; e) 1 and 5; f) 1 and 6.

When the rats were kept on an atherogenic diet, their cholesterol concentration in the liver and plasma was considerably increased. The increase affected the levels of both free and esterified cholesterol. The increase in the concentration of the latter was particularly marked. The hypercholesteremia was accompanied by an appreciable increase in bile secretion. In the old rats the increase in the cholesterol concentration in the blood plasma and liver was rather greater than in the adult rats. The increase was significant for the levels of cholesterol esters and total cholesterol in the blood plasma.

The bile-secreting function of the liver of the adult and old rats, kept on a normal and an atherogenic diet also was studied in another series of experiments in which bile was collected for 4 h. Since the results of these experiments agreed with those described above, details of them will not be given in this paper.

There is evidence in the literature of marked decrease in the excretion of cholesterol and bile acids with the bile and feces during aging [9, 11] and a sharp increase in their excretion during cholesterol loading [6]. However, in the investigations cited above, sexually immature (2-3 months old) and adult (14-18 months old) rats were compared. According to the results of the present experiments, the changes in bile secretion in rats in the older age period are not significant. Meanwhile, the excretion of bile acids was significantly increased by both adult and old rats kept on an atherogenic diet.

The increased excretion of bile acids with the bile was due to the presence not only of bile acids in the diet but also of cholesterol, as the results of the experiments of group 5 show.

A sharp decrease in the level of esterified cholesterol in the liver of the adult (0.09 mg/g) and old (0.08 mg/g) control rats should be noted after excretion of bile for 24 h. In the adult rats undergoing the mock operation this index was 0.55 mg/g [4]. These facts confirm the view that cholesterol esters, which are used in the synthesis of bile acids, perform a transport and reserve function [4, 5, 8].

The absence of difference in the bile secreting function of the liver between the adult and old rats kept on an ordinary diet, observed in these experiments, agrees with data of a recently published investigation [12], in which no change was found in the cholesterol-7-hydroxylase activity in the liver of rats between 18 and 24 months old.

Analysis of the results suggests that during alimentary cholesterol loading processes of cholesterol esterification, the synthesis of bile acids, and their excretion from the body are activated. If bile or bile and 6-methylthiouracil are given together with cholesterol, inhibition of bile acid synthesis by the feedback principle and accumulation of cholesterol esters in the liver tissue and blood are observed. Bile acids, whose excretion with the bile is intensified under these circumstances, may perhaps be predominantly exogenous rather than endogenous in origin. It must also be remembered that simultaneous administration of bile acids and cholesterol leads to better absorption of the latter in the intestine and thereby leads to the development of hypercholesteremia and lipoidosis of the liver.

The higher blood cholesterol level in the old rats than in the adult animals kept on an atherogenic diet is difficult to explain by the state of the bile secreting function of the liver at this age, for the quantity of bile acids

excreted with the bile of the old experimental animals did not differ significantly from its value in the adult rats. It can only be suggested that these special features of the response of old rats to an atherogenic diet are connected either with increased reabsorption of bile acids in the intestine in old age or with changes in other pathways of cholesterol catabolism with age.

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*Literature citation 5 omitted in Russian original – Consultants Bureau.