THE ACTION OF THYROID EXTRACT ON CHOLESTEROL METABOLISM IN ALIMENTARY ATHEROSCLEROSIS ASSOCIATED WITH EXPERIMENTAL LIVER DAMAGE

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The ability of the thyroid hormones to delay development of alimentary hypercholesteremia and lipoidosis of the aorta is largely dependent on stimulation of the activity of the liver in excreting cholesterol [2, 3]. To confirm this hypothesis, it is useful to study the action of thyroid extract during artificial liver damage, when the possibility of stimulating liver function is limited.

As a result of the toxic action of carbon tetrachloride (CCl_4), necrotic and fibrous changes develop in the liver, mainly in the central zones of its lobules [6]; it is in the central portion of the lobules that the intensity of metabolic processes is highest [10].

Concerning the action of CCl_4 against the background of alimentary atherosclerosis, there is only a brief report [9] describing a decrease in the concentration of lipoproteins of the Sg 12-10 and Sg 20-40 classes, and a paper by V. I. Metelitsa [1] showing that in rabbits poisoned with CCl_4 the development of hypercholesteremia and lipoidosis of the aorta is inhibited.

EXPERIMENTAL METHOD

Experiments were carried out on 40 rabbits weighing 2.5-3 kg, divided into five equal groups. Group 1 consisted of control rabbits, receiving an ordinary diet, group 2 of animals receiving cholesterol with the diet in a daily dose of 0.2 g/kg; group 3 of animals receiving cholesterol together with thyroid extract in a dose of 0.06 g/kg body weight; group 4 of animals receiving cholesterol and CCl_4 as intramuscular injections of a 40% solution in peach oil in a dose of 0.1 ml per rabbit every four days; group 5 of animals receiving a combination of cholesterol, thyroid extract, and CCl_4 in the same dosage. The experiment lasted 45 days. At the beginning and end of the experiment, the cholesterol concentration in the blood of the animals was determined by Bloor's method and the content of unsaturated fatty acid was estimated by the method of alkaline isomerization in ethylene glycol [8], followed by spectrophotometric analysis. The lipolytic activity was also determined by the extent of splitting of free fatty acid. Incubation with milk took place at the rate of 0.2 g lipids/ml blood serum. The free fatty acids were determined by Baretto's method [5].

At the end of the experiment the cholesterol concentration was determined in the aorta and liver. In addition, the content of unsaturated fatty acids was investigated in liver homogenates. Cholesterol was

TABLE 1. Effect of Thyroid Extract and Carbon Tetrachloride on Cholesterol Content in the Tissues, M ± m

Groups of rabbits	Blood cholesterol (in mg%)	Cholesterol in the aorta (in mg/100 mg dry weight)	Cholesterol in the liver (in mg/100 mg dry weight)
Healthy	100 ± 8.1	0.075 ± 0.007	0.25 ± 0.03
Receiving cholesterol	1,280 ± 124.2	0.345 ± 0.06	1.80 ± 0.2
Receiving cholesterol + thyroid	344 ± 18.8	0.213 ± 0.04	2.85 ± 0.5
Receiving cholesterol + CCl_4	320 ± 21.1	0.275 ± 0.04	1.54 ± 0.2
Receiving cholesterol + thyroid + CCl_4	1,125 ± 112.4	0.360 ± 0.06	1.72 ± 0.2

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TABLE 2. Effect of Thyroid Extract and Carbon Tetrachloride on Blood Concentration of Unsaturated Fatty Acids (in mg%), M ± m

Groups of Rabbits	Linoleic acid	Linolenic acid	Arachidic acid	Pantothenic acid	Ratio of linolenic acid / cholesterol
Healthy	72±10,1	10±0,8	6,5±0,8	3,0±0,3	0,72
Receiving cholesterol	164±10,0	22±3,9	12,0±1,5	5,1±1,0	0,13
Receiving cholesterol + thyroid	84±12,1	11±3,5	7,4±1,1	2,5±0,4	0,24
Receiving cholesterol + CCl_4	122±13,2	23±2,8	11,9±1,7	4,0±0,8	0,38
Receiving cholesterol + thyroid + CCl_4	185±14,5	24±2,9	16,0±1,8	3,9±0,6	0,16

TABLE 3. Effect of Thyroid Extract and Carbon Tetrachloride on Content of Unsaturated Fatty Acid in the Liver (in mg/100 mg dry weight of Liver), M ± m

Groups of Rabbits	Linoleic acid	Linolenic acid	Arachidic acid	Pantothenic acid	Ratio of linolenic acid/ cholesterol
Healthy	0,450±0,03	0,090±0,01	0,119±0,02	0,041±0,002	1,4
Receiving cholesterol	0,490±0,05	0,091±0,01	0,071±0,007	0,016±0,001	0,27
Receiving cholesterol + thyroid	0,320±0,06	0,058±0,005	0,055±0,004	0,012±0,001	0,11
Receiving cholesterol + CCl_4	0,697±0,09	0,121±0,06	0,116±0,03	0,024±0,001	0,45
Receiving cholesterol + thyroid + CCl_4	0,460±0,08	0,116±0,05	0,078±0,006	0,025±0,001	0,27

extracted with a chloroform–methanol mixture. The fatty acids were extracted by an acid mixture of ethanol with ether. The results obtained were calculated per 100 mg constant dry weight of organ.

EXPERIMENTAL RESULTS

The results of these experiments confirmed previous findings [2, 3] that thyroid extract inhibits the development of hypercholesteremia and of cholesterol infiltration of the aorta and increases the cholesterol content in the liver of rabbits (Table 1). Under the influence of CCl_4 a decrease in the alimentary hypercholesteremia and a decrease in the content of cholesterol in the aorta and liver were observed. Special interest was attached to the group of animals receiving cholesterol, thyroid, and CCl_4 simultaneously. In the rabbits of this group the cholesterol content in the blood, aorta and liver was very little different from that in these same tissues of animals receiving cholesterol alone (Table 1). The CCl_4 apparently prevented the action of thyroid on the cholesterol metabolism in the animals with experimental atherosclerosis. Evidently the experimental liver damage prevented the thyroid extract from influencing the cholesterol metabolism. This confirms yet again the view that the hormones of the thyroid gland exert their influence on cholesterol metabolism through the liver.

When the content of fatty acids was analyzed, besides their absolute content attention was directed also to the ratio between the linoleic acid and cholesterol. This index is important because, when there is a large difference between the cholesterol content in animals of different groups, the changes in the absolute content of fatty acids may not reflect their true deficiency or excess, and it is the relationship between cholesterol and the unsaturated fatty acids which largely determines the transformation of cholesterol in the body.

The absolute content of unsaturated fatty acids in the blood, to judge by the linoelic acid content (Table 2), increased when the animals were fed on cholesterol alone. The addition of thyroid extract or CCl_4 prevented this increase. However, the relative content of unsaturated fatty acids in the blood fell considerably during the reproduction of alimentary atherosclerosis. Administration of thyroid extract and, in particular, of CCl_4 reduced the development of a relative deficiency of unsaturated fatty acids, accompanying alimentary atherosclerosis.

When cholesterol, thyroid, and CCl_4 were administered together, both the absolute and the relative content of unsaturated fatty acids in the blood differed only slightly from these indices in the animals receiving cholesterol alone.

The absolute content of linoleic acid in the liver (Table 3) increased during the development of experimental atherosclerosis. Thyroid extract inhibited the increase in linoleic acid, while CCl_4 led to more marked accumulation of this substance in the liver. The relative content of unsaturated fatty acids in the liver was lower in the animals of all groups than in the healthy animals. A particularly sharp decrease was observed in the group of animals receiving cholesterol together with thyroid extract. Hence, during the development of atherosclerosis, the addition of thyroid extract to a high-cholesterol diet lowered both the absolute and the relative content of unsaturated fatty acids. Stimulation of liver function by thyroid hormones is evidently accompanied by an increase in the utilization of the unsaturated fatty acids, and this in turn promotes the accumulation of cholesterol in the liver.

Comparison of the absolute and relative content of unsaturated fatty acids in the blood and liver of the animals receiving cholesterol and the animals receiving a combination of cholesterol, thyroid extract, and CCl_4 shows that the difference between them is not significant. Hence, in the presence of liver damage by CCl_4, the action of thyroid extract was not manifested, either on the cholesterol metabolism or on the content of unsaturated fatty acids in the blood and liver.

The results of the study of the activity of the blood lipolytic enzymes showed that during reproduction of atherosclerosis this activity fell on the 45th day of cholesterol feeding (from 0.5 ± 0.06 to 0.14 ± 0.01 meq/liter). Administration of thyroid extract along with the cholesterol led to some degree of stimulation of lipolytic activity (0.28 ± 0.05 meq/liter). Damage to the liver of rabbits with experimental atherosclerosis by CCl_4 prevented the depression of lipolytic activity, and its level was actually slightly higher than in the controls (0.59 ± 0.04 meq/liter). Even higher lipolytic activity was found after the simultaneous administration of cholesterol, thyroid extract, and CCl_4 (0.88 ± 0.06 meq/liter).

In the presence of liver damage, including that caused by CCl_4, inactivation of the lipolytic enzymes is known to be inhibited [7], and in patients with liver damage the blood heparin concentration rises [4].

Possibly the decreased production of inhibitors of the lipolytic enzymes in the presence of liver damage and their increased stimulation by heparin may have brought about the observed effect in the present experiment.

It may be concluded from the experimental results described that the changes in lipid metabolism in atherosclerosis are mainly dependent on the state of the liver, and the thyroid hormones exert their influence on lipid metabolism through their action on the liver.

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