Thyroid Hormones: Effect on Aortic Acid Mucopolysaccharides and Atherosclerosis in Rabbits[1,2,3] (37077)

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Hypothyroidism and myxedema are characteristically associated with elevated serum cholesterol levels (1). Thyroid hormones reduce the serum cholesterol level not only in hypothyroid patients but also in hypercholesteremic subjects (2, 3). In view of the intimate correlation between hypercholesteremia and atherosclerotic cardiovascular disease (4), it has been generally assumed that thyroid hormones play an important role in atherogenesis (1–3).

In man, the extent and severity of atherosclerosis observed at autopsy, however, does not seem to have been influenced by the presence of either hypo- or hyperthyroidism (1, 5). Furthermore, animal experiments have led to divergent results. Removal or inhibition of thyroid function facilitates the development of atheromata in otherwise resistant dogs (6) and rats (7). In various birds fed cholesterol, thyroid hormones diminish hypercholesteremia and retard atherogenesis (8), but thyroidectomy alone does not enhance the spontaneous development of atherosclerosis in white Carneau pigeons (9). In rabbits, the administration of thyroxine or its analogues has been said by different authors to lessen (10–12) or to augment (13–15) the effect of cholesterol feeding upon the development of atherosclerosis. One of the purposes of the present study is, therefore, to clarify this ambiguity of thyroid effect on experimental atherogenesis in rabbits.

The importance of the aortic acid mucopolysaccharides (AMPS) in the development of atherosclerosis, especially in its early stage, has been emphasized (16, 17). The relationship between thyroid hormones and atherosclerosis may not be linked through the change of serum cholesterol levels but through the alteration of the aortic AMPS. The experiment is thus also designed to study the effect of thyroid hormones on the aortic AMPS and their relationship to atherosclerosis.

Materials and Methods. Forty young adult New Zealand white female rabbits, weighing an average of 2500 g at the beginning of the study and housed in individual cages in a well-ventilated animal facility, were divided into three groups: (a) Control group—12 rabbits received neither thyroxine nor thiouracil and were in a euthyroid state. (b) Thyroxine treated group—14 rabbits were fed levo-thyroxine dissolved in their drinking water. The concentration of levo-thyroxine in the drinking water was 0.02 mg/100 ml at the beginning and increased gradually (0.02 mg/100 ml every two weeks) up to 0.10 mg/100 ml over a period of ten weeks and then maintained on this concentration

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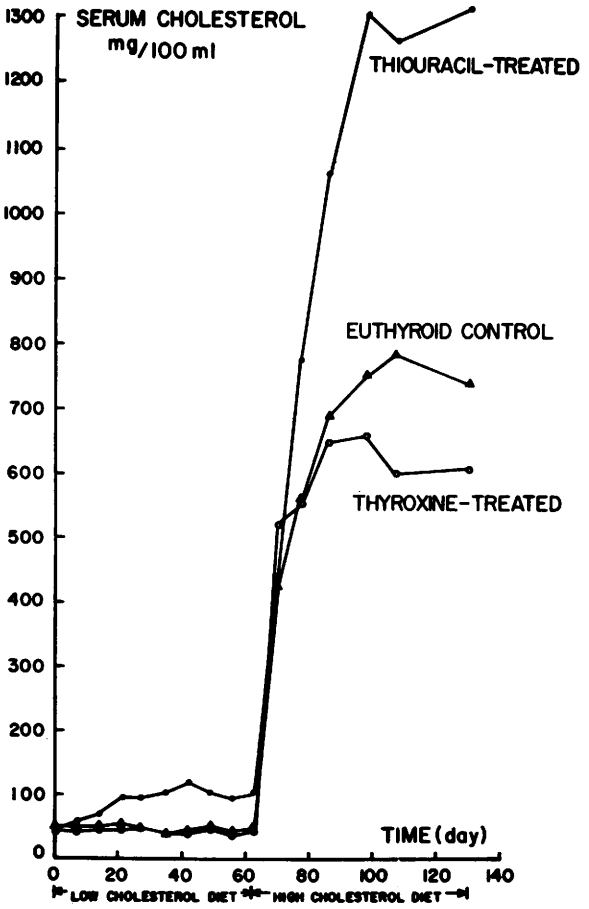

FIG. 1. Changes of serum cholesterol levels of euthyroid, thyroxine-treated, and thiouracil-treated rabbits during low- and high-cholesterol dietary periods.

for the rest of the experiment period. The thyroxine-containing water was given *ad libitum,* and each rabbit, on an average, consumed about one pint of water each day. (c) Thiouracil-treated group—14 rabbits were fed thiouracil in the drinking water. The concentration was also increased gradually from the initial 33 mg/100 ml to a final 66 mg/100 ml over a period of ten weeks and maintained at this concentration thereafter. The rabbits of this group consumed a lesser amount of water than the other two groups.

During the first 9 weeks of the experiment, the rabbits of all three groups were fed, *ad libitum,* regular commercial Rockland rabbit pellets (Monmouth, Illinois). A 3-ml of blood sample was taken from the central artery of the ear of each rabbit weekly for determination of (a) serum cholesterol by Zak *et al.* (18) method modified for automatic analysis, (b) serum phospholipid by the method of Goodwin *et al.* (19), and (c) serum protein-bound hexosamine by modified Boas' method (20). Half the animals in each group were sacrificed at the end of the 9th week of the experiment. The entire aorta of each animal was removed for analysis of its cholesterol content by the method of Ho *et al.* (21) and for isolation and quantitation of individual aortic AMPS contents (22, 23). Four major aortic AMPS, namely hyaluronic acid (HA), heparitin sulfate (HS), chondroitin sulfate-B (CS-B), and chondroitin sulfate-C (CS-C), were isolated by the method of Yukio and Gore (22). The amounts of AMPS were determined by the method of Gatt and Berman

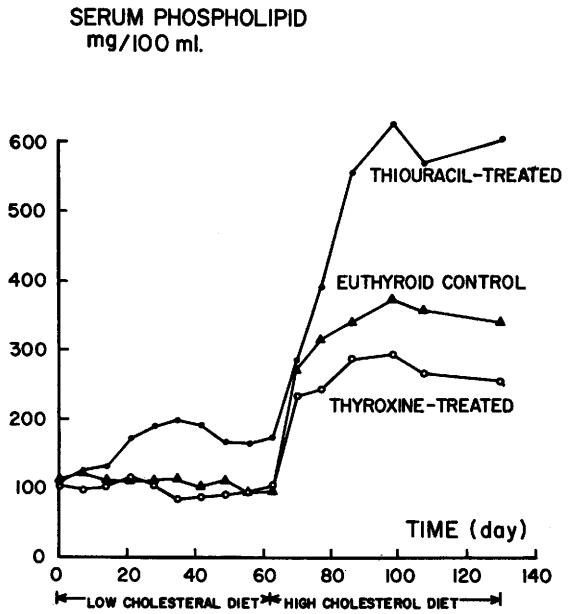

FIG. 2. Changes of serum phospholipid levels of euthyroid, thyroxine-treated, and thiouracil-treated rabbits during low- and high-cholesterol dietary periods.

(23) and expressed as micrograms of hexosamine.

The other half of the rabbits, having been maintained at their same level of thyroid hormone activity, either euthyroid, hyperthyroid, or hypothyroid, and on the regular Rockland rabbit pellets for 9 weeks, were then fed, *ad libitum,* the same diet supplemented with 2% crystalline cholesterol dissolved in an equal weight of peanut oil. The hormonal treatments remained the same for each group throughout the rest of the experiment. Serum cholesterol, phospholipid and protein-bound hexosamine were also determined on a weekly basis. One rabbit in each group was sacrificed on the following dates after the commencement of cholesterol feeding: day 25, 40, 54, 68, 82, 96, and 110. The aortas were removed for chemical determination of their cholesterol and AMPS contents by the same methods described above.

Results. Changes of serum cholesterol levels. (a) Low cholesterol dietary period: The regular Rockland rabbit pellet contains a very small amount of cholesterol, only 0.06 mg of cholesterol per gram of chow as determined previously by our laboratory (24). During the 12 weeks of such a low cholesterol dietary intake, the serum cholesterol levels of the thyroxine-treated group were not different from those of the control group, whereas the serum cholesterol levels of the thiouracil-treated group increased steadily and reached a plateau after 20 days of treatment. At this time, their serum cholesterol levels were twice as high as those of the controls (100 *vs* 50 mg/100 ml, Fig. 1).

(b) High cholesterol dietary period: The serum cholesterol levels of all groups of rabbits increased sharply after the starting of cholesterol feeding (Fig 1). A new plateau was attained in each group after 40 days of cholesterol feeding. At this time, the serum cholesterol levels of the thyroxine-treated group tended to be slightly lower than those of the control group, whereas the serum cholesterol levels of the thiouracil-treated group were still almost twice as high as those of the controls (1300 *vs* 750 mg/100 ml, Fig. 1).

Changes of serum phospholipid levels. The serum phospholipid levels of these three groups of rabbits exhibited changes parallel to their serum cholesterol levels (Fig. 2). The serum phospholipid levels of the thiouracil-treated rabbits were always twice as high as

TABLE I. Comparison of the Regression Equations Relating Aortic Cholesterol Content (y in mg % of Cholesterol/g of Dry Defatted Aortic Tissue) and the Duration of Exposure to Hypercholestermia (x in days) of Euthyroid Control, Thyroxine-Treated and Thiouracil-Treated Rabbits.

	Exponential Regression	γ	p	*SEE*
Euthyroid Control	$y = 4.94e^{0.024x}$	0.998	<0.001	1.04
Thyroxine-treated	$y = 4.55e^{0.029x}$	0.992	<0.001	1.13
Thiouracil-treated	$y = 5.49e^{0.016x}$	0.957	<0.001	1.18
	Linear Regression			
Euthyroid Control	$y = -0.846 + 0.440x$	0.959	<0.001	3.980
Thyroxine-treated	$y = -10.284 + 0.820x$	0.955	<0.001	8.815
Thiouracil-treated	$y = 4.482 + 0.212x$	0.990	<0.001	1.053

		Exponential Regression		*Linear Regression*	
t-test:	Control vs Thyroxine	$t = -1.55$	$p > 0.1$	$t = -1.99$	$p > 0.05$
	Control vs Thiouracil	$t = 2.33$	$p < 0.05$	$t = 3.15$	$p < 0.01$
	Thyroxine vs Thiouracil	$t = 3.69$	$p < 0.01$	$t = 4.11$	$p < 0.01$

those of the controls either in the low- or high-cholesterol dietary period. The serum phospholipid levels of the thyroxine-treated rabbits were not different from those of the controls during the low cholesterol period, but tended to be lower during high-cholesterol period (Fig. 2).

The ratios of serum cholesterol levels to serum phospholipid levels of the same sera were more related to the dietary cholesterol intake than to the thyroid activity. During the low-cholesterol dietary period the cholesterol:phospholipid ratios were 1:2 for all three groups of rabbits, whereas these ratios were reversed to 2:1 during cholesterol feeding.

Changes of serum protein-bound hexosamine. The concentrations of serum protein-bound hexosamine, which represents the serum mucoprotein or mucopolysaccharide, stayed quite constant in all three groups of rabbits throughout the experiment. The mean hexosamine levels of the control, the thyroxine-treated, and the thiouracil-treated rabbits were 61.3 ± 3.0 (mean ± SD), 57.9 ± 3.3, and 59.7 ± 3.5 mg/100 ml, respectively, during the low-cholesterol dietary period and were 62.2 ± 1.3, 63.1 ± 1.8, and 61.6 ± 2.1 mg/100 ml, respectively, during the high-cholesterol feeding period. They were not different statistically from each other. Therefore, the serum protein-bound hexosamine levels were not affected by either changes of thyroid activity or cholesterol feeding.

Changes of aortic cholesterol content. At the end of 9 weeks of low-cholesterol feeding and hormonal treatment, the cholesterol contents of the aortas of thyroxine-treated and thiouracil-treated rabbits were 4.40 ± 0.80 (mean ± SD) and 4.44 ± 0.67 mg/g of dry defatted tissue, which were not different from one another and also not different from those of the control rabbits (4.83 ± 0.50 mg/g dry defatted tissue). Apparently, when the dietary cholesterol intake was small or negligible, the aortic cholesterol contents of the thiouracil-treated rabbits were not influenced by their slightly elevated serum cholesterol levels in spite of the fact that their levels were double those of the normal rabbits.

The aortic cholesterol concentrations of all three groups of rabbits increased rapidly during the period of cholesterol feeding. Since the animals were sacrificed sequentially during this period, it is possible to perform regression analyses of the relationships between aortic cholesterol contents and the duration of exposure to hypercholesteremia. Such analyses revealed an excellent correlation between these two parameters in the fashion of either exponential or arithmetically linear increase (Table I). For the control and the thyroxine-treated groups, the curves fit the exponential equation better than the arithmetically linear equation, but for the thiouracil-treated group the reverse is true.

By careful examination of the regression equation shown in Table I, the following interesting results were found:

(a) The thiouracil-treated rabbits, in spite of having higher serum cholesterol levels,

TABLE II. Changes of Aortic AMPS in Euthyroid Control, Thyroxine-Treated, and Thiouracil-Treated Rabbits during Low-Cholesterol and High-Cholesterol Dietary Periods.

Diet	Hormonal state	Number	HA	HS	CS-C	CS-B	Total AMPS
Low Cholesterol	Euthyroid	6	1026 ± 210[a]	645 ± 139	1183 ± 326	259 ± 115	3113 ± 582
	Thyroxine-treated	7	733 ± 241[b]	611 ± 113	1097 ± 251	306 ± 130	2747 ± 412
	Thiouracil-treated	7	974 ± 206[c]	967 ± 239[b,c]	1241 ± 310	377 ± 164	3559 ± 680[c]
High Cholesterol	Euthyroid	6	1948 ± 590	1306 ± 235	2505 ± 389	415 ± 209	6137 ± 1084
	Thyroxine-treated	7	1739 ± 636	1318 ± 212	2207 ± 218	339 ± 104	5603 ± 755
	Thiouracil-treated	7	2220 ± 289	1509 ± 301	2210 ± 264	455 ± 107	6393 ± 375[c]

[a] Mean ± SD in micrograms of hexosamine per gram of dry defatted tissue.

[b] Significant difference from the control ($p < 0.01$ by t test).

[c] Significant difference from the thyroxine-treated group ($p < 0.01$ by t test).

HA: hyaluronic acid, HS: heparitin sulfate, CS-C: chondroitin sulfate-C, and CS-B: chondroitin sulfate-B.

showed the least amounts of cholesterol accumulation in their aortas. The rate of cholesterol accumulation (or the slope of the regression line) of this group of rabbits was much slower and always less than half of the other two groups. Furthermore, the initial cholesterol accumulation (or the constant term of the regression equation) seemed to be greater in this group than in the other two groups; this made the regression line more arithmetically linear than exponential.

(b) The thyroxine-treated group, on the other hand, having a tendency to have their serum cholesterol levels lower than those of the control group, exhibited a faster rate of cholesterol deposition in their aortas than the control group. However, this difference was only of statistically borderline significance. Since the aortic cholesterol contents correlate very well with the severity of dietary-induced atherosclerosis in rabbits, we may conclude that thyroxine augmented the aortic atherogenesis while thiouracil retarded this process in rabbits.

Changes in aortic AMPS. (a) Low-cholesterol dietary period: The changes of aortic AMPS during the period of low-cholesterol feeding in this study may be considered as pure effects of the thyroid hormones. The total AMPS content of the aortas from thyroxine-treated rabbits tended to be lower and that from the thiouracil-treated rabbits tended to be higher than the control value (Table II). However, because of large individual variations, these differences were of no statistical significance ($p > 0.1$). Direct comparison between the total aortic AMPS of thyroxine-treated and thiouracil-treated rabbits revealed, on the other hand, a statistically significant difference ($p < 0.01$).

The changes of individual AMPS were also not remarkable except for a decrease of aortic hyaluronic acid content in thyroxine-treated rabbits ($p < 0.05$) and an increase of heparitin sulfate content of the thiouracil-treated rabbits ($p < 0.01$) (Table II). The aortic contents of CS-B and CS-C were essentially unchanged by alteration of thyroid activity.

(b) High-cholesterol dietary period: Individual fractions of the aortic AMPS of all three groups of rabbits increased rapidly in the early period of cholesterol feeding and decreased slightly and slowly after reaching a plateau in one to two months. This pattern of change of individual aortic AMPS was similar in all three groups of rabbits, and their mean values were not statistically different from each other (Table II). There was no simple statistical relationship between aortic AMPS contents and duration of cholesterol feeding or aortic cholesterol contents. Although high cholesterol feeding in rabbits tended to mask the effects of thyroid hormones, still the total aortic AMPS of thiouracil-treated rabbits was significantly lower than those of the thyroxine-treated group ($p < 0.05$).

Comment. Paradox of serum cholesterol levels and aortic cholesterol contents: The aortic cholesterol contents of the thyroxine-treated and thiouracil-treated rabbits were not different from those of the euthyroid controls when they were on a low cholesterol diet. However, during cholesterol feeding, the

thyroxine-treated rabbits developed hypercholesteremia, lower than that of the thiouracil-treated group, but accumulated more cholesterol in their aortas. Such a paradoxical phenomenon was also observed by Gore (15) and Felt and Benes (25), but not by others (10, 11). The possible mechanisms for this unique effect of thyroid hormones will be discussed below.

In relation to cholesterol metabolism. Hypercholesteremia in hypothyroid subjects has been shown to be associated with a slower turnover of body cholesterol (26), while thyroxine administration enhances the fecal excretion of both neutral and acid sterols (3, 27). On the other hand, it has been shown by either kinetic analysis (25) or direct chemical measurements (28), that thyroxine causes a redistribution of cholesterol from the rapidly exchangeable pool, such as serum, to a slower turnover tissue compartment including the aorta. The aforementioned paradox might be the result of the combined effects of enhanced sterol excretion and redistribution caused by thyroid hormones.

In relation to changes of aortic AMPS. The basic mechanisms relating to the thyroxine-induced redistribution of cholesterol in the body are not known. One may postulate that the change of tissue affinity for circulating cholesterol or lipoprotein may account for such shifting. In this study we have directed our attentions particularly to the AMPS components of the aortic tissue. A small but significant decrease of aortic hyaluronic acid content was found in thyroxine-treated rabbits, whereas in thiouracil-treated rabbits, an increase of heparitin sulfate was noted. The reduction of HA by thyroxine treatment was also observed by Józsa and Luszitg (29) and might make the vascular wall more permeable to the circulating lipoproteins. Heparitin sulfate was considered to be an intermediate in the synthesis of heparin and belongs to the group of heparinoids (30) which are capable of activating lipoprotein lipase and promoting endogenous fibrinolysis. The increase of aortic HS content of the thiouracil-treated rabbits might lessen the amount of cholesterol accumulated in their aortas and the severity of atherosclerosis.

Cholesterol feeding, however, abolished such differences in aortic AMPS contents observed during low-cholesterol dietary period. Therefore, the true significance of the changes of the individual AMPS in hyper- or hypothyroid state in relation to atherogenesis is only speculative and still not confirmed.

In relation to other factors. The lack of significant association between the severity of coronary atherosclerosis and hypothyroidism in man was interpreted by Jones *et al.* (1) by the difference in their serum lipoprotein composition. Patients with idiopathic hypercholesteremia, which is very often associated with coronary atherosclerosis, have a significantly higher beta-lipoprotein fraction. In this study, the ratio of serum cholesterol to phospholipid was 1:2 during the low-cholesterol dietary period and was reversed to 2:1 during the high-cholesterol feeding period. The fact that all three groups of rabbits showed exactly the same changes indicated that their changes of serum lipoprotein composition were similar in all three groups and were more influenced by the dietary cholesterol intake than by the thyroid activity.

Thyroxine has been shown to potentiate epinephrine-induced medial arteriosclerosis in rabbits which renders the vessels more susceptible to cholesterol-induced atherosclerosis (14). Although no exogenous epinephrine was given to the animals in this study, the administration of thyroxine may make the animals more sensitive to endogenous sympathetic discharges and more prone to develop vascular lesions.

Summary. The effects of thyroid hormones on the serum lipids, aortic atherosclerosis and acid mucopolysaccharides were studied in 40 New Zealand white rabbits in two successive dietary periods: low and high contents of cholesterol. Thiouracil-treated rabbits had higher serum cholesterol levels but lesser cholesterol accumulation in the aorta than the euthyroid controls. On the other hand, thyroxine-treated rabbits tended to have serum cholesterol levels lower than those of the controls during cholesterol feeding but exhibited a greater degree of cholesterol accumulation in the aorta. Such a paradox could only be

partially explained by a decrease of aortic hyaluronic acid in the thyroxine-treated group and an increase of heparitin sulfate in the thiouracil-treated group. These differences in aortic AMPS contents were only observed during low-cholesterol feeding periods and were abolished by cholesterol feeding.

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