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Cholesterol Balance Studies in Mice with Modified Thyroid Activities.* (21237)

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It was observed recently in this laboratory (1), that in cholesterol-fed rats, thyroid administration significantly reduces plasma and liver cholesterol levels, in agreement with earlier findings(2-4). These effects cannot be attributed to a decrease in the rate of endogenous cholesterol formation, as thyroid hormone stimulates synthesis of the sterol(5-7). Therefore, the possibility was considered that mechanisms leading to an increased rate of sterol disposal are enhanced in the hyperthyroid state. Our work was undertaken to investigate, whether, in the mouse, the thyroid hormone accelerates the catabolism of cholesterol.

Methods. The total cholesterol balance was determined essentially as described recently (8). In the first experiment, female mice, 5-6 weeks old, were used.‡ All animals were fed purina chow supplemented with 1% sulfasuxidine and 0.05% streptomycin§ for one day prior to the experimental run, to prevent or reduce cholesterol destruction by the intestinal flora(8). During the entire balance period of 16 days, all mice of both experimental groups were kept individually in metabolism cages, and fed a diet containing the components mentioned above, 0.65% cholesterol, and other supplements as indicated.‖ In addition, the animals of the hyperthyroid group were given, at onset, one intraperitoneal injection of 50 μg of thyroxine, and then, fed 0.4% desiccated thyroid USP with their diet during entire balance period. In a second experiment, male mice (Swiss Colony Strain)¶ 5-6 weeks old were used. Prior to the experimental run, all animals were fed Purina Chow supplemented with 0.5% thiouracil** and the mentioned

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‡ Mice were obtained from California Caviary, Los Angeles.

§ Sulfasuxidine—Sharp and Dohme, Division of Merck and Co., Streptomycin hydrochloride—E. R. Squibb and Sons.

‖ Experimental diet: Purina chow (ground), 81.35%; yeast (strain G, Anheuser-Busch), 10%; dextrin, 2%; Wesson oil, 4.5%; cholesterol, .65%; desiccated ox bile (USP), .25%; sulfasuxidine, 1%; streptomycin, .25%. Cholesterol was dissolved in Wesson oil before being mixed with the diet.

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TABLE I. Effects of Thyroid and Thiouracil on the Total Cholesterol Balance of Mice under Different Dietary Conditions.

Exp.	Feeding conditions	Cholesterol content of diet, %	Endocrine supplement	No. of mice	Total cholesterol balance: Avg input, mg	Avg recovery* mg	Avg recovery* %	P for difference
1	Restricted	.65	Thyroid	8	313	250	80 ± 5	<.02
	"	"	None	6	320	302	94 ± 1	
2	"	"	Thyroid + thiouracil	10	290	268	92 ± 3	<.001
	"	"	Thiouracil	7	269	297	110 ± 1	
3	*Ad libitum*	.15	Thyroid + thiouracil	8	92	115	125 ± 4	<.001
	"	"	Thiouracil	7	83	85	103 ± 3	

* The stand. errors of the means were calculated using the formula: $S_M = \sqrt{\frac{\Sigma(x^2)}{n(n-1)}}$.

x = deviation from the mean; n = No. of animals. The P values were calculated using Fisher's Table of t.

antibacterial drugs for 12 days, and then divided into 2 groups. One group continued on the same regimen, while the other was fed 0.4% thyroid powder (USP) with the thiouracil containing diet, for a further pretreatment period of 3 days. During the balance period of 10 days, the mice were fed diets described for the second pretreatment period, further supplemented with cholesterol and the other supplements mentioned.‖ In both experiments, all animals received the same amount of diet twice daily. In a third experiment, animals used, and endocrine treatments were the same as in Exp. II. The dietary regimen, however, was different, *i.e.*, a diet containing only 0.15% cholesterol was fed *ad libitum* for 4 days. During that time the food intake was determined. In all 3 experiments, excreta were collected during the balance period, and the mice were then sacrificed. The carcasses and excreta were hydrolysed in 30% (w/v) KOH solution in 50% (v/v) alcohol, and then extracted with diethyl ether. The solid residues remaining after hydrolysis and extraction of feces were re-extracted for 10 hours in Soxhlet apparatus, first with 95% (v/v) alcohol and then with ether. The total cholesterol content of extracts was then determined using a modified Schoenheimer-Sperry procedure(9). The values obtained are usually considered to represent essentially cholesterol, although a contribution of other unsaturated sterols cannot be completely excluded(10). In some instances, the livers were excised at autopsy and their cholesterol content was determined separately. The values for the overall cholesterol balance were calculated from the total cholesterol input (carcass at onset + food consumed) and the total cholesterol recovery (carcass at end + excreta). The carcass cholesterol contents at onset were computed, using body weights of experimental animals at beginning of balance period, and the average carcass cholesterol concentrations of additional animals ("onset controls") treated in identical manner and sacrificed at same time. Four to 6 mice were used per group as onset controls; individual variations within groups were insignificant.

Results. The thyroid treatment produced symptoms typical of hyperthyroidism. *E. g.*, the physical activity, nervousness, water consumption and mortality, and, in Exp. 3, the food intake and fecal excretion were significantly increased.

The results obtained for the total cholesterol balance are shown in Table I. In Exp. 1 and 2, hyperthyroid mice were compared with normal and hypothyroid animals, when pair-fed restricted amounts of a diet containing 0.65% cholesterol. In the groups supplemented with thyroid, the cholesterol output was significantly smaller than the sterol input, and also significantly smaller than the recovery in the respective controls. In Exp. 2, the overall cholesterol recovery of the animals fed thiouracil exceeded 100%. The liver

TABLE II. Effects of Thyroid and Thiouracil on Cholesterol Content of Carcass and Excreta of Mice under Different Dietary Conditions.

Exp.	Feeding conditions	Cholesterol content of diet, %	Endocrine supplement	Avg total cholesterol content: Of carcass* At onset,† mg	Of carcass* At end,† mg	Of food eaten per 24 hr, mg	Of excreta per 24 hr,† mg/g body wt
1	Restricted	.65	Thyroid	38 ± 2	32 ± 1	17.2	1.29 ± .09
	"	"	None	36 ± 2	37 ± 2	17.8	1.44 ± .03
2	"	"	Thyroid + thiouracil	85 ± 4	74 ± 4	20.5	.85 ± .02
	"	"	Thiouracil	62 ± 3	88 ± 2	20.7	1.03 ± .01
3	*Ad libitum*	.15	Thyroid + thiouracil	62 ± 3	64 ± 2	7.5	.53 ± .02
	"	"	Thiouracil	66 ± 4	59 ± 2	4.4	.29 ± .01

* In Exp. 2, mortality rate was relatively high in the group supplemented with thyroid; somewhat heavier mice, obtained as replacements for the animals lost in this way, survived better; as a consequence, the avg body wt was somewhat greater for the hyperthyroid group.
† For calculation of the stand. errors see Table I.

cholesterol values are not shown separately as their contribution to the values for the overall balance was of minor extent.

In Exp. 3, the cholesterol balance of hyperthyroid mice was compared with that of thiouracil-treated animals under a different dietary regimen, *i.e.*, when fed *ad libitum* a diet relatively low in cholesterol. The results indicate that alteration of the nutritional state of the mice profoundly modified the action of the thyroid hormone on the cholesterol balance. In the hyperthyroid group, the cholesterol recovery was significantly larger than the sterol input, and larger than that for hypothyroid animals, in contrast to the data recorded for Exp. 1 and 2, where reverse relationships were observed.

When the effects of thyroid on the cholesterol contents of carcass and excreta are examined separately, a similar trend is observed (Table II). Under conditions of a restricted feeding of a high cholesterol diet, the hyperthyroid groups showed a decrease in rate of cholesterol excretion, as compared to normal (Exp. 1) or hypothyroid (Exp. 2) mice. The carcass cholesterol content was only slightly decreased by thyroid administration; in the hypothyroid animals, however, this value was found significantly increased under these dietary conditions (Exp. 2). Apparently, the thyroid treatment prevented such an accumulation of tissue cholesterol, in spite of a relatively high cholesterol intake.

When a diet relatively low in cholesterol was fed *ad libitum*, the hyperthyroid animals excreted cholesterol at a markedly increased rate, as compared to the mice treated with thiouracil alone (Exp. 3). The carcass cholesterol levels, however, were not significantly modified under these conditions.

Discussion. The results of the first 2 experiments were interpreted to indicate that the thyroid hormone can stimulate the degradation or modification of cholesterol. This effect was observed under conditions of a restricted intake of a diet relatively high in cholesterol. The authors are aware of only one report dealing with similar observations, an abstract of a paper by Hurxthal and Perkin(11). However, no experimental data were given in this paper and the dietary conditions employed were different. On the basis of turnover studies Rosenman *et al.* also concluded that the thyroid hormone enhances cholesterol destruction(12).

Investigators have shown that administration of thyroid hormone causes a reduction of tissue cholesterol concentrations elevated as a consequence of cholesterol feeding(1-4). The above mentioned results suggest that the mechanisms responsible for this phenomenon include reactions leading to the destruction or chemical modification of cholesterol, perhaps in addition to those stimulating the excretion of the sterol(12).

The results of the third experiment, on the other hand, were interpreted to indicate that, under conditions of *ad libitum* feeding of a diet relatively low in cholesterol, the thyroid hormone stimulated primarily endogenous

cholesterol synthesis and cholesterol excretion, in agreement with earlier observations(5-7, 12).

It is obvious, therefore, that the thyroid hormone may influence different phases of cholesterol metabolism and that the experimental conditions, in particular, the dietary regimen determine which effect predominates in a particular case.

Under the conditions of the first 2 experiments, the total caloric intake was inadequate for the requirement of a hyperthyroid animal; therefore, a condition of relative starvation or semi-starvation existed. During starvation, the rate of endogenous cholesterol synthesis is markedly reduced, as shown by Hutchens, *et al.*(13), Tomkins and Chaikoff(14) and Frederickson, *et al.*(15). Furthermore, the cholesterol intake was relatively high, and this also tended to reduce endogenous cholesterol synthesis, in line with reports by Gould and Taylor(16), Tomkins and co-workers(17) and Frantz, *et al.*(18). Thus 2 factors operated which are known to depress mechanisms responsible for cholesterol synthesis, and any effect which thyroid hormone would otherwise have exerted in this direction, was apparently inhibited by these circumstances. Therefore, the experimental conditions were favorable for a demonstration of another action of this hormone, *i.e.*, its influence on cholesterol destruction or conversion, and this effect predominated.

In the third experiment, on the other hand, the total caloric intake was more nearly adequate in the hyperthyroid group (*ad libitum* feeding), and the cholesterol intake was relatively low. Therefore, an entirely different metabolic pattern was observed, *i.e.*, the major action of the hormone was directed towards stimulating endogenous cholesterol synthesis and cholesterol excretion, and these effects predominated, obscuring any influence the hormone might have had on cholesterol destruction.

Summary. The total cholesterol balance was determined in mice fed 0.4% thyroid, in normal controls, and in mice fed 0.5% thiouracil. When a diet containing 0.65% cholesterol was offered in restricted amounts (paired feeding), the thyroid hormone stimulated destruction or chemical conversion of cholesterol. However, when a diet low in cholesterol was fed *ad libitum*, the thyroid hormone stimulated primarily the endogenous synthesis and the excretion of the sterol. The relation of the nutritional state of the animal to the mode of action of the thyroid hormone on the metabolism of cholesterol is discussed.

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