Influence of Thyroactive Compounds on Serum and Liver Cholesterol in Rats.* (26907)

DAVID KRITCHEVSKY, JANET L. MOYNIHAN AND MARVIN L. SACHS
The Wistar Institute of Anatomy and Biology and Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia

The effects of thyroid hormones on cholesterol metabolism have recently been reviewed (1) and the chemistry and physiology of these hormones have been discussed by Pitt-Rivers and Tata(2). There is now convincing evidence that compounds which affect thyroid function may exert an extrathyroidal effect on cholesterol metabolism. Cutting, Rytand and Tainter(3) demonstrated that effects on basal metabolic rate (BMR) were dissociated from effects on serum cholesterol levels; and more recently Duncan and Best (4) and Boyd(5) have shown that thiouracil exerts an effect on cholesterol metabolism in the athyreotic rat.

There have recently become available a wide variety of thyroxine analogs in which the distribution of nuclear iodine atoms or the structure of configuration of the side chain has been altered. These compounds are being tested in many laboratories to ascertain whether they exert an effect on cholesterol metabolism that is divorced from other thyroid hormone effects. Recent publications(6-10) have described the effects of representative members of this growing series of thyroxine analogs on the serum and liver lipids, heart weights and oxygen consumption of normal and cholesterol-fed rats. Boyd(11) has reviewed the more recent studies with the iodothyronines.

This report deals with the effects of 3 thyroactive compounds: 3,5,3′-triiodothyropropionic acid (Triprop), D-thyroxine (DT_4) and L-thyroxine (LT_4). Actually, this report covers 2 series of experiments, a comparison of 2 dosage levels of Triprop and a comparison of D- and L-thyroxine. The experiments were carried out simultaneously and both series were compared with the same set of controls. Most studies compare the effects of thyroactive compounds upon rats fed either normal or cholesterol-rich diets. We have studied the 3 compounds listed above in rats fed diets high in fat with and without added cholesterol and cholic acid.

Materials and methods. Groups of male Wistar rats, 100-120 g, were maintained for 3 weeks on the diets outlined in Table I. Rats were given daily subcutaneous injections of 2 concentrations of Triprop (0.03 mg/100 g or 0.15 mg/100 g body weight), DT_4 (0.05 mg/100 g body weight) and LT_4 (0.005 mg/100 g body weight. Solutions of the thyroactive compounds were prepared by suspending the compound to be used in a small amount of 95% ethanol, adjusting the pH to 10.0-10.5 with 0.1 N NaOH and diluting with distilled water so that the proper dosage could be administered in 0.10 ml. Fresh solutions were prepared each week. A water-ethanol blank, pH 10.0, was used for

TABLE I. Diets Fed to Rats on Thyroactive Drugs.

	Fat diet	Fat-cholesterol diet
Lard	25	25
Casein	20	20
Salt mix*	4	4
Dextrose	50.5	48
Choline chloride	0.5	0.5
Cholesterol†	—	2.0
Cholic acid	—	0.5

* Salt Mix #2, USP XIII (Nutritional Biochemicals Corp.): Calcium biphosphate (13.58), calcium lactate (32.70), ferric citrate (2.97), magnesium sulfate (13.70), dibasic potassium phosphate (23.98), sodium acid phosphate (8.72), sodium chloride (4.35).

† Lard contained 1% cholesterol, therefore fat diet contained approximately 0.25% cholesterol and fat-cholesterol diet about 2.25%.

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TABLE II. Effect of Thyroactive Compounds on Serum and Liver Cholesterol Levels of Rats Fed Fat or Fat-Cholesterol Diets.

	Fat diet					Fat-cholesterol diet				
Group*	No.†	Avg wt gain (g)	Avg liver wt (g)	Avg liver cholesterol (mg/g)	Avg serum cholesterol (mg %)	No.†	Avg wt gain (g)	Avg liver wt (g)	Avg liver cholesterol (mg/g)	Avg serum cholesterol (mg %)
Exp. 1										
T3P-3	4/5	58	8.79	5.22 ± .80‡	99.0 ± 7.4	5/5	34	9.67	56.0 ±14.6	197.0 ± 22.9
T3P-15	5/5	25	6.80	3.63 ± .40	110.1 ±10.9	5/5	26	7.72	43.6 ± 6.6	227.0 ± 35.4
C	5/5	54	9.07	2.96 ± .32	132.0 ±33.9	5/5	61	12.20	45.4 ± 3.6	185.0 ± 20.3
Exp. 2										
T3P-3	3/5	44	10.82	1.72 ± .55	54.8 ± 3.0	4/5	42	10.20	16.3 ± 3.2	80.9 ± 3.3
T3P-15	4/5	13	7.73	3.12 ± .34	51.7 ± 1.0	2/5	14	7.83	21.1	68.6
C	3/5	80	8.68	4.24 ±1.32	68.3 ± 3.4	4/5	46	9.68	29.2 ± 4.6	128.0 ± 8.9
DT4	5/5	−4	6.55	3.10 ± .14	64.1 ± 3.5	3/5	29	8.80	14.4 ± 1.6	78.3 ± 3.7
LT4	3/5	2	7.62	4.10 ± .24	60.8 ± .5	3/5	12	7.51	34.6 ± 1.6	78.2 ± 5.0
Exp. 3										
T3P-3	9/10	− 6	5.27	Lost	83.2 ± 6.8	9/10	—	6.85	Lost	178.7 ± 20.4
T3P-15	7/10	−20	5.53	"	99.1 ± 9.7	6/10	−23	6.74	"	168.0 ± 12.8
C	10/10	22	5.61	"	70.6 ± 4.5	10/10	14	7.47	"	368.5 ± 26.2
DT4	9/10	− 8	5.10	"	82.0 ± 7.6	9/10	−15	6.66	"	157.2 ± 14.5
LT4	8/10	−11	5.07	"	79.1 ± 4.0	10/10	− 1	7.10	"	110.3 ± 11.4
Exp. 4										
T3P-3	10/10	113	11.41	17.90 ±1.7	141.9 ±12.5	10/10	114	13.27	50.1 ± 2.9	184.9 ± 19.8
T3P-15	9/10	57	8.59	7.89 ±1.2	152.7 ±11.2	10/10	66	11.30	58.7 ± 2.1	161.0 ± 18.2
C	9/10	109	12.29	12.80 ±1.4	126.7 ±16.3	10/10	114	13.00	35.4 ± 3.5	206.1 ± 24.2
DT4	10/10	105	12.24	7.23 ±1.9	126.1 ± 7.9	10/10	116	13.31	34.1 ± 3.0	203.7 ± 12.6
LT4	10/10	113	11.13	7.33 ±1.0	143.1 ± 7.8	9/10	114	13.32	33.1 ± 3.4	176.5 ± 12.9
Exp. 5										
T3P-3	3/6	65	9.05	3.97 ± .23	70.0 ± 3.6	6/6	24	11.55	36.2 ± 7.7	69.0 ± 9.2
T3P-15	4/6	13	7.72	4.55 ± .47	65.3 ± 5.3	3/6	−23	8.07	32.9 ± 4.9	128.0 ± 21.9
C	6/6	42	9.43	4.75 ± .34	56.0 ± 5.4	4/6	31	12.40	37.7 ± 2.5	79.5 ± 7.7
DT4	6/6	26	7.75	4.44 ± .40	52.0 ± 9.9	5/6	7	9.79	21.0 ± 6.6	60.0 ± 11.6
LT4	6/6	21	8.32	10.00 ±3.01	49.0 ± 4.3	6/6	18	11.24	36.4 ± 7.4	53.6 ± 13.7

* T3P-3 — Triprop, .03 mg/100 g/day
T3P-15— Triprop, .15 mg/100 g/day
DT4 — D-Thyroxine, .05 mg/100 g/day
LT4 — L-Thyroxine, .005 mg/100 g/day
C — Control

† No. survivors/No. starters

‡ Stand. error

the control rats. After 3 weeks the rats were killed and their blood taken for analysis. Livers were weighed and homogenized in chloroform-methanol 2:1. The liver extract was dried over sodium sulfate and an aliquot used for cholesterol assay. Cholesterol analyses were carried out by the Trinder method (12). Five separate experiments were carried out.

Results and discussion. The results are summarized in Table II. The 5 experiments were carried out during April, July, December, June and October respectively, of a 2-year period. The livers of all the animals in the third experiment were lost in a laboratory accident.

In the experiments involving the high fat diet, the thyroactive compounds had little consistent effect on serum or liver cholesterol values. This was true for the 2 Triprop groups as well as for those on D- or L-thyroxine. On the high fat-high cholesterol diet, administration of Triprop generally lowered serum cholesterol levels; liver cholesterols were also lower in these groups than in the controls. The 2 thyroxine treated groups also showed generally lower serum and liver cholesterol levels than were observed in the controls. At the dosages administered serum cholesterol levels were lower in the L-thyroxine group while liver cholesterol levels were lower in the D-thyroxine groups.

The thyroid state of the rat does not appear to affect total body cholesterol(13,14). We attempted to calculate the total serum-liver cholesterol pool to ascertain if this value might not offer more evidence concerning the effects of the various treatments we were studying. Total liver cholesterol was calculated by multiplying the average liver weights by average liver cholesterol values. The serum cholesterol pool was calculated on the assumption that the average blood volume of the rat is 6% of his body weight(15). Assuming 50% of the blood to be serum, this volume, usually between 4 and 6 ml, was multiplied by the serum cholesterol concentration (mg/ml). The serum cholesterol contribution to the liver-serum cholesterol pool is almost negligible for the groups fed the fat-cholesterol diet, varying between 0.5% and 2% of the pool. Average values are summarized in Table III. There was no consistency in these values. When liver weight and body weight are considered, as they are in these calculations, lower serum or even liver concentrations do not always reflect lower serum-liver cholesterol content.

TABLE III. Average Liver-Serum Cholesterol Pool Size (mg) of Rats Fed Fat and Fat-Cholesterol Diets and Treated with Thyroactive Compounds.

Group*	Fat diet	Fat-cholesterol diet
Exp. 1		
T3P-3	34.1	551.4
T3P-15	29.6	347.8
C	34.1	564.1
Exp. 2		
T3P-3	21.6	169.8
T3P-15	26.4	167.8
C	41.1	288.8
DT4	23.0	130.5
LT4	34.0	264.2
Exp. 4		
T3P-3	214.2	677.8
T3P-15	76.2	672.2
C	151.4	474.6
DT4	97.7	468.2
LT4	99.7	453.3
Exp. 5		
T3P-3	40.5	421.9
T3P-15	38.4	270.0
C	47.9	471.9
DT4	37.0	208.3
LT4	85.7	411.8

* See legends—Table I.

Ruegamer(7) found that in rats fed a high fat diet, Triprop treatment tended to lower serum cholesterol values but neither Triprop (0.03 mg/100 g) or L-thyroxine (0.04 mg/100 g) significantly altered liver cholesterol levels. Duncan and Best(6) found that L-thyroxine (0.0003 mg/100 g) caused a slight increase in serum cholesterol levels and a slight drop in total liver cholesterol; neither were significant. In a longer term experiment, the feeding of L-thyroxine (0.0009 mg/100 g or 0.0006 mg/100 g) lowered serum cholesterol levels while raising liver cholesterol content.

Our experiments indicate that thyroactive drugs can affect serum and liver cholesterol levels in rats fed high fat or high fat-high cholesterol diets. It is of importance to consider the total body cholesterol content of

these animals. Clearly, there are no standard data in the literature regarding the effects of thyroactive compounds on cholesterol metabolism, since dosages used, routes, and duration of administration have varied from study to study.

Summary. In a series of 5 separate experiments, rats were fed high fat and high fat-high cholesterol diets and were treated with various thyroactive compounds. Two dosages of 3,5,3′-triiodothyropropionic acid (Triprop) (0.03 mg and 0.15 mg/100 g body weight) were administered subcutaneously and, in general, were found to lower serum and liver cholesterol levels when compared with controls. Administration of D-thyroxine (0.05 mg/100 g body weight) and L-thyroxine (0.005 mg/100 g) also lowered serum and liver cholesterol levels. Among individual experiments there was a variation in magnitude, and in a few instances in the direction of effect.

1. Kritchevsky, D., *Metabolism,* 1960, v9, 984.

2. Pitt-Rivers, R., Tata, J. R., *The Thyroid Hormones,* Pergamon Press, London, 1959.

3. Cutting, W. C., Rytand, D. A., Tainter, M. L., *J. Clin. Invest.,* 1934, v13, 547.

4. Duncan, C. H., Best, M. M., *Am. J. Physiol.,* 1958, v194, 351.

5. Boyd, G. S., in Pincus, G., *Hormones and Atherosclerosis,* Academic Press, Inc., New York, 1959, p49.

6. Duncan, C. H., Best, M. M., *Endocrinology,* 1958, v63, 169.

7. Ruegamer, W. R., Alpert, M. E., Silverman, F. R., *ibid.,* 1960, v66, 160.

8. Boyd, G. S., Oliver, M. F., *J. Endocrinol.,* 1960, v21, 25.

9. Cuthbertson, W. F. J., Elcoate, P. V., Ireland, D. M., Mills, D. C. B., Shearley, P., *ibid.,* 1960, v21, 45.

10. Best, M. M., Duncan, C. H., *Am. J. Physiol.,* 1960, v199, 1000.

11. Boyd, G. S., *J. Atherosclerosis Res.,* 1961, v1, 26.

12. Trinder, P., *Analyst,* 1952, v77, 321.

13. Vignes, H., Glomaud, G., *Compt. rend. Soc. biol.,* 1938, v127, 585.

14. Fleischmann, W. B., Shumacker, H. B., Jr., *Bull. Johns Hopkins Hosp.,* 1942, v71, 175.

15. D'Amour, F. E., Blood, F., *Manual for Laboratory Work in Mammalian Physiology,* Experiment 3, Univ. Chicago Press.

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Decline in Rate of Cholesterol Synthesis During Maturation of Chicken Aorta.* (26908)

SEYMOUR DAYTON† (Introduced by Morton I. Grossman)

Medical Service, Veterans Administration Center, and Department of Medicine, University of California, Los Angeles

While most of the cholesterol of the normal artery wall appears to arrive there by transfer from plasma, there has been reason to believe that local synthesis may contribute significantly(1). The experiments described here indicate, among other things, that cholesterol synthesis by avian aortic tissue falls off rapidly as the animal matures.

Materials and methods. White Leghorn cockerels, maintained on a commercial feed, were sacrificed by exsanguination. Aortas were removed, immersed in chilled buffer, freed of clots and connective tissue, and opened longitudinally. In some experiments the aorta was cut into thoracic and abdominal segments, and each of these was split longitudinally into 2 portions, one for incubation with acetate as substrate and the other with mevalonate. Each artery or piece of artery was weighed and placed into an incubation vessel containing 3.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 0.1 ml of 5% dextrose, and 0.1 ml of either sodium acetate-1-

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