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Effects of L- and D-Thyroxine on Cholesterol Synthesis and Turnover in the Chick.* (28956)

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The recent interest in cholesterol-lowering agents and the well-known alterations in serum cholesterol with L-thyroxine have stimulated a search for a calorigenically less active analog with similar effects on blood cholesterol levels. It has been postulated that L-thyroxine stimulates hepatic cholesterol synthesis and also the rates of biliary excretion and/or degradation, the net result being a reduction in serum level(1). D-thyroxine, by various animal assays estimated to be about 20% as active physiologically as the L-form (2), has been reported to have liver and serum cholesterol-lowering effects in the rat (1), produces marked reduction of liver fat in the chick(3), and has minimal effect on basal metabolic rate or on the electrocardiogram in myxedematous patients(4). Acetate carbons have been shown to be utilized in the biosynthesis of cholesterol(1). The liver, being the major site of cholesterol synthesis (5), has been the tissue studied by various investigators of mammalian cholesterol synthesis. The present studies were undertaken to compare the pharmacological effects of L- and D-thyroxine on rate of cholesterol synthesis from acetate-2-C^{14} both *in vivo* and *in vitro* and the disappearance of cholesterol-4-C^{14} in radioiodine thyroidectomized chicks.

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Materials and methods. Day-old White Leghorn cockerels were divided into 2 groups of 24 animals each and were fed chick grower ration.† Control and experimental groups received this ration throughout the experimental period. Birds were housed in individual cages with feed and water *ad libitum*. The chicks of the experimental group received radioiodine (350 μc) at the end of the first and third week to ablate the thyroid. Prior to each radioiodine dose the chicks were injected intraperitoneally daily for 5 days, with 0.1 unit of beef thyrotropin (TSH)‡ to increase thyroidal uptake. Thyroidal uptake (48 hours) ranged from 12 to 25% after the first I^{131} dose, while after the second dose, uptake was 1 to 7%. At 6 weeks of age the chicks were judged to be thyroidectomized by the lack of comb growth, rise in serum cholesterol, and lack of thyroid tissue in the neck in a few sacrificed at this time. The serum protein-bound iodine level dropped from a range of 1 to 1.5 μg per 100 ml to 0.6 to 1.0, and the serum cholesterol levels had risen from a range of 150-175 mg per 100 ml serum to 200-235 mg.

† The chick grower ration contained 5% hydrolyzed animal fats, 23% protein; with additional iodide salts omitted, iodine content averaged 1 mg per kg.

‡ Thytropar (Armour).

The thyroidectomized chickens at 6 weeks of age were divided into 3 groups as follows:

THX, basal diet without thyroxine supplement

THX-L, basal diet supplemented with 4 mg of Na-L-thyroxine§ pentahydrate per kg of diet

THX-D, basal diet supplemented with 20 mg of Na-D-thyroxine§ pentahydrate per kg of diet

In addition there was a group, *EU*, euthyroid controls, untreated chickens on basal diet.

The various studies to be described were performed at 9-11 weeks of age.

(a) *Studies on cholesterol synthesis from acetate-2-C^{14} in intact chicks.* Three chicks from each group (THX, THX-L, THX-D and EU) were injected intravenously with acetate-2-C^{14} (50 μc/kg body wt) and blood samples were removed by cardiac puncture at 1, 2 and 4 hours. Serum cholesterol, extracted with acetone-absolute ethanol (1:1, using 9 ml of solvent per ml of serum was determined on the centrifuged extract(6). Cholesterol was precipitated as a digitonide (7) and the precipitate was washed with ethanol, ether, and suspended in water, plated and counted.

(b) *Studies with liver slices.* Liver slices were prepared as described previously(8,9), using 3 chicks from 4 different treatment groups; slices in duplicate, were incubated in a Krebs-Ringer bicarbonate medium equilibrated with 95% O_2 and 5% CO_2. Acetate-2-C^{14} was added to the medium to give an initial concentration of 1 mg/ml. Approximately 1.0 g of wet liver slices, weighed on a Roller Smith torsion balance, was incubated in 6 ml of medium containing from 4 to 4.5 $\times$ 10^6 cpm of labeled substrate. After 90 minutes' incubation tissues were analyzed for C^{14} activity in cholesterol. Cholesterol was extracted from the tissue slices after hydrolyzing the tissue with alcoholic sodium hydroxide and then extracting with ethyl ether. Carrier cholesterol was added and the digitonide complex was precipitated, washed, and counted.

(c) *Studies with cholesterol-4-C^{14}.* Three chicks from each group were injected with 3.89 μc of cholesterol-4-C^{14}. Blood samples were removed by cardiac puncture at 1, 2, 6 and 26 hours. Labeled serum cholesterol was measured in 0.1 ml serum dried on a planchet without isolation and calculated as counts per minute per ml.

(d) *Radioactivity measurement.* Radioactivity was assayed with a Tracerlab proportional gas flow counter which was calibrated with 6 mg of purified cholesterol-digitonide on a planchet. The results were corrected for background. The counting time was such as to keep counting statistics to a 1% probable error.

(e) *Blood chemistries.* Serum and liver cholesterol was determined by the Searcy method(6); the serum protein-bound iodine (PBI), by chloric acid digestion technique (10) with brucine end point stabilization (11).

(f) *Statistical analysis.* Significance of differences between 2 trends was evaluated by the F-test(12); differences were considered significant at $p<0.05$ level by Tukey studentized range test(12).

Results. The effect of L- and D-thyroxine on various parameters observed at 10 weeks of age is summarized in Table I. No differences in weight gain were observed in thyroidectomized animals treated with L- or D-thyroxine. However, thyroidectomized and treated groups showed a slow growth rate when compared to the euthyroid group. Liver weights and liver cholesterol levels were unchanged in these groups; but serum cholesterol levels were significantly higher in thyroidectomized as compared to normal animals. Administration of L- or D-thyroxine lowered serum cholesterol levels to normal. PBI levels were low in the EU, as previously reported(13), and in THX, but became elevated with both L- and D-thyroxine. This is of interest in view of the reported lack of

§ Both Na-L- and Na-D-thyroxine (pentahydrate) were generously supplied by Baxter Laboratories, Morton Grove, Ill. Estimating daily food intake during the study period to average 64 g (range 49-79), average daily dose of Na-L-thyroxine was calculated as 235 μg, for Na-D-thyroxine 1165 μg, or 23.5 and 116.5 μg per 100 g body weight, respectively.

TABLE I. Effect of L- and D-Thyroxine on Liver Weight, Body Weight, Cholesterol, and PBI Levels in the Chick.

Group	Treatment	Body wt gain (g)	Liver		Serum	
			Wt (g)	Cholesterol (mg/100 g)	PBI (μg/100 ml)	Cholesterol (mg/100 ml)
Thyroidectomy*	None	376 ± 45 (11)	21.8 ± 7.5 (7)	361 ± 62† (3)	.6 ± .3 (10)	228 ± 40‡ (14)
" *	L-Th_4	367 ± 59 (10)	19.9 ± 4.8 (7)	275 ± 45† (6)	9.2 ±5.6 (10)	151 ± 22 (11)
" *	D-Th_4	346 ± 54 (9)	22.9 ± 4.9 (6)	256 ± 51† (4)	26 ±8 (10)	152 ± 23 (11)
Euthyroid	None	509 ± 73 (10)	22.6 ± 6.3 (7)	329 ± 49† (5)	.9 ± .1 (8)	155 ± 25 (15)

These data were obtained in 10-wk-old chicks which had been observed or were on treatment for 4 wk. Mean values ± standard deviation; No. of animals for the various observations in parentheses.

* Radiothyroidectomy with I^{131}.

† Differences between groups not significant.

‡ Differences from other groups significant, $p < .001$.

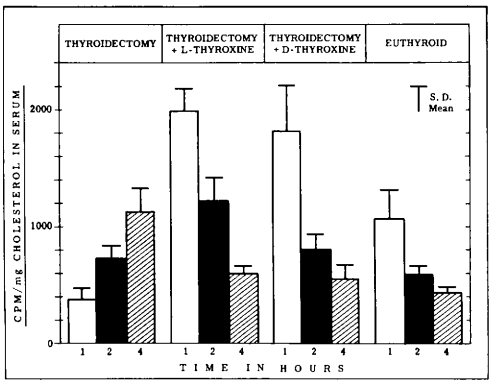
specific serum thyroxine carrier proteins in chicks as compared to man, with albumin being the major site of binding in the chick (14). The short biological half-life of the injected L-thyroxine resulting in the decreased binding by serum proteins in the chick(14) may be the cause for the low PBI in this species as compared to mammals.

Fig. 1 summarizes the data on incorporation of acetate-2-C^{14} into cholesterol *in vivo*. Radioactivity in serum cholesterol increased with time in the thyroidectomy group whereas in normal and in THX-L and THX-D groups peak radioactivity was reached in the first hour with a gradual decline over the next 3 hours. At one hour, labelled cholesterol levels were highest in THX-L and THX-D animals, and these were significantly different from the other groups. The relative trends from 1 to 4 hours were not significantly different in EU, THX-L, or THX-D. At 4 hours the blood levels of cholesterol-C^{14} synthesized in untreated thyroidectomized chickens were higher than in other groups.

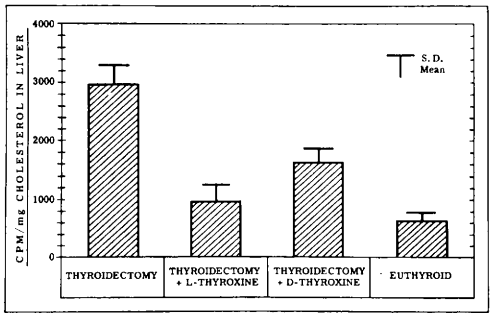
Data on incorporation of acetate-2-C^{14} into cholesterol by liver slices are shown in Fig. 2. Liver slices from thyroidectomized chicks, after 90 minutes' incubation, contained more radioactivity as cholesterol when compared to normal groups. The specific activity of cholesterol in the THX-L and EU group was not significantly different. However, in the THX-D treated group the specific activity was greater than in THX-L and EU, but lower than in THX. No differences were observed in the supernatant liquid.

The rate of disappearance of labeled cholesterol from serum is shown in Fig. 3. Thyroidectomized animals showed slower disappearance rates of cholesterol-C^{14} from the blood stream; no significant trend differences were observed between EU, THX-L or THX-D animals although the disappearance rates of these 3 groups were more rapid than THX.

Discussion. The data presented are consistent with the thesis that L-thyroxine and D-thyroxine in the thyroidectomized chick stimulate liver cholesterol synthesis, release, and peripheral turnover in a manner comparable to that found in the euthyroid animal. The incorporation of acetate-2-C^{14} into serum cholesterol when administered *in vivo* indicates a rapid liver synthesis and release as well as peripheral turnover when either agent is used. The higher than normal specific activities at 1 hour with both L- and D-thyroxine may indicate that the quantities administered were in a pharmacological dose range. The lack of weight gain in the iodoamino acid groups as well as decreased testicular size and comb growth, coupled with the elevated PBI levels indicate that the dose of the agents administered was quite high. Since no oxygen consumption studies were performed it is not clear whether a hyperthyroid state existed. The *in vitro* studies with liver slices further support the view that



(1)



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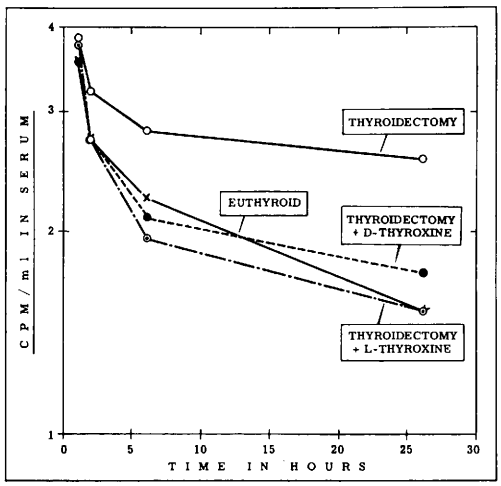

(3)

FIG. 1. Specific activity of serum cholesterol after injection of acetate-2-C^{14} in chicks over 4 hr period (mean values for 3 animals per group). The thyroidectomized treated groups (THX-L and THX-D) are not significantly different at 1 hour at the 0.05 level (difference 145 or more in specific activity had to be present for the significance), while other groups were significantly different. The lower specific activity of THX group may indicate some dilution of radioactivity in a larger cholesterol pool. Relative trends, i.e., proportional losses from 1 to 2 to 4 hours, do not differ significantly for THX-L, THX-D, and EU, but are obviously different between THX and EU.

FIG. 2. Incorporation of acetate-2-C^{14} into cholesterol by liver slices *in vitro* (90 min incubation, mean values for animals per group).

FIG. 3. Serum radioactivity over 26 hr after injection of cholesterol-4-C^{14} (mean of 3 animals per group). Trends of radioactivity do not differ significantly in EU, THX-L or THX-D, but THX is significantly different from THX-L at $p<0.05$ level.

these agents stimulated synthesis and release. The high specific activity of liver cholesterol in the thyroidectomized chicks and its progressive rise in serum after *in vivo* injection of acetate-2-C^{14}, indicated a reduced release rate which was accelerated by both iodoamino acids.

Slower disappearance of the administered labeled cholesterol was noted in the thyroidectomized group, like that demonstrated in myxedema patients(15) and hypothyroid mice(16). Both L- and D-thyroxine were effective in causing these disappearance rates to return to normal.

Some variability in effects of thyroid states and medications in various species was noted (1).

The recently accepted mode of action of thyroxine and its analogs pinpoints the site in cholesterol metabolism at biliary excretion and/or degradation. Calculations of bile acid synthesis and pools in the intact rat indicate that when D- and L-triiodothyronine are administered the total biliary acid synthesis increases as a result of increased chenodeoxycholic acid synthesis(17). This occurs without alteration in cholic acid. The effect of the D-isomer is produced with negligible calorigenic action.

Comparison of the effects of the levo and dextro isomers cannot be readily utilized to explain the mode of action of these thyroactive compounds on cholesterol synthesis in man since chicken serum lacks a specific thyroxine binding carrier(14); in man competitive binding by thyroxine analogs for serum thyroxine protein carrier sites has been postulated as the mode of action of these agents (Ballantine and Oliner, unpublished observations). However, these studies with thyroid-

ectomized chicks indicate that the D-isomer has a similar action on cholesterol synthesis and turnover as does L-thyroxine.

Summary. The effect of L- and D-thyroxine on cholesterol synthesis and turnover in thyroidectomized and in normal untreated chicks has been compared using acetate-2-C^{14} and cholesterol-4-C^{14}. (a). Although liver weights and liver cholesterol levels were not significantly different between the normal (EU) and iodoamino acid treated thyroidectomized chicks and those thyroidectomized without treatment, serum cholesterol levels were elevated only in the last group. (b). Appearance of labeled serum cholesterol after acetate-2-C^{14} injection, cholesterol synthesis after incubation of liver slices with acetate-2-C^{14}, and cholesterol-4-C^{14} turnover in these various groups indicate that both L-thyroxine and D-thyroxine in the quantities used can stimulate cholesterol biosynthesis and turnover in a manner comparable to that found in the normal. (c). The data are discussed in the light of present concepts of the mode of action of thyroid substances on cholesterol metabolism, and the applicability of the findings to mammalian species.

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Variables Influencing Uptake of Radioactive Triiodothyronine from Plasma by Erythrocytes of Cattle.* (28957)

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The *in vitro* uptake of tracer amounts of radioactive triiodothyronine (T_3-I^{131}) from plasma by red blood cells (RBC) has been suggested as a simple and rapid method for evaluating thyroid function in humans(1). This procedure has been shown to possess diagnostic accuracy in humans comparable to other standard tests such as: a) basal metabolic rate, b) chemical protein bound iodine, and c) thyroid I^{131} uptake(2-5). Many stud-

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