It has been demonstrated that thyroid hormone and its analogues reduce plasma cholesterol levels both in hypo- and in eu-thyroid individuals, and also that D-thyroxine is much less toxic and less calorigenic than L-thyroxine. Katz and Pick reported that thyroid hormone given to chicks on an atherogenic diet, either as desiccated thyroid or thyroxine, suppressed hypercholesterolemia, but the effects on atherogenesis were inconsistent. Although numerous reports describe the effect of D-thyroxine on plasma cholesterol levels, there are at this time few studies of its effect on atherosclerosis.

The present report is an attempt to evaluate the effects of both L- and D-thyroxine on atherogenesis as well as on cholesterolemia in cockerels fed a cholesterol-oil supplemented diet.

**Methods**

Three series (S63A, 68, 71) of experiments were done involving a total of five groups and 180 birds. The established procedures of our department for experiments on chronic atherosclerosis in chickens were used throughout. All the cockerels were from a Hy-line hybrid strain obtained at one day of age from a commercial hatchery. They were raised in a battery brooder on commercial chick starter mash (protein content 20%) until they were 11 to 12 weeks of age when the experimental regimens were started. For the next five weeks all groups were fed the same atherogenic diet, consisting of the addition of 1% cholesterol and 5% cottonseed oil to the mash, plus the various types and amounts of the thyroid analogues detailed below. All the cockerels were killed at the end of this five-week experimental period.

Experiment 1 compared the effects of L- and D-thyroxine. The cockerels were divided into three groups in all three series: Group 1, the control group, received no medication. Group 2 received 1 mg of L-thyroxine daily five days a week for five weeks. Group 3 received 1 mg of D-thyroxine on the same schedule.

Experiment 2 evaluated the effects of dosage of D-thyroxine. In addition to a control group (group I), three other groups were given D-thyroxine in daily doses (of graded magnitude) five days per week for five weeks as follows: group II, 1 mg; group III, 2 mg; and group IV, 4 mg.

The commercial L- and D-thyroxine used were prepared for intramuscular injection in strengths of 1, 2, and 4 mg per ml by dissolving 100, 200, and 400 mg, respectively, in 100 ml of 0.9% NaCl. The pH of the thyroxine solution was then adjusted by adding concentrated NaOH solution to pH 11.0, at which point the solution cleared. Concentrated H2SO4 was then added to bring the pH back to 8.0 and the preparation was refrigerated. Each solution was used for a period of no more than five days. Fresh solutions were prepared every five days.

In addition to concentration of total plasma cholesterol, gross atherosclerosis of the thoracic aorta and microscopic atherosclerotic involvement of the coronary arteries, the other parameters studied were body weight, food intake, comb index (height x width of the comb), and thyroid and testes weights. Each bird was weighed at the start and at two and five weeks of the experiment.

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experimental period. Food intake was determined weekly for each group. Plasma cholesterol levels were determined by the method of Sperry and Webb.

All birds were autopsied at the end of the experimental period. Aortic atherosclerosis was evaluated according to established methods of this department by grading from 0 (no lesion) to 4+ (most severe). The extent of coronary atherosclerosis was determined by examining one frozen section stained with Sudan IV from each of 2 blocks obtained from each heart and then calculating the percentage of atherosclerotic arteries and arterioles.

Results

The food intake was slightly higher than in the controls in groups on L- and D-thyroxine in experiment 1 (table 1) and was comparable to the controls in all groups on D-thyroxine in experiment 2 (table 2). The amount of weight gain was reduced by 1 mg of L-thyroxine but was less affected by 1 mg of D-thyroxine; it was decreased progressively with doses of D-thyroxine above 1 mg. The cockerels gained less weight on 4-mg doses of D-thyroxine than on 1-mg doses of L-thyroxine.

Both L- and D-thyroxine decreased thyroid weight compared to controls. The effect of L-thyroxine was more pronounced than that of D-thyroxine. No further decrease in thyroid weight was noted with increased dosage of D-thyroxine above the 1 mg level.

The testes weighed least in the L-thyroxine group, the next lowest weight being in the 4-mg D-thyroxine group. The weight of testes became progressively greater as the dose of D-thyroxine decreased. The comb index in the various groups paralleled the change in testes weights.

The effects on plasma cholesterol levels and atherosclerosis are shown in tables 1 and 2. As table 2 shows, the average concentration of plasma cholesterol was 707 mg% (se ± 38) in the 58 birds studied. Both L- and D-thyroxine in 1-mg doses in experiment 1 (table 1). However, in experiment 2, no significant effect of D-thyroxine was seen on thoracic aorta atherosclerosis regardless of the dose used (table 2). This variability of results between the two experiments confirms that seen in previous studies with thyroid hormones.

Coronary atherosclerosis was not suppressed significantly by L- or D-thyroxine at any dose level used, despite the significant lowering of plasma cholesterol concentrations. In fact, chicks treated with 4 mg D-thyroxine had the highest score of coronary vessels with lesions.

The possibility exists that the concentrations of plasma cholesterol are so high as to obscure an effect on coronary atherosclerosis which might be attained at lower cholesterol levels. In our experience, the critical point at which this might be expected in the chicken is around 800 mg%. Therefore, this lack of effect cannot be attributed to the levels of plasma cholesterol in the treated and untreated birds. In unpublished results, we have found that, aside from estrogens, there is a fair correlation between plasma cholesterol level attained at the end of a five-week period in the chicken and the degree of coronary atherosclerosis. However, this relationship shows a curvilinear trend. Equal increments of plasma cholesterol are less effective at higher than at lower concentrations. Thus, a difference of 100 mg% produces a greater effect on atherogenesis in the 200 to 800 range than in ranges over 800 mg%.

Discussion

The failure of thyroid and of D- and L-thyroxine to protect against diet-induced coronary atherosclerosis in the chick, reported here or in previous studies, despite some suppression of hypercholesterolemia, is significant because of its clinical implications. In rabbits, Kritchevsky noted that D- and L-thyroxine exerted a hypocholesterolemic effect on cholesterol-fed rabbits and also
TABLE 1

Effect of L- and D-Thyroxine in 1-mg Doses on Cockerels Fed on Atherogenic Diet* (S63A, 68, 71)

<table>
<thead>
<tr>
<th>Group</th>
<th>Medication</th>
<th>Number of birds in group</th>
<th>Food intake</th>
<th>Gain in body weight</th>
<th>Thyroid Weights</th>
<th>Testes</th>
<th>Comb index</th>
<th>Plasma cholesterol</th>
<th>Thoracic aorta atherosclerosis</th>
<th>Coronary arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no.</td>
<td>g/day</td>
<td>g</td>
<td>0.155 ± 0.006</td>
<td>6.35 ± 0.65</td>
<td>73.3 ± 3.5</td>
<td>707 ± 38</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>58</td>
<td>97</td>
<td>477 ± 21†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>L-thyroxine, 1 mg†</td>
<td>29</td>
<td>125</td>
<td>275 ± 46</td>
<td>0.045 ± 0.006</td>
<td>0.69 ± 0.19</td>
<td>39.4 ± 3.1</td>
<td>451 ± 52</td>
<td>86</td>
<td>0.70 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>D-thyroxine, 1 mg†</td>
<td>54</td>
<td>103</td>
<td>399 ± 15</td>
<td>0.068 ± 0.006</td>
<td>3.74 ± 0.54</td>
<td>62.8 ± 3.8</td>
<td>542 ± 38</td>
<td>89</td>
<td>0.84 ± 0.07</td>
</tr>
</tbody>
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P values

<table>
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<tr>
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<th>&lt;.001</th>
<th>&lt;.001</th>
<th>&lt;.001</th>
<th>&lt;.01</th>
<th>ns§</th>
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<tbody>
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<td>1 vs. 3</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
<td>&lt;.01</td>
<td>ns</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

*All chicks received chick starter mash supplemented with 1% cholesterol and 5% cottonseed oil.
†± standard error of the mean.
‡Injected intramuscularly daily 5 days/week for 5 weeks.
§ns: not significant.
### TABLE 2

**Effect of Various Dose Levels of \( \text{d-Thyroxine} \) on Cockerels Fed an Atherogenic Diet* (S71)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Medication</th>
<th>Number of birds in group</th>
<th>Food intake</th>
<th>Gain in body weight</th>
<th>Weights</th>
<th>Combs index</th>
<th>Plasma cholesterol mg %</th>
<th>% with lesions</th>
<th>Average grade</th>
<th>Per cent of birds with lesions</th>
<th>Per cent incidence</th>
<th>Per cent of positive vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>19†</td>
<td>91</td>
<td>452 ± 22‡</td>
<td>0.169 ± 0.008</td>
<td>5.66 ± 0.95</td>
<td>72.4 ± 3.2</td>
<td>832 ± 64</td>
<td>100</td>
<td>1.00 ± 0.09</td>
<td>100</td>
<td>22.0 ± 2.0</td>
</tr>
<tr>
<td>II</td>
<td>( \text{d-Thyroxine, 1 mg} )</td>
<td>20†</td>
<td>88</td>
<td>470 ± 17</td>
<td>0.071 ± 0.006</td>
<td>5.59 ± 0.81</td>
<td>73.6 ± 3.2</td>
<td>643 ± 69</td>
<td>95</td>
<td>0.91 ± 0.12</td>
<td>100</td>
<td>23.5 ± 2.2</td>
</tr>
<tr>
<td>III</td>
<td>( \text{d-Thyroxine, 1 mg} )</td>
<td>20</td>
<td>95</td>
<td>392 ± 20</td>
<td>0.071 ± 0.004</td>
<td>3.00 ± 0.55</td>
<td>69.7 ± 1.9</td>
<td>498 ± 45</td>
<td>95</td>
<td>0.99 ± 0.12</td>
<td>100</td>
<td>22.8 ± 2.3</td>
</tr>
<tr>
<td>IV</td>
<td>( \text{d-Thyroxine, 1 mg} )</td>
<td>19</td>
<td>95</td>
<td>202 ± 22</td>
<td>0.067 ± 0.006</td>
<td>1.23 ± 0.31</td>
<td>53.3 ± 3.9</td>
<td>535 ± 51</td>
<td>100</td>
<td>0.99 ± 0.13</td>
<td>100</td>
<td>27.3 ± 3.0</td>
</tr>
</tbody>
</table>

* \( P \) values

<table>
<thead>
<tr>
<th>Group</th>
<th>Medication</th>
<th>1 vs. II</th>
<th>ns**</th>
<th>&lt;.001</th>
<th>ns</th>
<th>ns</th>
<th>ns</th>
<th>ns</th>
<th>ns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 vs. III</td>
<td>0.01</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**All chicks received chick starter mash supplemented with 1% cholesterol and 5% cottonseed oil.**

†These birds are mentioned in table 1.

§Injected intramuscularly daily 5 days/week for 5 weeks.

‡± standard error of the mean.

**ns: not significant.**
tended to reduce the degree of aortic atheromatosis produced, but observations on coronary atherosclerosis were not reported in Kritchevsky’s paper. None of the thyro-active compounds fed by him to hypercholesterolemic rabbits, after cessation of cholesterol feeding, caused regression of atheromatosis; actually, an increase in the grade of atherosclerosis was observed. In the chicken, the same lack of regression of atherosclerosis with thyroid hormones was noted by Stamler et al.\textsuperscript{1,10} The present study fails to reveal any definite advantage of the D-isomer of thyroxine over the natural hormone in preventing atherogenesis.

This discrepancy in the effects of thyro-active compounds on plasma cholesterol levels and atherosclerosis may be due to an additional action of thyroid hormone which leads to vascular damage, and, therefore, predisposes the vessel to increased atheroma formation at mild hypercholesterolemic levels.\textsuperscript{11} This mechanism is also suggested by Lorenzen’s work on L- and D-thyroxine effects in rabbits.\textsuperscript{12-14} He demonstrated that L-thyroxine can induce alterations in the mucopolysaccharides of the aortic wall in rabbits\textsuperscript{13} and intensify epinephrine-induced alterations.\textsuperscript{12} D-Thyroxine had a similar effect on the aortic wall.\textsuperscript{14} On the basis of his histochemical data, Lorenzen postulated that this damaging effect on the arterial wall by D- and L-thyroxine was a direct peripheral action.\textsuperscript{14} Suppression of serum cholesterol levels is not always a reliable index of decreased atherogenesis particularly when dealing with hormones. Direct observation of blood vessel alterations is the only valid approach to the study of atherosclerosis.

**Summary**

In cockerels, L-thyroxine was significantly more active biologically than an equivalent dose of D-thyroxine, or even four times the dose, as demonstrated by the effects on body weights, organ weights and comb index. In cockerels fed an atherogenic diet, the plasma cholesterol concentrations in all the thyroxine-treated groups were significantly lower than in the control groups.

No consistent effect of D-thyroxine on thoracic aorta atherosclerosis was observed. However, L-thyroxine had some protective action in the one experiment done.

Despite suppression of hypercholesterolemia, no protection against coronary atherosclerosis was seen with the administration of either L- or D-thyroxine. In fact, with an increase in dose of D-thyroxine to 4 mg, there developed actually an increase in the percentage of coronary vessels showing atherosclerosis.

These observations confirm once again the importance of avoiding reliance exclusively on blood cholesterol levels when judging the effects of hormones on atherogenesis.

**Acknowledgment**

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Failure of D- and L-Thyroxine to Protect Cholesterol and Oil Fed Cockerels Against Coronary Atherogenesis

Savitri Jain, Ruth Pick, Philip J. Johnson and Louis N. Katz

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