Estrogen-Induced Regression of Coronary Atherosclerosis in Cholesterol-Fed Chicks

By Ruth Pick, M.D., Jeremiah Stamler, M.D., Simon Rodbard, M.D., Ph.D., and Louis N. Katz, M.D.

The present study demonstrates that estrogens reverse previously induced coronary atherosclerosis in cholesterol-fed cockerels, despite the continued presence of the atherogenic stimulus in the diet. Aorta atherogenesis remains unaffected. Hypercholesterolemia is moderately enhanced by estrogen administration; total cholesterol–lipid phosphorus (C/P) ratios are depressed to normal levels, consequent upon estrogen-induced hyperphospholipemia.

In previous studies it was shown that estrogen administration prophylactically inhibits the development of coronary atherosclerosis in cholesterol-fed cockerels. The data of the present experiment demonstrate that estrogenic hormone is also capable of reversing previously induced coronary lesions, despite continued cholesterol feeding.

Methods

Fifty 1 day old Hy-line cockerels were received from a commercial hatchery and reared in a battery brooder. They were fed commercial chick starter mash until they were 7 weeks of age. During the next five weeks, they received mash supplemented with 2 per cent cholesterol plus 5 per cent cotton-seed oil (2CO). At the conclusion of this period, 10 of the 50 birds were sacrificed; all 10 were found to have aorta and coronary artery lesions. The remaining 40 cockerels were then divided into four groups (table 1); all continued to receive the experimental 2CO diet. In the first pair of groups (groups 1 and 2), the effect of estrogen on atherogenesis was assayed during the age period 12 to 15 weeks (5 to 8 weeks of cholesterol feeding). In the second pair of groups, estrogen influence was studied during age 15 to 20 weeks (8 to 13 weeks of cholesterol feeding) (table 1). The chicks of groups 1 and 2 were sacrificed at 15 weeks of age, those of groups 3 and 4 at 20 weeks.

At each sacrifice (12, 15 and 20 weeks of age) plasmas were analyzed for total cholesterol and lipid phosphorus according to the methods of Schoenheimer and Sperry and Man and associates, respectively. Thus the plasmas of estrogen-treated birds were analyzed both before and after administration of estrogen was instituted.

At sacrifice the heart and great vessels were inspected for gross atherosclerotic plaques, and graded grossly from 0 to 4 according to criteria previously described. The hearts were then fixed in 10 per cent formaldehyde. Two blocks were cut from each heart. Two frozen sections were then cut from different levels of each block and stained with Sudan IV for lipids. When no coronary atherosclerotic lesions were found in these four sections, eight further blocks were cut from these hearts and treated similarly. After frozen sections were prepared, the blocks were embedded in paraffin and slides stained with hematoxylin and eosin and with van Gieson’s connective tissue stain. Absence of lesions in these 20 sections was the criterion for designating a heart as negative for coronary atherosclerosis. Further, regression of coronary lesions was identified, based upon the presence of fibrosis with little or no associated lipid deposit. An attempt was also made to assess the intensity of atherogenesis in the hearts of birds with lesions. Toward this end a count was made of the number of vessels exhibiting lesions, in relation to the total number of vessels seen in the first two sections from the first two blocks. This count gave an index of the incidence (degree, hence severity) of coronary atherogenesis in a given heart and in the hearts from a given group of birds (table 3).

† In this initial effort at grading of coronary lesions, the criterion for designating a vessel as positive for atherosclerotic involvement was presence of lipid in any layer of the vessel wall, whether intra-or extracellular, diffuse or focal, associated or unassociated with lipophage accumulation and fibroblast proliferation. This criterion was utilized, although it was
feeding, aorta atherogenesis became progressively more severe. Estrogen exhibition was completely without effect on this process. The only gross difference was the more vividly yellow color of lesions in the estrogen-treated birds. The microscopic appearance of aorta lesions from chicks of both groups was typical of cholesterol-induced atherosclerosis.

**Coronary Arteries.** The hearts appeared grossly normal. On microscopic examination all cholesterol-fed control (non estrogen-treated) animals showed a marked degree of coronary atherosclerosis, ranging from single-layer, foam-cell intimal cushions to huge lipid-laden atherosclerotic plaques. A high percentage of vessels was involved in the pathologic process. No signs of spontaneous regression of the lesions was noted in any of the animals. In the estrogen-treated groups a significantly modified picture was encountered. In group 2, treated with estrogens for only three weeks, the lesions tended to be very small, exhibiting only slight fibrosis and containing little or no stainable lipid. When present, this lipid deposition was located chiefly in the outer layers of the media and adventitia, leaving the intima lipid free. Concomitantly, a greater than usual accumulation of fat was frequently present in the perivascular connective tissue in close proximity to the adventitia.

At the 20-week sacrifice, the differences between control (group 3) and experimental (group 4, estrogen-treated for five weeks) were even more marked (table 3). Estrogen-induced regression of coronary lesions was almost com-

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**Table 1.** Experimental Regimens after Division into Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Regimen During 12th-15th Weeks of Age</th>
<th>Regimen During 15th-20th Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2CO</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2CO plus estrogen</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2CO</td>
<td>2CO</td>
</tr>
<tr>
<td>4</td>
<td>2CO</td>
<td>2CO plus estrogen</td>
</tr>
</tbody>
</table>

**Table 2.** Effect of Estrogens on Aorta Atherogenesis in Cholesterol-Fed Chicks

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at Sacrifice</th>
<th>Duration of Cholesterol Feeding</th>
<th>Duration of Estrogen Administration</th>
<th>Incidence of Aorta Lesions</th>
<th>Grade of Aorta Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>weeks</td>
<td>weeks</td>
<td>weeks</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Pre-Division</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>1. 2CO</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>100</td>
<td>3.5</td>
</tr>
<tr>
<td>2. 2CO plus estrogen</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>100</td>
<td>3.2</td>
</tr>
<tr>
<td>3. 2CO</td>
<td>20</td>
<td>13</td>
<td>0</td>
<td>100</td>
<td>4.2</td>
</tr>
<tr>
<td>4. 2CO plus estrogen</td>
<td>20</td>
<td>13</td>
<td>5</td>
<td>100</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Table 3.** Effect of Estrogens on Plasma Lipids and Coronary Atherogenesis in Cholesterol-Fed Chicks

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Birds</th>
<th>Age</th>
<th>Duration of Cholesterol Feeding</th>
<th>Duration of Estrogen Administration</th>
<th>Plasma Total Cholesterol*</th>
<th>Plasma C/P Ratio*</th>
<th>Birds with Coronary Lesions</th>
<th>Degree of Involvement of Coronary Vessels Count %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7</td>
<td>20</td>
<td>13 weeks</td>
<td>0 weeks</td>
<td>870§</td>
<td>42.7</td>
<td>100</td>
<td>119/197 = 60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(503-1697)</td>
<td>(27.8-98.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>20</td>
<td>13 weeks</td>
<td>5 weeks</td>
<td>1305</td>
<td>12.0</td>
<td>67</td>
<td>2/237 = 0.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(753-2188)</td>
<td>(8.5-15.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Bleeding at sacrifice.
† Cf. text for criterion of freedom from coronary lesions, i.e., absence of lesions in 20 sections from 10 blocks.
‡ Counts were made on two sections taken from the first two blocks; the numerator is the no. of vessels with lesions (including lipid infiltration, atheroma, atherosclerotic plaque—see text), the denominator is the total no. of vessels seen; both numerator and denominator are the sum for the particular group.
§ Values enclosed in parentheses are the ranges which determined the mean values.

**RESULTS**

1. **Pathologic Findings**

* Aorta. Gross aorta findings are summarized in table 2. With prolongation of cholesterol

recognized that mere presence of lipid, per se, in the vessel wall is not adequate to justify a pathologic diagnosis of atherosclerosis. Atherosclerosis, by definition, is a thickening of the arterial wall, resulting from a complex tissue reaction, from which lipid accumulation is only one component. In subsequent applications of this grading method, the vessels visualized were divided into three categories: normal, positive for lipid infiltration only, positive for atherosclerosis.
COMPLETE. Three birds were entirely free of lesions. Multiple sections of hearts of six other chicks revealed an occasional vessel which exhibited a residual plaque. These few remaining lesions were similar morphologically to those observed in the estrogen-treated birds at the 15-week sacrifice. Altogether, only four plaques were seen that exhibited the appearance of a well-developed lesion without signs of regression. In the lesions that showed regression, van Gieson's connective tissue stain revealed only a few strands of very fine young fibrous tissue.

2. Biochemical Findings

The mean value for total plasma cholesterol was similar in the control and the experimental groups before estrogen treatment was instituted (for example, in groups 3 and 4 at 15 weeks of age, a value of 1012 and 1015 mg. per 100 ml., respectively). After estrogen therapy the plasma total cholesterol in the treated group was moderately higher (table 3). The most significant change occurred in the lipid phosphorus levels. These rose, consequent upon estrogen-induced hyperphospholipemia, from an average of 17.9 mg. per 100 ml. before treatment to 108.7 mg. per 100 ml. after five weeks on estrogen. Therefore, the total cholesterol–lipid phosphorus (C/P) ratio fell from an average of 56.7 to 12.0 (table 3).

DISCUSSION

The foregoing findings demonstrate that estrogen effects regression of cholesterol-induced coronary atherosclerotic lesions in chicks, despite continued presence in the diet of the atherogenic stimulus. Thus, estrogen is effective against coronary atherogenesis in both prophylactic and therapeutic-type experiments. In accordance with our observations in the former study, estrogen was again completely without influence on aorta atherogenesis.

The results of this experiment confirm the thesis that atherosclerosis is a reversible process. Moreover, they represent a significant extension of our information concerning regression (healing, reversibility) of experimental atherosclerosis. Previous studies of regression involved the effects, principally on the aorta, of cessation of cholesterol feeding.\textsuperscript{9-11} Definite regression of the pathologic process is demonstrable under this circumstance. The present study further shows that: (1) regression may be effected during continued ingestion of cholesterol; (2) this estrogen-induced regression may be remarkably effective; (3) it may involve clearing of both lipidosis and fibrosis from the vessel wall; (4) it may have a high preferential selectivity for the coronary arteries.

The mechanism of estrogen-induced regression of coronary lesions remains obscure. Several possibilities suggest themselves: Conceivably—from the peculiar histologic picture of the lesions showing partial regression—estrogen may induce lipophage activity which effects movement of lipids towards the adventitia and the perivascular tissue. Likewise, estrogen may decrease permeability of the endothelium for lipids. In addition to these possible actions of estrogen on vascular tissue, the effects of the hormone on plasma lipid-lipoprotein complexes may be key factors altering atherogenesis. These possible mechanisms, obviously hypothetic at the present stage of knowledge, hardly seem to account for one significant phenomenon observed in these experiments: regression of coronary lesions without effect on aorta atherogenesis. This finding may be related to metabolic differences (qualitative and/or quantitative) between vascular tissue of the aorta and the heart. Further work is necessary to clarify these new problems posed by our data.

SUMMARY

1. Estrogen reverses coronary atherosclerosis in cockerels previously induced by cholesterol feeding, despite continued feeding of the cholesterol diet.

2. This estrogen-induced regression occurs in the presence of continued marked hypercholesterolemia, of reversal of previously elevated total cholesterol–lipid phosphorus (C/P) ratios to normal levels, and of persistent aorta atherogenesis.

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