The Inhibition of Coronary Atherosclerosis by Estrogens in Cholesterol-Fed Chicks

By R. Pick, M.D., J. Stamler, M.D., S. Rodbard, M.D., Ph.D., and I. N. Katz, M.D.

Chronic administration of estrogens parenterally inhibits coronary athereogenesis in cockerels fed a cholesterol-supplemented diet, although no such prophylactic effect was observed against aorta athereogenesis. This protection of the coronary vessels is associated with depression of the plasma total cholesterol–lipid phosphorus ratio toward normal levels consequent upon estrogen-induced hyperphospholipemia.

CORONARY athereogenesis occurs with significantly greater frequency in the human male than in the female. Little is known concerning the basis for this sex differential in susceptibility to coronary athereogenesis. In an attempt to clarify this problem experimentally, we studied the effects of estrogen administration on athereogenesis in the coronary arteries and aorta of cholesterol-fed cockerels. Plasma lipid patterns were simultaneously analyzed in order to ascertain whether any observed morphologic effects might be correlated with concomitant lipid alterations.

METHODS

Forty-five 1 day old Hy-line cockerels were obtained from a commercial hatchery and reared in a battery brooder. They were fed commercial chick starter mash until they were 7 weeks of age. They were then divided into three groups: Group 1 (control) received mash supplemented with 2 per cent cholesterol and 5 per cent cotton seed oil. Group 2 (experimental) received the same diet plus daily injections of 1 mg. estradiol benzoate (Progynon B, Schering).* Group 3 received plain a diet of mash without cholesterol supplement, and daily injections of 1 mg. estradiol benzoate.† Blood samples were taken and groups of chicks sacrificed at 12, 15 and 20 weeks of age (5, 8 and 13 weeks on the experimental regimens, respectively). Individual blood samples were analyzed for total cholesterol and for lipid phosphorus by the methods of Schoenheimer and Sperry* and of Man and co-workers, respectively. At autopsy the hearts and great vessels were inspected for gross athereosclerotic plaques. The lesions were recorded graphically on special forms and graded grossly 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 these were stained with Sudan IV for lipids. When no coronary athereomatous lesions were found in these sections, eight further blocks were cut from these hearts and treated similarly. The hearts were described as either positive or negative for microscopic athereosclerosis. No attempt was made to grade the lesions. A heart was described as negative when no artery in 20 examined sections showed a lipid-containing intimal lesion.‡

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Some of the data of these experiments were presented before the September, 1950 meeting of the American Physiological Society† and the November, 1951 meeting of the American Society for the Study of Atherosclerosis.‡

* We are grateful to Dr. Abbott W. Allen of the Schering Corp., Bloomfield, N. J., for the generous quantities of Progynon B which he kindly supplied for these experiments.

† This latter group confirmed previous findings concerning the effects of estrogenic substances in birds fed normal diets.‡ These data are not relevant to the present study.

‡ At the 20 week sacrifice (13 weeks on the experimental regimen), slight lipid accumulations were present in the media of several coronary arteries of three birds of group 2. This lipid was uniformly confined to the media; in no case was it present in the intima. The intima appeared normal, no fibrotic changes were observed in the media, the vessel was not thickened and the lumen was not narrowed. These lipid accumulations, therefore, were not considered to be athereomas. hearts with an occasional artery exhibiting only this medial change were considered negative for coronary athereosclerosis.
RESULTS

1. Pathologic Findings

The gross and microscopic findings at the three periods of sacrifice were essentially similar and are therefore described together. Atrophic lesions virtually occluding the lumen of the vessels. Some lesions also showed sclerotic changes, that is, fibrosis and calcium deposition. In contrast to these findings, only 4 of 12 birds in group 2 (cholesterol-fed, estrogen-injected) exhibited coronary lesions.

Aorta: Gross atherosclerosis in the aorta was present in 100 per cent of birds of both groups. The mean grading for gross aorta lesions was 3.1 and 3.9 for groups 1 and 2 respectively. Thus there were no significant differences between the two groups in incidence and severity of aorta atherosclerosis. In both groups, the gross and microscopic appearance of these lesions was typical of cholesterol-induced atherosclerosis. 11 The only noticeable gross difference was a more vividly yellow color of the lesions in the estrogen-treated chicks.

Coronary Arteries: The hearts appeared grossly normal. On microscopic examination, however, a significant difference was noted between the two groups. All group 1 chicks (receiving cholesterol without estrogens) showed a marked degree of coronary atherosclerosis. Lesions varied from early, subendothelial lipid infiltrations to massive atheromata.

![Figure 1](http://circ.ahajournals.org/)

**Fig. 1.** The relationship of the plasma cholesterol (C) and lipid phosphorus (P) to the presence of coronary atherosclerosis. Values are simultaneous (C and P) at the time of sacrifice. Ordinates represent the total plasma cholesterol level in mg. per 100 cc. The abscissas represent the lipid phosphorus in mg. per 100 cc. The U's represent chicks fed 2% cholesterol but not treated with estrogens. The E's represent chicks on the same diet but receiving 1 mg. estradiol benzoate intramuscularly daily. The circled E's are cases without coronary atherosclerosis. The diagonal line represents a C/P ratio of 20. Symbols above the line have a ratio greater than 20, while those below the line have a ratio less than 20.

**Table 1.**—Effects of Estrogen Administration on Plasma Lipids and on Coronary Atherosclerosis in Cholesterol-fed Chicks

<table>
<thead>
<tr>
<th>Group 1: 2C-O§</th>
<th>No. of chicks</th>
<th>Total cholesterol (mg%)</th>
<th>Lipid P (mg%)</th>
<th>C/P ratio</th>
<th>% Coronary lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13</td>
<td>895†</td>
<td>17</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>380-1800</td>
<td>8-25</td>
<td>32-77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: 2C-O + estrogen</th>
<th>No. of chicks</th>
<th>Total cholesterol (mg%)</th>
<th>Lipid P (mg%)</th>
<th>C/P ratio</th>
<th>% Coronary lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>742</td>
<td>47</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>129-1800</td>
<td>6-109</td>
<td>9-25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Two birds in the 2C-O group and 3 birds in the 2C-O + estrogen group died during the experiment. No plasma lipid determinations were done, therefore they are not included in the series.

† Lipid P = lipid phosphorus.

‡ C/P ratio = cholesterol/lipid phosphorus ratio.

§ 2C-O = diet of mash supplemented with 2 per cent cholesterol and 5 per cent cottonseed oil.

¶ Mean value.

|| Range.
2. Biochemical Findings

The data on plasma lipids are summarized in table 1. The mean value for total cholesterol in group 1 (895 mg. per 100 cc.) was slightly but not significantly higher than in group 2 (742 mg. per 100 cc.). In contrast to the similar plasma cholesterol levels, the two groups exhibited significant differences in plasma lipid phosphorus concentrations. Whereas the mean lipid phosphorus level in group 1 was 17 mg. per 100 cc., in group 2 (cholesterol-fed, estrogen-injected) it was 47 mg. per 100 cc. Correspondingly, the total cholesterol-lipid phosphorus (C/P) ratios were 47 and 16,* respectively, that is, the estrogen-treated, cholesterol-fed birds had C/P ratios near the normal level, whereas the group 1 cockerels had markedly elevated ratios.

3. Pathologic-Biochemical Correlations

A close relationship was found between C/P ratio and the occurrence of coronary atherosclerosis (fig. 1). Thus, all birds of group 1, with markedly elevated C/P ratios, had coronary atherosclerosis.* In group 2, chicks with C/P ratios less than 20 were uniformly free of lesions. Whenever the dosage of estrogen was inadequate to keep the C/P ratio depressed below 20,† coronary atherosclerosis was present (fig. 1). This occurrence of coronary atherosclerosis was not related to the level of total cholesterol (table 1). This fact emerges even more clearly when data on individual chicks of group 2 are considered. Thus, one estrogen-treated bird with a cholesterol level of 1800 mg. per 100 cc., but a C/P ratio of only 16 had no coronary lesions; another injected chick with a cholesterol of only 460 mg. per 100 cc., but a relatively high C/P ratio of 25 had distinct coronary lesions.

Discussion

The data of these experiments demonstrate clearly that estrogens may prevent coronary atherogenesis in cholesterol-fed cockerels*. These findings are particularly significant in view of the well-known sex difference in human susceptibility to coronary atherosclerosis. Thus, especially before age 40, human males are victims of coronary disease far more frequently than females.‡  ‡ In the latter (postclimacteric) decades of life, the incidence of coronary atherosclerosis in the two sexes becomes similar. Hitherto these findings have been unexplained. The present experiment indicates that estrogenic activity may play an important role in protecting the human female against coronary atherogenesis.

Our findings further suggest that this prophylactic effect of estrogens may depend on their ability to restore cholesterol-phospholipid ratios to normal despite persistent hypercholesterolemia. Data obtained by other workers support this possibility. Thus, studies on patients with chronic biliary xanthomatosis led Ahrens and Kunkel to the conclusion that elevated C/P ratios are key factors in atherogenesis.‡  ‡ Gertler and co-workers found that coronary atherosclerosis in young men is correlated with elevation of C/P ratios.‡  ‡ Further, Eilert recently demonstrated that estrogens can lower C/P ratios of humans.‡  ‡ These clinical findings, although fragmentary, all support the thesis that elevated C/P ratios have significance in the pathogenesis of atherosclerosis. Further, they suggest that estrogenic activity, by depressing C/P ratios to normal levels, may be a factor in the prophylaxis of coronary atherosclerosis in sexually mature females.

Laboratory evidence supporting this concept is also available. Thus, Kellner and associates and Duff and Payne showed that aorta atherogenesis is inhibited in hypercholesterolemic rabbits with normal C/P ratios. In

* The normal C/P ratio for cockerels is 12-13.
† The depression of the C/P ratio by estrogens is a consequence of the pattern of hyperlipemia they induce, that is, predominantly a hyperphospholipemia, in contrast to the predominant hypercholesterolemia of cholesterol feeding.
‡ This decreased susceptibility to coronary atherosclerosis is not observed in women with diabetes mellitus.
contrast to these findings in rabbits, we observed no correlation between C/P ratios and aorta atherosclerosis in cockerels.\footnote*{In addition to the present study, previous experiments yielded similar negative results.} However, a correlation between C/P ratios and coronary lesions was definitely demonstrated in the present experiment.

Finally, one additional significant fact clearly emerges from this experiment, namely, that no necessary relationship exists between atherogenesis in the aorta and coronary arteries. The prevention of lesions in the coronary arteries by estrogens, in the absence of any prophylactic effect against aortic atherosclerosis, makes this principle clear. It becomes apparent that the coronary arteries differ from the aorta in susceptibility to atherosclerosis. This difference in susceptibility of the various vascular beds is also present in man.\footnote{STAMLER, J., AND KATZ, L. N.: The plasma cholesterol/phospholipid ratio and atherosclerosis in the chick. Am. J. Physiol. 163: 752, 1950.}

Hence, it may be concluded that atherogenesis does not proceed according to the same biologic laws in different vascular beds.

These facts warrant the further conclusion that experimental studies of atherosclerosis should not be confined to aorta pathology. In order to elucidate the laws governing atherogenesis in the coronary and cerebral arteries (sites of lesions causing greatest human morbidity and mortality), these particular vessels must be studied experimentally. It can no longer be assumed that laws of atherogenesis in the aorta apply equally well to the coronary and/or cerebral vessels.

Summary

1. Estrogen administration inhibited coronary atherogenesis in cockerels fed a cholesterol-containing diet.

2. This protection of the coronary vessels by estrogen was associated with changes in total cholesterol - lipid phosphorus (C/P) ratios. Thus, all estrogen-treated, cholesterol-fed birds with C/P ratios below 20 were uniformly free of lesions. In all chicks with a C/P ratio above 20, coronary atherosclerosis was present, whether or not estrogen was injected.

3. Estrogen failed to exert any prophylactic effect against atherosclerosis of the aorta in any of the birds.

4. Since the two arterial beds examined in this experiment showed different atherogenic responses to a given procedure (cholesterol feeding plus estrogen injection), it becomes apparent that atherogenesis does not proceed according to the same biologic laws in different vascular beds.

Acknowledgments

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References


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