Prevention of Coronary Atherosclerosis by Estrogen-Androgen Administration in the Cholesterol-Fed Chick

By Jeremiah Stamler, M.D., Ruth Pick, M.D., and Louis X. Katz, M.D.

Estrogens are highly effective both prophylactically and therapeutically against cholesterol-induced coronary atherogenesis in cockerels. The relative immunity of premenopausal women to coronary sclerosis may be related to the presence of these ovarian hormones. Therefore, estrogens may be useful in the treatment of human coronary atherosclerosis. With this object in mind, the elimination of the feminizing effects of estrogens would be highly desirable. The present experiments prove that concomitant exhibition of androgens and estrogens maintain masculine sex characteristics without interfering with the beneficent effect of estrogens on the coronary arteries in cockerels.

Recent studies in this department demonstrate that estrogens are highly effective both prophylactically and therapeutically against cholesterol-induced coronary atherogenesis in cockerels. These findings indicate that estrogens may be a key factor in the relative immunity of premenopausal women to clinical coronary sclerosis. Further, they suggest that estrogens may be useful in the treatment of human coronary lesions.

In any attempt to assess this latter possibility, it would be highly desirable to avoid likely feminizing effects of estrogens on male subjects. One possible way to attain this objective would be through combination of estrogen and androgen therapy. With this in mind, the present experiment was preliminarily undertaken, assessing the influence of this combined hormonal regimen on the experimental lesions in cockerels. It attempted to supply adequate androgen for masculinization either exogenously or endogenously, by administration of testosterone or chorionic gonadotrophin respectively.

This study also entailed an exploration of the mechanism of estrogen inhibition of cholesterol-induced coronary atherosclerosis. By analyzing the effects of gonadotrophic and androgen on estrogen-treated cholesterol-fed chicks, it permitted an evaluation of the possibility that estrogen inhibition of coronary atherogenesis is related to the concomitant estrogen-induced suppression of pituitary gonadotrophin-testicular androgen secretion.

Finally, this experiment was designed to determine the effects of exogenous testosterone and chorionic gonadotrophin, per se, on cholesterol-induced atherogenesis, in the absence of concomitant estrogen exhibition.

Methods

Two series of experiments were completed, utilizing a total of 100 chicks, in accordance with procedures established in this department. In both, one day old Hy-line cockerels were obtained from a commercial hatchery and reared in a battery brooder. Prior to initiation of the experimental regimen, birds were maintained on a commercial chick starter mash and tap water ad lib. The first series was divided into four experimental groups (10 chicks per group) at the age of 19 weeks; the second, into six groups at 8 weeks of age (table 1). The experimental diet ingested by all groups was: in series I, mash supplemented with 2 per cent cholesterol plus 5 per cent cottonseed oil (2 CO); in series II, mash supplemented with 1 per cent cholesterol plus 5 per cent cottonseed oil (1 CO). The duration of the experiment was: in series I, five weeks (age 19 to 24 weeks); in series II, seven weeks (age 8 to 15 weeks). Records of feed intake and body weight were...
maintained throughout. The hormonal regimens are summarized in table 1. Serial comb indexes were obtained to estimate over-all androgenic activity (exogenous and/or endogenous).4

In both series, chicks were bled serially from a wing vein or by cardiac puncture. Individual samples of heparinized plasma were analyzed for total cholesterol and lipid phosphorus by the methods of Schoenheimer and Sperry5 and Man and Peters6 respectively. Samples of sera from individual birds were also analyzed ultracentrifugally to determine concentrations of various classes of lipoproteins.7 * At the time of sacrifice, complete autopsies were performed. Aortas and hearts were evaluated for atherogenesis by established gross and microscopic methods.1,2

### Table 1. Hormonal Regimens

<table>
<thead>
<tr>
<th>Series</th>
<th>Group</th>
<th>Estradiol Benzoate mg./bird/day</th>
<th>Testosterone Propionate mg./bird/day</th>
<th>Pregnant Mare's Serum l.U./chick/day</th>
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<td>0</td>
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</tr>
<tr>
<td>I</td>
<td>4</td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
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<td>2</td>
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<tr>
<td>II</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>100-400</td>
</tr>
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</table>

* Series I: cockerels age 19 to 24 weeks, 10 per group, fed 20 diet.
Series II: cockerels age 8 to 15 weeks, 10 per group, fed 100 diet.
† Parenterally, in oil.
‡ Parenterally, in saline.

### Results

All three groups of cockerels receiving estrogen were remarkably free of coronary atherosclerosis (groups 2, 4 and 6, series I and II, table 2).† This estradiol inhibition of cholesterol-induced coronary atherosclerosis persisted when either testosterone or chorionic gonadotrophin was concomitantly administered (groups 4 and 6). In series II, the dosage of testosterone was adequate to maintain masculinization of secondary sex characteristics (combs and wattles) throughout the experiment (series II, group 4, table 2).† Nevertheless, estrogen anti-atherogenesis continued to prevail. The two usual effects of estradiol—feminization of combs and inhibition of coronary atherogenesis—became dissociated, with neutralization of the former and persistence of the latter.

The estrogen-inhibition of coronary atherosclerosis cannot be attributed to differences in cholesterol intake, since all groups were similar with respect to feed intake and rate of weight gain. For instance, the thoracic aortas were graded 1.2, 1.8, 0.9, 1.7 respectively for gross cholesterol-induced atherosclerosis; in the six groups of series II, they were graded 1.3, 1.3, 1.4, 1.3, 1.3 and 1.4 respectively. Despite a greater percentage of cholesterol in the series I diet (2 per cent vs. 1 per cent), coronary atherogenesis was significantly less in the non-estrogen treated groups of series I, as compared with their counterparts in series II. This finding is in accord with previous observations of an age-conditioned relative immunity of 20 to 26 week old cockerels to cholesterol-induced atherogenesis.8

† This was the only estrogen-treated group in which masculinization was successfully maintained. Both the smaller doses of testosterone used in series I (group 4, table 3) and the level of gonadotrophin administered in series II (group 6, table 3) did not achieve this objective. Further, in both series, neither testosterone nor gonadotrophin reversed the feminizing effect of estrogen on the cloaca. In all estrogen-treated cockerels (groups 2, 4, and 6) the testes were small (table 3).
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gain. Neither is the immunity to coronary atherogenesis in the estrogen-treated groups attributable to differences in degree of hypercholesterolemia, since similar levels of plasma total cholesterol prevailed throughout the experiment in all groups of a given series (table 4).

In accord with previous findings, estrogen administration effected a marked elevation of plasma phospholipid levels, with consequent depression of the plasma total cholesterol/lipid phosphorus (C/P) ratios toward normal despite the concomitant hypercholesterolemia (table 4). This estrogen influence on hyperphospholipemia tended to persist in the groups given testosterone or gonadotrophin together with estradiol (groups 4 and 6, table 4). However, in the androgen-estrogen treated chicks of series II (group 4), plasma phospholipid levels tended to fall (with resultant rises in C/P ratios) during the latter weeks of the experiment, when testosterone dosage attained the level of 2.0 to 3.0 mg/bird/day. Thus, the mean plasma lipid phosphorus levels of the group 4 cockerels were 57.7 and 15.3 at the third and seventh experimental weeks respectively, and the C/P ratios were 17 and 27 respectively. Only this group exhibited this apparent testosterone-induced reversal of estrogen hyperphospholipemia. Further work is needed to verify this phenomenon and assess its significance, particularly in relation to the problem of the role of C/P ratios in coronary atherogenesis.

Exhibition of testosterone or gonadotrophin alone, without estrogen, had no significant effect on plasma lipid levels or atherogenesis (coronary or aorta) (groups 3 and 5, tables 2 and 4). In both series, exogenous testosterone exhibition apparently effected an overall hyperandrogenism, judging from the data on comb indices (group 3, table 3). These high comb indices in the testosterone-treated cockerels were associated with large testes in series I and small testes in series II (table 3). It is not clear from the data available whether this divergence in results is related to chick age, testosterone dosage and/or other factors. Chorionic gonadotrophin alone produced anticipated effects, that is, increases in both comb indices and testes weights (group 5, table 3). As already indicated, the hormone-induced hyperandrogenism in groups 3 and 5 (primarily exogenous in the former, endogenous in the latter) was without effect on plasma lipid patterns or atherogenesis.

**DISCUSSION AND CONCLUSIONS**

Previously, it was shown that estrogen is effective both prophylactically and therapeutically against cholesterol-induced coronary atherogenesis in cockerels. The findings of the present experiment are additional confirmation...
of the estrogen prophylactic effect. Further, they demonstrate that coronary lesions are also inhibited by combined administration of estrogen and androgen, a regimen that avoids the usual feminizing effects of estrogen on secondary sex characteristics. Studies are now in order to assess the possible utility of both regimens—estrogen alone, and estrogen plus androgen—for the prophylaxis and/or therapy of coronary atherosclerosis in man. Such an investigation is now being pursued.

Previously we presented the tentative conclusion—based on the demonstration of estrogen inhibition of experimental coronary atherosclerosis—that estrogen secretion may be a key factor responsible for the relative immunity of premenopausal women to coronary atherosclerosis. This concept poses the question: Is the relative susceptibility of the human male to coronary atherosclerosis due to absence (relative) of estrogen or to presence of androgen, or to both (that is, a low ratio estrogen/androgen)? The findings of the present study, together with other observations on castrated cockerels, may afford a partial answer to this problem. They demonstrate that in the absence (relative) of estrogen, cholesterol-induced coronary atherogenesis proceeds in cockerels, whether they are hyperandrogenic, eunuchoid, or hypoa-androgenic. Therefore the presence of estrogen, per se, with a relatively high ratio of estrogen/androgen, appears to be the sine qua non for anti-atherogenic activity. Prior to age 50, coronary atherogenesis is more common in males than in females, apparently because men are lacking (relatively) in estrogens and have a low estrogen/androgen ratio—and not primarily because of their relatively high level of androgen secretion. Further experimental and clinical investigation evaluating this concept is essential.

An additional proposition is suggested by the findings of the present experiments: Anti-atherogenesis is present in all estrogen-treated groups, regardless of concomitant male hormone administration. With adequate doses of testosterone, it is possible to overcome the hypo-androgenism due to estrogen inhibition of anterior pituitary gonadotrophin-testicular androgen secretion. Nevertheless, coronary atherogenesis continues to be inhibited. Therefore, estrogen anti-atherogenesis apparently is not a consequence of estrogen suppression of anterior pituitary gonadotrophin-testicular androgen secretion. It is not a consequence of estrogen-induced hypoandrogenism. The mechanism of estrogen anti-atherogenesis remains to be elucidated.

Finally, one further tentative conclusion: Since it is apparently possible with androgen to effect a dissociation of the feminizing and anti-atherogenic actions of estrogen, the mechanisms of these two influences—as already indicated—are apparently different. Therefore, we may anticipate the eventual acquisition of an estrogen-like compound which produces minimal feminization and maximal inhibition of coronary atherogenesis.

**SUMMARY**

1. The phenomenon of estrogen prophylactic inhibition of coronary atherogenesis is further confirmed.
2. The combined administration of estrogen and androgen, with resultant prevention of estrogen feminization, is at least as effective as estrogen alone in the prophylactic inhibition of cholesterol-induced chick coronary atherogenesis.
3. Neither androgen nor chorionic gonadotrophin alone has a significant influence on cholesterol-induced coronary or aorta atherogenesis in intact cockerels.

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