Inhibition of Cholesterol-Induced Coronary Atherogenesis in the Egg-Producing Hen

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

Mature egg-producing hens, intact or after oviduct ligation, are markedly resistant to cholesterol-induced coronary atherogenesis. This inhibition of coronary atherosclerosis is apparently effected by the endogenous estrogen secretion of these female birds.

Previous work in this department demonstrated that oral or parenteral administration of estrogens inhibits coronary atherogenesis in cholesterol-fed cockerels.1-3 The present study was undertaken to determine whether the endogenous estrogen secretion of the egg-producing hen has a similar prophylactic effect.

Methods

The experimental designs and technics were generally in accord with the standardized procedures developed by the department's atherosclerosis research group.4 Altogether, four series of chronic experiments were completed, varying in duration from 5 to 11 weeks, and utilizing a total of 129 fully grown, sexually mature chickens, age 24 to 34 weeks at the onset of the study (table 1). Essentially, a comparison was made of the plasma lipid and atherogenic (aortie and coronary artery) response to cholesterol feeding in mature female vs. male chickens. All hens were laying eggs before the experiment began; they exhibited the typical plasma lipid pattern resulting from endogenous estrogen secretion by the ovary associated with egg production (table 2).3,4 In series BC 22, based on the plasma lipid findings in the earlier series (table 3), a greater dietary atherogenic stimulus was imposed upon the hens than upon the roosters, by feeding the females a mash containing twice as much cholesterol (2 per cent) as that given the males (1 per cent) (table 1). In series BC 20 and BC 22, the removal from the body of large quantities of cholesterol and lipid via egg-laying was counteracted in one group of females by ligation of the oviduct. These hens continued to produce egg yolks which were deposited from the ovary into the peritoneal cavity. It is probable that at least partial absorption and recirculation of the lipoprotein material occurred.

The specific nutritional, biochemical, physiologic and pathologic methods employed in studying these birds were detailed in a previous paper.4

Results

Plasma Lipids. When fed the same diet as roosters, intact hens consistently exhibited lower plasma total cholesterol levels despite essentially similar feed intakes and despite the hyperlipemic influence of the endogenous estrogen secretion (series BC 15 and BC 18, table 3). Intact females eating a 2 per cent cholesterol diet (2 CO) exhibited plasma total cholesterol levels essentially similar to those of males ingesting a 1 CO mash (groups 1 and 2, series BC 20 and BC 22, table 3). Thus, to achieve levels of hypercholesterolemia in egg-laying hens equal to those in roosters, females had to ingest twice as much cholesterol (since levels of feed intake were practically identical). This less marked hypercholesterolemic response of the hen to cholesterol feeding was apparently a by-product of egg laying, since it was virtually abolished by oviduct ligation, which significantly enhanced hypercholesterolemia in both series BC 20 and BC 22 (group 4, table 3; data from these two series are combined in this table). Administration of estrogens to roosters markedly intensified hypercholesterolemia in this experiment (group 3, series BC 22, table 3).

In all series, hens (both intact and with ligated oviducts) exhibited grossly increased plasma lipid phosphorus levels with low or
INHIBITION OF CORONARY AtherosGENESIS

Table 1.—Experimental Regimens

<table>
<thead>
<tr>
<th>Series</th>
<th>Group</th>
<th>Number of Birds</th>
<th>Sex</th>
<th>Age Weeks</th>
<th>Diet</th>
<th>Other Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC 15</td>
<td>1</td>
<td>11</td>
<td>Male</td>
<td>34-44</td>
<td>2 CO*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>Female</td>
<td>34-44</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td>BC 18</td>
<td>1</td>
<td>15</td>
<td>Male</td>
<td>28-33</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24</td>
<td>Female</td>
<td>28-33</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1A</td>
<td>22</td>
<td>Male</td>
<td>28-39</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>13</td>
<td>Female</td>
<td>28-39</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td>BC 20</td>
<td>2</td>
<td>4</td>
<td>Female</td>
<td>28-33</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>Female</td>
<td>28-33</td>
<td>2 CO</td>
<td>Oviduct ligation</td>
</tr>
<tr>
<td>BC 22</td>
<td>1</td>
<td>8</td>
<td>Male</td>
<td>24-29</td>
<td>1 CO*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>Female</td>
<td>24-29</td>
<td>2 CO</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>Male</td>
<td>24-29</td>
<td>1 CO</td>
<td>Estrogens†</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>Female</td>
<td>24-29</td>
<td>2 CO</td>
<td>Oviduct ligation</td>
</tr>
</tbody>
</table>

* 1 CO and 2 CO are chick starter mash supplemented with 5% cottonseed oil (O) and 1%, 2% cholesterol respectively.
† 20-30 mg./chick/day of mixed conjugated equine estrogens (Premarin) in drinking water.

normal plasma total cholesterol–lipid phosphorus (C/P) ratios. This plasma lipid pattern, resulting from endogenous estrogen secretion in egg-producing females, was also noted in males receiving oral estrogens (group 3, series BC 22, table 3).

Coronary Artery and Aorta AtherosGenesis. In all series, hens (intact and oviduct-ligated) exhibited marked inhibition of coronary artery atherosclerosis. In accord with previous findings,1,3 estrogen-treated roosters also exhibited this phenomenon (group 3, series BC 22, table 3). All groups—untreated males, estrogen-treated males, intact females and oviduct-ligated females—exhibited similar incidences and gradings of cholesterol-induced thoracic aorta atherosclerosis.

Discussion and Conclusions

Sexually mature chickens exhibit a significant sex difference in susceptibility to experimental cholesterol-induced coronary...

Table 2.—Plasma Lipid Levels in Mature Male and Female Chickens Fed Plain Mash, without Cholesterol or Oil Supplement

<table>
<thead>
<tr>
<th>Series</th>
<th>Sex</th>
<th>Age</th>
<th>Number of Birds</th>
<th>Total Cholesterol mg.%</th>
<th>Lipid Phosphorus mg.%</th>
<th>C/P Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC 15</td>
<td>Male</td>
<td>33</td>
<td>6</td>
<td>166 ± 31.5‡</td>
<td>45.5 ± 9.0</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td></td>
<td>71-261‡</td>
<td></td>
<td>16.7-70.7</td>
<td>3.4-4.2</td>
</tr>
<tr>
<td>BC 18</td>
<td>Male</td>
<td>28</td>
<td>6</td>
<td>98 ± 8.9</td>
<td>9.7 ± 0.4</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>28</td>
<td>8</td>
<td>88 ± 6.1</td>
<td>14.6 ± 2.3</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63-108</td>
<td></td>
<td>8.7-27.0</td>
<td>8.8-11.1</td>
<td>3.1-9.6</td>
</tr>
<tr>
<td>BC 20</td>
<td>Male†</td>
<td>28</td>
<td>6</td>
<td>98 ± 8.9</td>
<td>9.7 ± 0.4</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>26</td>
<td>4</td>
<td>124 ± 20.2</td>
<td>24.1 ± 5.5</td>
<td>5.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83-169</td>
<td></td>
<td>8.8-34.1</td>
<td>8.8-11.1</td>
<td>3.8-9.4</td>
</tr>
</tbody>
</table>

* C/P Ratio is the ratio of plasma total cholesterol to plasma lipid phosphorus.
† Range.
§ Standard error of the mean.
† Data from previous experiment, BC 18
The marked resistance of hens to coronary atherosclerosis apparently cannot be attributed to disposal ("clearing") of cholesterol from the body via egg laying, since it is present in oviduct-ligated, as well as in intact females.

This sex difference in coronary atherosclerosis is not present in immature chicks. Previous studies in this department demonstrated that sexually immature male and female chicks exhibit an identical susceptibility to experimental cholesterol-induced coronary atherosclerosis.7, 8

The resistance to coronary atherosclerosis in egg-producing, cholesterol-fed hens is associated with enhancement of hyperphospholipidemia, lowering of plasma total cholesterol–lipid phosphorus ratios to normal levels and susceptibility to aorta atherosclerosis. This response pattern in hens is identical with that produced by oral or parenteral exhibition of exogenous estrogens to cholesterol-fed cockerels, roosters and immature female chicks.1-3, 6, 7

All these findings indicate that inhibition of cholesterol-induced coronary atherosclerosis in hens is effected by endogenously secreted ovarian estrogens.

In our original study on the effects of estrogens on cholesterol-induced atherosclerosis, an empiric method was utilized to establish

### Table 3.—Results: Plasma Lipids and Atherogenesis

<table>
<thead>
<tr>
<th>Series</th>
<th>Groups</th>
<th>Sex</th>
<th>Total Cholesterol mg. %</th>
<th>C/P Ratio*</th>
<th>Gross Thoracic Aorta Lesions</th>
<th>Microscopic Coronary Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Birds with Lesions %</td>
<td>Grade of Lesions %</td>
</tr>
<tr>
<td>BC 15</td>
<td>1</td>
<td>Male</td>
<td>418 ± 38.4†</td>
<td>34.8 ± 2.4</td>
<td>86 1.2 ± 0.5</td>
<td>0.0-4.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Female</td>
<td>232 ± 24.2</td>
<td>24.4-50.4</td>
<td>60 0.4 ± 0.1</td>
<td>0.0-0.8</td>
</tr>
</tbody>
</table>

** C/P Ratio is the ratio plasma total cholesterol/plasma lipid phosphorus.
† BC 20 and BC 22 combined (12 hens).
‡ Estrogen-treated group.
§ Oviduct ligated groups, BC 20 and BC 22 combined (9 hens).
¶ Coronary count is the ratio vessels with lesions / total vessels examined.

Plasma lipid data are the means of values obtained at the following experimental periods:
BC 15—Weeks 1, 3, 5, 9 and 10
BC 18—Groups 1 and 2—Weeks 1, 3 and 5
BC 18—Groups 1A and 2A—Weeks, 1, 3, 5, 6 and 11
BC 20—Weeks 1, 3 and 5
BC 22—Weeks 1 and 5

** Range.
†† Standard error of the mean.
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the dosage of 1 mg. per chick per day of parenteral estradiol. A preliminary acute experiment on cockerels fed plain mash was carried out in which a range of estradiol dosages was tested for their effects on plasma lipids. A dosage of 1 mg. per chick per day was selected because this was the minimum amount of estradiol capable of reproducing the plasma lipid pattern of the egg-laying hen. It was estimated that this dosage approximated the physiologic daily estrogen secretion in the mature female. The results of the present experiment on hens—both with respect to plasma lipid patterns and atherogenesis—reinforce the foregoing conclusion from our original assay, based exclusively on plasma lipid responses in the cockerel fed plain mash. Physiologic (not so-called pharmacological) dosages of estrogen, exogenous or endogenous, are capable of protecting the coronary arteries of male and female chickens against cholesterol-induced atherogenesis.

It is noteworthy that mature, gonadally-active chickens and humans both exhibit a marked sex differential—in favor of the female of the species—in susceptibility to coronary atherosclerosis. The findings of the present experiment lend further support to the presumptive conclusion from our previous studies1-3 that estrogens may play a key role in protecting premenopausal women against coronary atherogenesis.

SUMMARY

Mature egg-producing hens, intact or with ligated oviducts, are markedly resistant to cholesterol-induced coronary atherogenesis. This inhibition of coronary atherosclerosis is apparently effected by the endogenous estrogen secretion of these female birds.

SUMARIO Español

Gallinas adultas productoras de huevos, intactas o luego de la ligadura del oviducto, son marcadamente resistentes a la aterogénesis coronaria inducida por colesterol. Esta inhibición de la ateroesclerosis coronaria es aparentemente producida por la secreción endógena de estrógeno de estas aves hembras.

ACKNOWLEDGMENTS

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* Deborah V. Dauber Research Assistant.
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