Testicular Action and Structural Relationships of Compounds with Antiatherogenic Activity

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In a study comparing the antiatherogenic action of estradiol-17α and of estradiol-17β in cholesterol-fed chickens, it was observed that, although both isomers were able to induce testicular atrophy at the dosage used, only estradiol-17β prevented coronary atherosclerosis. Similarly, in men active estrogens but not estradiol-17α are able to influence blood lipids. The mechanisms involved in the prevention of atherosclerosis by estrogens have not been elucidated. The theories suggested to explain these effects include blood lipid changes as well as direct effects of the hormones on the arteries. The importance of concurrent endocrine changes has not been totally explored, although thorough studies related to gonadal, thyroid, and pancreatic functions have already been conducted. A relevant point to determine in this connection is whether the antiatherogenic activity of estrogens, as observed in male animals, is related to induced atrophy of the testicles. In rats and in rabbits, prevention of experimental atherosclerosis by estrogens is apparently not related to a generalized endocrine effect. As a further attempt to define the intimate mechanism in hormonal prevention of atherosclerosis, two simultaneous studies, here reported, have been carried out: (1) a correlation between testicular changes and atherosclerosis in cholesterol-fed cockerels under estrogenic or androgenic therapy; (2) an attempt to correlate molecular structure and antiatherogenic activity.

Methods

Six-week-old, white Leghorn cockerels were housed in pens measuring 100 × 100 × 50 cm. and fed commercial chick starter mash during one week. They were then fed 1 per cent cholesterol added to the diet and divided into several groups of 15 birds each: (1) vehicle-injected controls; injected with (2) estradiol; (3) diethylstilbestrol; (4) estriol; (5) androstanolone; (6) Δ5-androstenediol; (7) androstanediol; each one of these drugs was given at a 1-mg dosage (see fig. 1). Two further groups were injected with 0.5 mg., i.e., groups (8) estriol and (9) androstanolone. Birds were injected subcutaneously five times a week; volumes were kept at 1 ml.

All birds were sacrificed after eight weeks on this experimental regimen, when one animal from each group was selected daily for study. Blood was withdrawn from the heart; the comb was removed following the procedures of Frank et al. Testicles, adrenals, and hypophysis were removed, carefully trimmed and blotted on filter paper, weighed, and fixed in 10 per cent formaldehyde. The tissues were embedded in paraffin, and the sections were studied with hematoxylin-eosin, Masson's trichrome, and P.A.S. MacManus stains as described by Gomori.

Testicles were classified as "abnormal" when spermatocytes and spermatozoa were absent. An attempt was made to determine the cause of the atrophy. Testicles from the controls were larger than those from the treated birds.

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204

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As can be seen in Table 1, initial body weight

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Comb index (Gm./100 Gm.)</th>
<th>Testicles (Gm./100 Gm.)</th>
<th>Adrenals (mg./1000 Gm.)</th>
<th>Pituitary (mg./1000 Gm.)</th>
<th>Initial weight (Gm.)</th>
<th>Final weight (Gm.)</th>
<th>% increase in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vehicle-injected</td>
<td>12</td>
<td>17.54 ± 1.973</td>
<td>3.217 ± 0.367</td>
<td>127.7 ± 5.2</td>
<td>4.254 ± 0.478</td>
<td>492 ± 18.700</td>
<td>1404 ± 35.000</td>
<td>185.36</td>
</tr>
<tr>
<td>2. Estradiol 1 mg.</td>
<td>12</td>
<td>5.217 ± 1.003</td>
<td>1.564 ± 0.678</td>
<td>141.7 ± 9.1</td>
<td>4.820 ± 0.367</td>
<td>437 ± 27.200</td>
<td>1424 ± 62.000</td>
<td>225.85</td>
</tr>
<tr>
<td>3. Diethylstilbestrol 1 mg.</td>
<td>9</td>
<td>1.438 ± 0.270</td>
<td>0.293 ± 0.028</td>
<td>133.8 ± 36.1</td>
<td>3.152 ± 0.165</td>
<td>465 ± 22.053</td>
<td>1490 ± 94.015</td>
<td>220.43</td>
</tr>
<tr>
<td>4. Estradiol 1 mg.</td>
<td>12</td>
<td>13.725 ± 2.345</td>
<td>2.385 ± 0.577</td>
<td>146.5 ± 30.0</td>
<td>5.393 ± 0.526</td>
<td>386 ± 25.807</td>
<td>1314 ± 40.410</td>
<td>240.41</td>
</tr>
<tr>
<td>5. Androstanolone 1 mg.</td>
<td>12</td>
<td>25.537 ± 1.443</td>
<td>2.090 ± 0.594</td>
<td>143.3 ± 22.8</td>
<td>4.944 ± 0.336</td>
<td>426 ± 21.900</td>
<td>1218 ± 189.000</td>
<td>185.91</td>
</tr>
<tr>
<td>6. D5-Androstenediol 1 mg.</td>
<td>12</td>
<td>19.770 ± 1.323</td>
<td>4.060 ± 0.850</td>
<td>130.4 ± 9.90</td>
<td>4.015 ± 0.670</td>
<td>420 ± 22.900</td>
<td>1307 ± 47.000</td>
<td>211.19</td>
</tr>
<tr>
<td>7. Androstenediol 1 mg.</td>
<td>12</td>
<td>19.664 ± 2.105</td>
<td>3.770 ± 1.154</td>
<td>144.9 ± 27.4</td>
<td>4.309 ± 0.682</td>
<td>405 ± 19.341</td>
<td>1158 ± 206.000</td>
<td>185.92</td>
</tr>
<tr>
<td>8. Estradiol 0.5 mg.</td>
<td>12</td>
<td>22.111 ± 2.971</td>
<td>3.538 ± 0.518</td>
<td>132.5 ± 7.70</td>
<td>4.060 ± 0.425</td>
<td>465 ± 16.560</td>
<td>1319 ± 45.300</td>
<td>183.60</td>
</tr>
<tr>
<td>9. Androstanolone 0.5 mg.</td>
<td>12</td>
<td>22.844 ± 0.633</td>
<td>3.900 ± 0.800</td>
<td>119.4 ± 6.00</td>
<td>5.034 ± 0.436</td>
<td>495 ± 21.590</td>
<td>1414 ± 62.000</td>
<td>185.65</td>
</tr>
</tbody>
</table>

*Average ± standard error.

*P < .02

*P < .001
was very similar in all birds, although random differences were present in some of the groups. The chickens gained weight normally; it was maximal in those injected with 1 mg. of estrogens.

Aortic Macroscopic Atherosclerosis

Neither estrogens nor androgens modified aortic atherosclerosis, with the exception of diethylstilbestrol, which increased the incidence of lesions (table 2).

Coronary Microscopic Atherosclerosis

As shown in table 2, estradiol and diethylstilbestrol decreased coronary atherosclerosis, while the other injected substances, when considered individually, were without effect.

Chemical Determinations

Cholesterol determinations in plasma and in the aortic wall are shown in table 2. The only significant difference is seen in estradiol-treated birds having higher aortic cholesterol content and in the diethylstilbestrol group having higher cholesterolemia than their corresponding controls.

Pituitary and Adrenal Findings (Table 1)

No significant changes were induced by the injected drugs on pituitary and adrenal weights, nor on their histology.

Testicular Findings

Testicles, when blindly classified as normals or abnormals according to criteria stated under "Methods," differed considerably in their relative weights ($t = 8.074; P < .001$). Consequently, it was possible to study the effects of drugs simply by recording the weight of the glands. As shown in table 1, only estradiol and diethylstilbestrol were able to induce testicular atrophy.

Correlation of Several Parameters

As seen in table 3, birds were also classified under three headings: (a) "noninjected," including 34 animals given no injections or receiving saline or vehicle; (b) "estrogens," including 45 chickens from groups 3, 4, 5, and 9, i.e., those injected with estradiol, diethylstilbestrol or estriol; (c) "androgens," 48 cockerels from groups 6, 7, 8, and 10, i.e., those injected with androstanolone, Δ5-androstenediol and androstanediol. The inclusion of animals injected with hormonal preparations at dosages lower than those effective, such as estriol, for instance, provided intragroup controls for the determination of the "r" coefficient of correlations. As expected, a positive correlation was present between comb index and testicular weight in "noninjected" and in "estrogen" groups, but not in the "androgen"-treated group, since in these birds only the weight of the comb, but not of the testicles,

*These animals were the controls of another experiment performed simultaneously with the present one and they are not shown in the previous tables.
was increased. When testicular or comb weights were correlated with coronary lesions, it is seen that no correlation was found between these parameters.

Blood cholesterol levels showed great variability and were not significantly altered by either medication. Loose correlation was present between blood cholesterol levels and coronary atherosclerosis in "noninjected" and in "androgen" treated birds. In the "estrogen" group, no correlation was found between these parameters.

Discussion

Our results confirm previous reports showing that estriol is able to prevent coronary atherosclerosis in cholesterol-fed birds;\(^\text{13,14}\) negative results seen with another estrogen, namely estriol, might probably be related to low dosage, since coronary lesions have been prevented with this hormone.\(^\text{15}\) The other steroids tested here, although still having a C\(_{17}\) \(\beta\)-OH group, did not prevent coronary atherosclerosis. In spite of the fact, then, that estradiol-17\(\beta\) is active, and estradiol-17\(\alpha\) inactive in this respect,\(^\text{12}\) it is clear that the C\(_{17}\) \(\beta\)-OH group, per se, does not confer antatherogenic activity on a given steroid, which evidently must be related to structural configuration including the rest of the molecule. Furthermore, even the steroid nucleus itself is not necessary for such an action, since diethylstilbestrol, a stilbene derivative, was also very effective in preventing coronary atherosclerosis.

Consequently, it can tentatively be indicated that at the dosage used here: (1) antatherogenic activity is seen with estrogens having a \(\beta\)-oriented group at the steroid C\(_{17}\); (2) other nonestrogenic steroids having this group may not show such an action; (3) antatherogenic activity may be exhibited by molecules without steroid configuration.

The experiments here presented also indicate that prevention of coronary atherosclerosis may be effected by estrogens, even in animals without disturbed endocrine activity; in fact, no changes in adrenal or in pituitary weights were induced, although it is probable that more subtle methods of study might detect endocrine changes not shown by mere weighing of the organs. In connection with the relationship of the arterial changes induced by estrogens and its feminizing activity, the present experiments apparently show that the two effects can be separated. The evidence pointing to this conclusion is included in tables 1 and 3. In table 1, it is seen that when the animals were considered as a group, estradiol and diethylstilbestrol induced testicular atrophy and decreased comb index. Nevertheless, when the primary and secondary male characteristics were correlated against coronary atherosclerosis in birds individually, it is clear that no correlation existed between these parameters, as shown in table 3. Consequently, these findings showing that the endocrine and the arterial action of estrogens may be separated under appropriate conditions are opposite to those expressed preliminarily by Peck at al.,\(^\text{19}\) although they confirm our own previous reports.\(^\text{1,2,5,7,8}\)

### Summary

Cholesterol-fed chickens have been injected subcutaneously for eight weeks with the
following drugs: estradiol, diethylstilbestrol, estriol, androstanolone, Δ5-androstenediol and androstanediol. Aortic and coronary atherosclerosis, blood and aortic cholesterol, as well as several endocrine structures have been studied. Coronary atherosclerosis has been prevented by estradiol and by diethylstilbestrol, while the other steroids have been ineffective at the dosage used here. When birds were considered individually, no correlation was found between antiatherogenic activity and endocrine changes, thus confirming that testicular depression is not necessary for arterial protection. An attempt to correlate molecular structure and arterial changes has been carried out.

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