Testicular Action and Structural Relationships of Compounds with Antiatherogenic Activity

By Manuel René Malinow, M.D., Jaime A. Moguilevsky, M.D., Baltazar Lema, M.D., Grato E. Bur, M.D., and Aaron Erenfreyd, M.D.

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; 1, 2 similarly, in men active estrogens 3 but not estradiol-17α 4 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. 3 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. 6 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, 7 and in rabbits, 8 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-androstanediol; (7) androstenediol; 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 simultaneously 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. 9 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 trichromie, and P.A.S. MacManus stains as described by Gomori. 10 Testicles were classified as "abnormal" when sper.

*Manufacturer's composition: proteins 19 per cent; fats 5 per cent; carbohydrates 50 per cent; salts 10 per cent; rouphage 6 per cent; vitamin A 7,000U/100 Gm.; vitamin D 1,300U/100 Gm.; aureomycin 250 mg./100 Gm.
†Estradiol, Δ 1, 3, 5(10) estratriene, 3, 17, β-diol; diethylstilbestrol, 4, 4'-dihydroxy-a, β-diethyl- stilbene and androstanolone, androstane 3 one 17 βol, were obtained through the kindness of Dr. Esteban Montuori, from the laboratories of Dr. Gador, Buenos Aires; estriol, Δ 1, 3, 5(10) estratriene, 3, 16α 17 β-triol, was kindly obtained from Dr. Julio Ricci, from Schering Argentina, Buenos Aires; Δ 5 androstenediol, Δ 5 androstene, 3, 17 β diol, from Mann Research Laboratories; androstenediol, androstane, 3, 17 β diol, obtained from Ciba, Ltd., Basle. All drugs were processed into sterile vials through the cooperation of Dr. Esteban Montuori, from the laboratories of Dr. Gador, Buenos Aires.
Compositions with Antiatherogenic Activity

matogenesis was absent, or when they showed atrophy of the germ cells, or of the interstitial cells of Leydig. The aorta and the great vessels were examined macroscopically; the heart was fixed in 10 per cent formaldehyde and two segments parallel to the atrioventricular groove were totally cut into 10-μ frozen sections; five sections of each segment were chosen at random, stained with hematoxylin-Sudan IV and all arteries greater than 30 μ, clearly showing all structures, were classified as follows: grade 0.5, initial endothelial and/or subendothelial infiltration of isolated sudanophilic granules; grade 1.0, medium endothelial and/or subendothelial infiltration of sudanophilic granules; grade 1.5, marked endothelial and/or subendothelial sudanophilic infiltration forming a total or partial arterial obstruction; grade 2.0, initial endothelial and/or subendothelial cellular proliferation of isolated cells; grade 2.5, medium endothelial and/or subendothelial cell proliferation forming small plaques; grade 3.0, marked endothelial and/or subendothelial cell proliferation forming partial or total obstructions. All graded arteries were added in each segment; negative arteries were recorded and results expressed as grade per 100 arteries. A statistical study of 10 against 2, 4, 6, or 8 sections showed that results agreed in all cases; consequently, in the present paper, lesions found in 2 sections are recorded.

Cholesteryl was determined with Sperry and Webb's method in plasma and in an aortic extract obtained after continuous boiling in acetic-acetone for 12 hours. All observations were performed "blindly"; results were compared with Student's t-test, with the Chi square method, or with the correlation coefficient "r", according to the tables of Fisher and Yates.

Results

Body Weight

As can be seen in table 1, initial body weight...
Table 2
Atherosclerotic Lesions and Chemical Determinations in Chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Coronal macroscopic atherosclerosis</th>
<th>No. of birds</th>
<th>Blood cholesterol mg./100 ml.</th>
<th>No. of birds</th>
<th>Aorta cholesterol mg./100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vehicle-injected</td>
<td>None to minimal</td>
<td>12</td>
<td>10 278 ± 25.4</td>
<td>12</td>
<td>224.33 ± 27.53</td>
</tr>
<tr>
<td>2. Estradiol 1 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>11 242 ± 25.5</td>
<td>11</td>
<td>322.72 ± 36.68*</td>
</tr>
<tr>
<td>3. Diethylstilbestrol</td>
<td>None to minimal</td>
<td>9</td>
<td>8 341 ± 12.7*</td>
<td>8</td>
<td>277.00 ± 25.24</td>
</tr>
<tr>
<td>4. Estradiol 1 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>12 222 ± 16.3</td>
<td>9</td>
<td>322.33 ± 44.72</td>
</tr>
<tr>
<td>5. Androstanolone 1 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>9 233 ± 25.8</td>
<td>9</td>
<td>249.11 ± 21.85</td>
</tr>
<tr>
<td>6. Δ5-Androstenediol 1 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>12 280 ± 22.9</td>
<td>9</td>
<td>256.66 ± 28.86</td>
</tr>
<tr>
<td>7. Androstanediol</td>
<td>None to minimal</td>
<td>12</td>
<td>12 280 ± 30</td>
<td>10</td>
<td>229.20 ± 16.73</td>
</tr>
<tr>
<td>8. Estriol 0.5 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>9 238 ± 15</td>
<td>9</td>
<td>242.20 ± 21.34</td>
</tr>
<tr>
<td>9. Androstanolone 0.5 mg.</td>
<td>None to minimal</td>
<td>12</td>
<td>12 246 ± 26.1</td>
<td>11</td>
<td>290.40 ± 21.95</td>
</tr>
</tbody>
</table>

*P < .05
1P < .01

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 Macroscopic 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 estradiol; (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 estradiol, 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, was measured.

*These animals were the controls of another experiment performed simultaneously with the present one and they are not shown in the previous tables.
Table 3

Correlation Coefficient "r" Between Several Parameters in Cholesterol-Fed Chickens

<table>
<thead>
<tr>
<th>Group*</th>
<th>No. of birds</th>
<th>Combi index/ testicular weight</th>
<th>Testicular weight/coronary atherosclerosis</th>
<th>Comb index/coronary atherosclerosis</th>
<th>Coronary atherosclerosis/blood cholesterol levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninjected</td>
<td>34</td>
<td>0.44081</td>
<td>0.0519</td>
<td>0.0108</td>
<td>0.3901†</td>
</tr>
<tr>
<td>Estrogens</td>
<td>45</td>
<td>0.82221</td>
<td>0.0543</td>
<td>0.1566</td>
<td>0.0431</td>
</tr>
<tr>
<td>Androgens</td>
<td>48</td>
<td>0.2027</td>
<td>0.1519</td>
<td>0.1352</td>
<td>0.3377†</td>
</tr>
</tbody>
</table>

*(See text)

IPA < .02
IPA < .01

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 estradiol 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 anti-atherogenic 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) anti-atherogenic 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) anti-atherogenic 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 et al.,\(^\text{10}\) although they confirm our own previous reports.\(^\text{1, 2, 5, 7}\)

Summary

Cholesterol-fed chickens have been injected subcutaneously for eight weeks with the
following drugs: estradiol, diethylstilbestrol, estriol, androstanolone, \( \Delta^5 \)-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.

Acknowledgment

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References

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