The Effect of 19-Norandrostenolone on Experimentally-Induced Atheroma in Cockerels


The anabolic and weakly androgenic compound 19-norandrostenolone has been administered to cholesterol-fed cockerels in 2 dose levels. A marked fall in serum cholesterol occurred with the lower dose level and a significant reduction in the incidence of coronary artery lesion was noted with the higher dose level.

TESTOSTERONE has been found to inhibit coronary atherogenesis and to reduce plasma cholesterol, phospholipid, and cholesterol-phospholipid ratio in the cholesterol-fed pullet. In the male bird Katz et al. found neither regression of coronary artery lesion nor significant shift in serum lipids after administration of testosterone, although the dose levels of 0.2 to 1 mg. daily used by these workers were smaller than the 3 mg. per day level used by Cook and his co-workers.

In the human male patient with coronary disease, methyl testosterone was found to inhibit in part the lipid reducing effect produced by ethinyl estradiol. When given alone, it had no influence on plasma cholesterol or the C/P ratio.

The weaker androgen methyl oestrenolone will reduce plasma cholesterol in normal dogs, but disagreeable, if not alarming, side-effects have precluded its general use in man. The related compound 19-norandrostenolone phenyl propionate (NAPP) is anabolic and weakly androgenic, with the ability to conserve protein and calcium, and can be given to human subjects without significant side-effects. The experiment described below was designed to study the effects of NAPP on experimentally induced atheroma and such lipid changes as might occur in the blood.

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Supported by a grant from the Scottish Hospital Endowments Research Trust.

Received for publication July 23, 1959.

Methods

Sixty-four Golden Legbar X Light Sussex Cockerels, a breed previously tested for susceptibility to experimental atheroma, were obtained at the age of 8 weeks from The West of Scotland Agricultural College Poultry School. The birds were kept in individual cages and were fed ad libitum on the particular diet for 8 weeks, with the exception of 1 group which was killed at the end of 5 weeks to prove the establishment of lesions prior to treatment. The daily food intake and body weights were measured weekly (tables 1 and 2). The experiment included the following groups: (A)—8 birds fed commercial chick mash for 8 weeks; (B)—12 birds fed the atherogenic diet, consisting of commercial chick mash supplemented by 2 per cent cholesterol and 5 per cent cottonseed oil, for 5 weeks; (C)—12 birds fed the atherogenic diet for 8 weeks; (D)—12

Table 1

Food Intake (Gm./day)—Group Means

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Group A</td>
<td>80</td>
<td>107</td>
<td>111</td>
<td>114</td>
<td>133</td>
<td>122</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>80</td>
<td>104</td>
<td>111</td>
<td>116</td>
<td>122</td>
<td>122</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>80</td>
<td>105</td>
<td>109</td>
<td>109</td>
<td>117</td>
<td>118</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>80</td>
<td>105</td>
<td>116</td>
<td>117</td>
<td>117</td>
<td>119</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Group E</td>
<td>80</td>
<td>130</td>
<td>112</td>
<td>115</td>
<td>111</td>
<td>126</td>
<td>125</td>
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</tr>
</tbody>
</table>

Table 2

Body Weight (Gm.)—Group Means

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>824</td>
<td>944</td>
<td>1174</td>
<td>1364</td>
<td>1641</td>
</tr>
<tr>
<td>Group B</td>
<td>736</td>
<td>901</td>
<td>1149</td>
<td>1385</td>
<td>1536</td>
</tr>
<tr>
<td>Group C</td>
<td>712</td>
<td>901</td>
<td>1134</td>
<td>1353</td>
<td>1502</td>
</tr>
<tr>
<td>Group D</td>
<td>749</td>
<td>1001</td>
<td>1121</td>
<td>1455</td>
<td>1638</td>
</tr>
<tr>
<td>Group E</td>
<td>736</td>
<td>861</td>
<td>1122</td>
<td>1458</td>
<td>1510</td>
</tr>
</tbody>
</table>
Table 3
Mean values for plasma cholesterol (CHOL) mg./100 ml., plasma phospholipids (PH) mg./100 ml. and cholesterol-phospholipid ratio (C/P)

<table>
<thead>
<tr>
<th>Group</th>
<th>0 week</th>
<th>5 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHOL</td>
<td>PH</td>
<td>G/P</td>
</tr>
<tr>
<td>A</td>
<td>129</td>
<td>8.1</td>
<td>17.0</td>
</tr>
<tr>
<td>B</td>
<td>167</td>
<td>7.7</td>
<td>22.8</td>
</tr>
<tr>
<td>C</td>
<td>156</td>
<td>7.7</td>
<td>21.4</td>
</tr>
<tr>
<td>D</td>
<td>87</td>
<td>5.5</td>
<td>17.7</td>
</tr>
<tr>
<td>E</td>
<td>85</td>
<td>6.1</td>
<td>14.2</td>
</tr>
<tr>
<td>F</td>
<td>117</td>
<td>8.4</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Table 3: Mean values for plasma cholesterol (CHOL) mg./100 ml., plasma phospholipids (PH) mg./100 ml. and cholesterol-phospholipid ratio (C/P).

Birds fed the atherogenic diet for 8 weeks; from the 6th to the 8th week inclusive they were given 5 mg. NAPP intramuscularly per day; (E)—12 birds treated as in Group D, except that NAPP was administered in a dose of 1 mg. intramuscularly per day; and (F)—5 birds fed commercial chick mash; from the 6th to 8th week inclusive they were given 1 mg. NAPP intramuscularly per day.

Blood was taken from an alar vein into heparinised tubes at the beginning of the experiment and at the end of the fifth and the eighth weeks. Each sample of blood was analysed for cholesterol by a modification of the Sperry-Schoenheimer method and for phospholipids by the method of King. Group B was killed at the end of the fifth week and at the end of 8 weeks.

The aortas and brachiocephalic vessels were examined for atheroma and the degree of atheroma was graded as follows: Slight atheroma implies focal lesions up to about 2 mm. in diameter, of white or pale cream colour, accompanied by minimal gross thickening of the intima. Moderate lesions consisted of plaques up to about 5 mm. in diameter, cream or ivory-yellow in colour, with distinct intimal thickening and longitudinal (“tree-trunk”) ridging. In severe cases, larger plaques of cream to yellow colour merged into diffuse atheroma. There was distinct thickening, ridging and sometimes distortion of the wall.

The hearts were fixed in 10 per cent formal saline and 3 transverse slices taken through mainly ventricular areas with the exclusion of the apex. One frozen section was prepared from each slice and stained by Sudan IV and haemalum. A microscopic count of atheroma in the coronary arteries was then carried out, any sudanophilic intimal thickening or plaque being regarded as a positive finding. Not infrequently the intima or media of a vessel retained some Sudan IV but showed no structural abnormality. This was regarded as a negative feature. The incidence of atheroma was expressed as a percentage for the group.

Results
Plasma Cholesterol, Phospholipid and Cholesterol-Phospholipid (C/P ratios)

Group mean values for plasma cholesterol, phospholipid and C/P ratios are presented in Table 3; the statistical methods employed were the analysis of variance and analysis of co-variance. At the end of 5 weeks, i.e., immediately prior to the commencement of drug treatment, no significant difference was found between the cholesterol-fed groups (B, C, D and E). In Group E (given 1 mg./day) a significant fall in the plasma cholesterol and C/P ratio occurred; at 8 weeks the values showed a highly significant difference from those of the cholesterol-fed controls (Group C). In contrast the values for plasma cholesterol, phospholipid and C/P ratios in the group which had received 5 mg. of the drug daily (Group D) were not significantly different from the cholesterol-fed controls. The fall in cholesterol level between the end of the fifth and eighth weeks in Group D is not statistically different from that in Group C.

Atheroma

The effects of NAPP on aortic and coronary arterial atheroma are summarized in Tables 4 and 5. A daily dosage of 1 mg. (Group E) diminished the severity of aortic atheroma. In addition, the incidence of coronary atheroma was reduced to 18.6 per cent as compared with 37.3 per cent in the control Group C, but this reduction was not statistically significant. On the other hand, in a daily dosage of 5 mg. (Group D) there was not only a reduction in the severity of aortic
Table 4
Incidence of Aortic Lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence of aortic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
</tr>
<tr>
<td>Group A</td>
<td>11</td>
</tr>
<tr>
<td>Group B</td>
<td>0</td>
</tr>
<tr>
<td>Group C</td>
<td>0</td>
</tr>
<tr>
<td>Group D</td>
<td>0</td>
</tr>
<tr>
<td>Group E</td>
<td>0</td>
</tr>
<tr>
<td>Group F</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 5
Incidence of Coronary Artery Lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of chicks with lesions</th>
<th>No. of vessels counted</th>
<th>% Coronary arteries with lesions (Group Means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>12</td>
<td>401</td>
<td>25.0</td>
</tr>
<tr>
<td>Group C</td>
<td>12</td>
<td>615</td>
<td>37.3</td>
</tr>
<tr>
<td>Group D</td>
<td>11</td>
<td>520</td>
<td>16.2</td>
</tr>
<tr>
<td>Group E</td>
<td>12</td>
<td>498</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Sexual Inhibition

In the cholesterol-fed group receiving the smaller dosage (Group E) 3 out of 12 birds (25 per cent) showed testicular hypoplasia. With the larger dose of hormone (Group D), hypoplasia affected 9 out of 12 birds (75 per cent) and was greater in degree. Growth of the secondary sex organs (comb and wattles) was inhibited in only one bird in each group.

Discussion

Several points of interest arise from this experiment. It would appear that NAPP in a daily dosage of 1 mg. per day has the capacity to lower plasma cholesterol in cockerels fed an atherogenic diet containing 2 per cent cholesterol. This reduction in serum cholesterol was associated with diminished severity of atherosomatic process in the aorta. In spite of the fall in the C/P ratio it did not produce a significant regression of lesions in the coronary arteries. This may be merely a question of degree because the reduced level of the C/P ratio, although significantly lower than the value for the control group receiving the atherogenic diet (Group C), was insufficient to bring the C/P ratio back to within normal limits. It is noteworthy that regression of coronary atheroma by potent estrogenic drugs is associated with a return of the C/P ratio to within normal limits, and the possibility exists that had this drug been given throughout the period of cholesterol feeding, i.e., as a prophylactic, a significant reduction in the incidence of coronary atheroma might have resulted.

The ability of a substance with weak androgenic properties to lower plasma cholesterol and reduce the severity of aortic atheroma in the male bird is contrary to the previous report on the effects of the androgenic substance testosterone. It may be that androgenicity is not the operative factor in this effect and the recent reports of Nishida et al and Stamler et al on the protective action of dietary protein on cholesterol-induced atheroma in chicks suggest an alternative explanation. These workers noted independently that an increased content of protein in the diet had a protective action against experimentally-induced atheroma in the chick and that this effect was associated with a lowering of plasma cholesterol. NAPP is a powerful anabolic substance with the ability to conserve protein and it is possible that its action was mediated through its effect on protein metabolism.

The results obtained with a daily dosage of 5 mg. are also of interest. In spite of apparently androgenic side-effects, it was able to bring about a significant regression of atheroma in the aorta and coronary arteries without affecting the plasma cholesterol level or cholesterol-phospholipid ratio.

It is not possible to give an adequate explanation for the different effects of NAPP, in the 2 doses employed. This drug is known to have anabolic and androgenic properties so that these effects must be considered in any explanation. It is possible that the ratio of anabolic to androgenic activity varies according to the dosage used and that this in part,
at least, explains the different effects in the 2 doses employed. It may be, however, that the action of the drug, in the higher dosage, is directly on the vessel wall and unrelated to its anabolic and androgenic actions. The effect of other anabolic substances, in high dosage, on experimental atheroma might help to answer this question. Whatever the explanation, the fact remains that a significant regression of atheromatous lesions induced by cholesterol feeding was achieved without a commensurate fall in the plasma cholesterol level or in the cholesterol-phospholipid ratio.

Summary

The effects of 19-norandrostenolone phenyl propionate on cholesterol-induced atheroma in cockerels are described. In a dosage of 1 mg. intramuscularly per day it produced a significant reduction in plasma cholesterol levels and C/P ratio. This was accompanied by a regression of aortic lesions and an appreciable, but not statistically significant regression of coronary artery lesions.

In a dosage of 5 mg. daily it produced a regression of aortic lesions and a statistically significant reduction in the incidence of coronary arterial lesions without any significant effect on plasma cholesterol and phospholipid levels.

Further work is necessary to elucidate the mechanism of these effects.

Acknowledgments

We are indebted to Dr. J. H. Wright and Professor J. W. Emusie for their guidance and helpful criticism, to Dr. Hewett of Organon Laboratories Ltd. for his kindness in supplying the drug and to Dr. R. A. Robb for his aid with the statistical analysis of results.

Summario in Interlingua

Es describite le effectos de propionato phenylic de 19-norandrostenolona super le atheroma inducito per cholesterol in gallettos. In un dosage de 1 mg per die, administrate per via intramuscular, le droga pro-
duceva un significative reduction del nivelllos de chol-
estero in le plasma etiam del proportion de chol-
estero a phospholipido. Isto esseva accompagnate per un regression del lesiones aortic e citam per un ap-
preciabile ben que statisticamente non significative regression del lesions in le arterias coronari.

In un dosage de 5 mg per die, le droga pro-
duceva un regression del lesiones aortic e un statisticamente significative reduction del incidentia de lesions del arteria coronari sin ull significative effecto super le

nivelllos plasmatic de cholesterol e de phospholipido.

Labores additional es necessari pro elucidar le me-
chanismo de iste effectos.

References

1. COOK, D. L., EDGREN, R. A., AND HARRIS, T. W.: Androgen inhibition of atherogenesis in pul-
2. KATZ, L. N., STAMLER, J., PICK, R., AND ROD-
BARD, S.: Effect of testosterone and chorionic
gonadotrophin on estrogen-induced inhibition
of coronary atherogenesis in cholesterol-fed
3. OLIVER, M. F., AND BOYD, G. S.: Influence of
sex hormones on the circulating lipids and
lipoproteins in coronary sclerosis. Circulation,
4. SCHOENHEIMER, R., AND SPERRY, W. M.: A micro-
method for the determination of free and com-
bined cholesterol. J. Biol. Chem. 106: 745,
1934.
5. KING, E. J., AND WOODS, I. D. P.: Micro-
analysis in Medical Biochemistry. London,
J. and A. Churchill Ltd., 1956, P. 57.
6. PICK, R., STAMLER, J., ROBBA, S., AND KATZ,
L. N.: Estrogen-induced regression of coronary
atherosclerosis in cholesterol-fed chicks. Circu-
lation, 6: 558, 1952.
7. NISHIDA, T., TAKENAKA, F., AND KUMMEROW, F.
A.: Effect of dietary protein and heated fat on
serum cholesterol and beta-lipoprotein levels,
and on the incidence of experimental athero-
sclerosis in chicks. Circulation Research, 6:
104, 1958.
8. STAMLER, J., PICK, R., AND KATZ, L. N.: Effects
of dietary protein and carbohydrate level on
cholesterolemia and atherogenesis in cockerels
on a high-fat, high-cholesterol mash. Circula-
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Circ Res. 1960;8:78-81
doi: 10.1161/01.RES.8.1.78

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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