Influence of Sex Difference and Hormones on Elastine and Collagen in the Aorta of Chickens

By José Cembrano, M.D., Manuel Lillo, Ph.G., José Val, Ph.G., and Jorge Mardones, M.D.

In previous papers, we have reported that the incidence of atheromatosis of the aorta induced in chickens by cholesterol-rich diet was significantly higher in males than in females. The results obtained in our laboratory in various experimental groups show that clear macroscopic lesions were observed in 41 out of 54 males and in 15 out of 69 females, maintained on a cholesterol-rich diet for 90 to 150 days. The \( \chi^2 \) calculated with these data is 35.9, meaning that the difference is highly significant. The comparison of cholesterol, total lipids and lipid phosphorus levels, observed in blood of chickens of both sexes fed with cholesterol-rich diet and with and without macroscopic atheromatosis of the aorta, showed that the differences were too small and inconsistent to explain the significant difference of the susceptibility to experimental atheromatosis observed in both sexes.

Buddecke studied the chemical composition of the aorta altered by atheroma in human beings and in hens. He reported that concerning nonlipid components, elastine was lower, and "ground substance" higher, in atheroma than in normal aorta. Collagen was higher in atheroma of the hens; but nonconclusive changes were observed in human beings. These structural changes appear to be the consequence of the atheromatosis.

On the other hand, it is possible that structural changes of the subintimal layers may induce different resistance to the diffusion of cholesterol through the arterial wall, and thus might play a role in the pathogenesis of atheromatosis.

In order to know whether or not the sex differences in susceptibility to atheromatosis are related to structural differences of subintimal layers of the aorta, we have studied the elastine and collagen content of this artery in adult chickens of both sexes fed with stock diet.

An initial study on normal chickens showed that the elastine content of the aorta was higher in males than in females, but the difference in collagen content was not significant. Experiments were then repeated in a second group of chickens in which the influence of gonadectomy and estradiol in males and of testosterone in females was also studied.

Methods

Two experiments were performed. In the first, collagen and elastine were estimated in the aorta of hybrid (Leghorn and Rhode Island) chickens of both sexes maintained in the laboratory fed with stock diet. In the second, the same study was performed on Leghorn chickens of both sexes fed with stock diet and submitted to various experimental conditions, namely: gonadectomy or estradiol treatment in males, testosterone treatment in females.

 Estradiol and testosterone were given in pellets of 25 mg., placed in the subcutaneous tissue of the higher part of the neck. Gonadectomy and pellets implantation were performed at the age of four months. Chickens were killed 1 or 2 months later, and the thoracic and abdominal aorta was obtained by dissection. All the surrounding tissues were carefully removed.

The total aorta was weighed and then minced with scissors and treated successively with acetone (15 ml., 6 hours, 2 times) and diethylether (15 ml., 6 hours, 2 times) at room temperature. Then it was dried to constant weight. To an aliquot weighing 12 to 13 mg. were added 4 ml. of water; it was heated in the autoclave at 15 pounds of pressure for 3 hours. This extraction was repeated 3 times and the extracts were pooled and dried in a boiling water bath under air current. This pool contained 95 to 98 per cent of the collagen. The residue was submitted again to an extraction with 4 ml. of water in the autoclave at 15 pounds for 3 hours; this extraction was rejected. The final residue contained the elastine and practically no collagen.

Collagen and elastine were estimated in the...
Table 1
Collagen and Elastine of the Aorta of Chickens of Both Sexes, Hybrids of Leghorn and Rhode Island, Six- to Seven-Months Old and Fed with Stock Diet

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Collagen % m ± s*</th>
<th>Range</th>
<th>Hydroxyproline from elastine % m ± s*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>8</td>
<td>33.7±2.8</td>
<td>25.3-45.5</td>
<td>12.4±0.06</td>
<td>0.7-1.6</td>
</tr>
<tr>
<td>Females</td>
<td>10</td>
<td>29.0±1.6</td>
<td>20.9-43.3</td>
<td>0.7±0.05</td>
<td>0.4-1.2</td>
</tr>
</tbody>
</table>

*A-Arithmetic mean ± its standard error.

Table 2
Collagen and Elastine of the Aorta of Leghorn Chickens, Five- to Six-Months Old, Submitted to Different Treatments and Fed with Stock Diet

<table>
<thead>
<tr>
<th>Sex treatment</th>
<th>N</th>
<th>Collagen % m ± s*</th>
<th>Range</th>
<th>Hydroxyproline from elastine % m ± s*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Control</td>
<td>14</td>
<td>22.8±0.89</td>
<td>18.7-29.1</td>
<td>1.03±0.02</td>
<td>1.2-1.5</td>
</tr>
<tr>
<td>F Control</td>
<td>11</td>
<td>17.6±0.89</td>
<td>12.7-23.1</td>
<td>0.01±0.04</td>
<td>0.7-1.1</td>
</tr>
<tr>
<td>M Gonadectomy</td>
<td>9</td>
<td>18.4±0.89</td>
<td>13.4-21.6</td>
<td>0.22±0.06</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td>M Estradiol</td>
<td>14</td>
<td>19.6±1.09</td>
<td>11.9-26.1</td>
<td>0.95±0.06</td>
<td>0.5-1.3</td>
</tr>
<tr>
<td>F Testosterone</td>
<td>12</td>
<td>20.9±0.87</td>
<td>17.2-26.9</td>
<td>1.19±0.06</td>
<td>1.0-1.7</td>
</tr>
</tbody>
</table>

*A-Arithmetic mean ± its standard error.

The collagen of the aorta is expressed in mg./100 nig. of dry lipid free aorta, using the factor 7.46 to convert the figures of hydroxyproline into those of collagen. The data available show that the hydroxyproline content of collagen is constant enough in the samples obtained from different tissues and animal species.

The elastine is expressed as milligram of hydroxyproline liberated by hydrolysis of this protein by 100 nig. of dry lipid-free aorta. Elastine from different sources contains variable proportions of hydroxyproline and in that obtained from the aorta of chickens, this proportion is not constant. Thus, it is not possible to convert with accuracy the figures of hydroxyproline into elastine.

Results

Sex Differences

The comparison of the elastine of the aorta of males and females (table 1 and control chickens in table 2) show that it was significantly higher in males than in females (t = 5.8 and 9.6, respectively; P < 0.001).

In the second experiment (table 2), the collagen content was significantly higher in males (t = 4.1; P < 0.001). The difference observed in the first experiment (table 1) was not significant (t = 1.9; P > 0.05), perhaps because of the dispersion of the data. The genetic heterogeneity of these chickens could explain this dispersion. Furthermore, they were older than those in the second experiment.

Influence of Gonadectomy in Males

Gonadectomized males exhibited significantly less elastine (t = 6.1; P < 0.001) and collagen (t = 3.5; P < 0.01) in the aorta, than control males (table 2). The figures were very similar to those exhibited by the control females.

Influence of Estradiol in Males

Males treated with estradiol showed a significantly lower elastine (t = 5.6; P < 0.001) and collagen (t = 2.3; P < 0.05) than control males. The figures were not different from those of gonadectomized males and control females (table 2). In every chicken treated with estradiol, an atrophy of the comb was present. Autopsy revealed that the testes weighed significantly less than those of the control males (2.33 ± 0.44 versus 8.53 ± 0.27 Gm.; t = 12.16; P < 0.001). Thus, the influence of the treatment with estradiol on the elastine and collagen of the aorta wall could be either a direct effect of the hormone, or an indirect one, mediated by reducing the activity of the testes.
Influence of Testosterone

In females treated with testosterone, the elastin and collagen were significantly higher than in control females (t = 4.1; P < 0.001 for elastin and t = 2.7 P < 0.02, for collagen). These data do not differ from those of control males (table 2).

Discussion

Our experimental results indicate that in chickens the proportion of the lipid-free aorta formed by elastin and collagen is higher in males than in females. This difference seems to be determined by the hormones of the testes, because 1 month after the removal of these organs, the proportion of elastin and collagen of the aorta wall was about the same as that observed in normal females. Furthermore, the administration of testosterone to females increased this proportion to the level observed in normal males.

The role played by the ovarian hormones is not clear. The effect of estradiol that reduced in males the proportion of elastin and collagen to the levels exhibited by females could be explained by the inhibition of testicular activity induced by this estrogen. The influence of gonadectomy in females should be studied in order to clarify this question.

The relationship between this secondary sex character and the sex difference in the susceptibility of chickens to experimental atheromatosis of the aorta induced by feeding cholesterol-rich diet, reported by Cembrano et al.1 deserves further study in order to establish whether or not structural changes inducing differences in the diffusion of cholesterol through the subintimal layers could be an important factor in the pathogenesis of atheromatosis.

As the sex differences of elastin and collagen proportion of the lipid-free aorta are in the same sense, it is not impossible that they could be the consequence of the increase of another substance, different from lipid and water.

Summary

Collagen and elastin of the aorta were estimated in normal chickens of both sexes in cockerels, gonadectomized or treated with estradiol, and in hens treated with testosterone.

The results showed that collagen and elastin were significantly higher in males than in females. Gonadectomy in males decreased significantly the content of collagen and elastin, so that the values became similar to those observed in females. The treatment of males with estradiol lowered the collagen and elastin content to values similar to those observed in females and in gonadectomized males. The treatment of females with testosterone increased significantly the collagen and elastin content of the aorta to levels similar to those observed in males.

Summario in Interlingua

Le collageno e la elastina del aorta esseva estimate in gallinas de ambe sexos, in juvane gallos che labeva essite gonadectomisate o tractate con estradiol, e in gallinas feiin che labeva essite tractate con testosterona. Le resultatos monstrava che le contento de collageno e de elastina esseva significativemente plus alto in masculos que in femininas. Gonadectomia in masculos reduceva significativamente le contento de collageno e de elastina, de maniera che le valores deveniva simile a illos observate in femininas. Le tractamiento de masculos con estradiol reduceva le contento de collageno e de elastina a valores simile a illos observate in femininas e in masculos gonadoc- tomisate. Le tractamiento de femininas con testosterona augmentava significativamente le contento de collageno e de elastina del aorta, usque a niveles simile al niveles observate in masculos.

References

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JOSÉ CEMBRANO, MANUEL LILLO, JOSÉ VAL and JORGE MARDONES

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