Coronary Arteriosclerosis in Dogfish
(Scyliorhinus canicula)
An Assessment of Some Potential Risk Factors

Lina García-Garrido, Ramón Muñoz-Chápuli, and Victoria de Andrés

Coronary myointimal lesions are described in the dogfish (Scyliorhinus canicula). These lesions are similar to those previously described in salmonids and are characterized by breaks in and disappearance of the inner elastic layer and intimal thickening as a result of inclusions of fibers and smooth muscle cells. Lesions are associated with all the branching points in the main subepicardial conal coronary arteries that supply the heart. Intimal thickenings were rare in other parts of these arteries. However, we found extensive lesions unassociated with branching points in two main intramyocardial ventricular arteries that supply the ventricular spongy myocardium. We carried out a statistical study of the incidence and severity of these intramyocardial lesions in relation to several potential risk factors. Intimal thickenings were present in 90.5% of the fish specimens and 40% of the histological sections. Sex, reproductive stage, plasma triacylglycerol, and cholesterol (total and related to high-density lipoproteins) were not significantly related to either the incidence or severity of lesions. Total fish length was significantly correlated with the lesion severity index ($r=0.33$, $p<0.01$). We also found significant differences in incidence related to the location of lesions. The middle areas of the intramyocardial branches, very close to the atrioventricular canal, were more affected than the cranial and caudal areas. The dorsal and ventral artery walls were also more affected than the lateral ones. The preferential location of the lesions in areas presumably subjected to mechanical stress because of a bifurcating bloodstream or the pulsatile flow throughout the atrioventricular canal suggests that coronary arteriosclerosis in dogfish is an age-related process, with hemodynamic factors playing a primary or secondary pathogenetic role. This disease seems not to be related to some factors suggested for salmonids, such as reproductive cycle, anadromous migration, river pollution, or plasma lipid concentration. (Arteriosclerosis and Thrombosis 1993;13:876–885)

KEY WORDS • coronary arteries • arteriosclerosis • myointimal hyperplasia • cholesterol • serum lipids • risk factors • dogfish • sharks • elasmobranchs

Coronary arteriosclerosis in fish has been known for three decades.1 Lesions reported in fish are characterized by thinning, breaking, or replication of the inner elastic layer and intimal proliferation of smooth muscle cells apparently proceeding from the muscular media layer. The smooth muscle cells are accompanied by extracellular collagen and elastin, generally without lipid deposition. The endothelial cell layer remains intact, although its cells appear highly vacuolated. The media is thinned, and its muscle fibers are disrupted.2–10 Several pathogenetic factors have been suggested. Since this disease was discovered and extensively studied in salmonids, physiological stress related to anadromous migration involving increased plasma 17-hydroxycorticosteroids has been proposed as a main causal mechanism.11 Other factors associated with developing coronary arteriosclerosis are alteration in sex hormone status during maturation,7,12 environmental mutagens and poisons,8,9 and high levels of apolipoprotein B (apo B)–containing lipoprotein, either spontaneous13 or induced by diet.14,15 It is uncertain whether some of these associations can be considered cause-and-effect relations.

Hemodynamic factors have only recently been considered in fish coronary arteriosclerosis. The reported absence of coronary arterial lesions in five species of elasmobranchs in relation to their high prevalence in salmonids has been attributed to the mechanical stress produced in the coronary artery of the latter by the distension of the bulbus arteriosus during ejection of blood from the ventricle.16,17 The importance of hemodynamic factors in early intimal thickening of human coronary arteries has been stressed.18–22 If intimal thickenings can be promoted in the fish artery wall by alterations in blood pressure and flow, their incidence and severity should be larger in branching points, ostia, curves, and taperings of the blood vessel or other definite areas subjected to mechanical stress.

We have described elsewhere the occurrence of coronary arteriosclerosis in dogfish (Scyliorhinus canicula)
with characteristics similar to those found in salmonids. This study reports on the incidence and severity of coronary arteriosclerosis in this fish in relation to such factors as sex, size, reproductive cycle, and serum lipids. We also compare the presence of lesions in subepicardial and intramyocardial vessels and investigate whether definite areas of the vessels are more prone to develop lesions.

**Methods**

One hundred five specimens of dogfish (52 males, 53 females) were used in our study. All were caught in Málaga Bay (western Mediterranean Sea) by commercial trawlers between April and September, 1987. The specimens were kept in tanks on board and anesthetized with 0.04% tricaine methane sulfonate (Sigma Chemicals, UK) when landed. The heart was dissected, rinsed, and fixed in 4% (wt/vol) formaldehyde neutralized with MgCO$_3$. Samples of blood were drawn from the pericardial cavity. The total length of the fish was measured, and the gonads were dissected and assessed to determine their reproductive stage. Three reproductive stages were established: immature, mature, and spawning. Immature specimens are characterized by claspers, undeveloped testes and ductus deferens (males), and the ovary lacking developed ovocytes (females). Specimens bearing large testes (males) and large ovocytes in the ovary (females) were considered mature. Spawning dogfish showed the same morphological characteristics as the mature ones, but males had sperm in seminal vesicles and females carried capsulated eggs in the ovisacs.

At the laboratory, blood was allowed to clot, and serum was obtained by low-speed centrifugation. Serum samples were stored at -20°C until analyzed a few days later.

All the analytical procedures were made with commercial kits supplied by Selavo (Siena, Italy). Total cholesterol was measured by the CHOD-PAP enzymatic-colorimetric method. High-density lipoprotein (HDL) cholesterol was isolated by selective precipitation of low- and very-low-density lipoproteins with Mg$^{2+}$/dextran sulfate and then measured by the CHOD-PAP method. Triacylglycerol was determined enzymatically by the Trigly-Cinet method.

The hearts were dehydrated and embedded in paraffin wax, and 8-μm sections were obtained from four different levels of the conus arteriosus and ventricle. A separation of 0.2–0.4 mm was allowed between two consecutive sampling levels, depending on the heart size. At least 50 serial sections were sampled from each level. Thus, more than 200 sections were examined from each heart. Sections were stained with Weigert's resorcin-fuchsin elastic stain.

Six large coronary arteries were studied (Figure 1). There are usually four subepicardial arteries on the conus arteriosus, two laterodorsal and two lateroventral. Sometimes the number of conal arteries was reduced to two or three because of either individual variation or the level of section, beyond the origin of some conal artery. Four arteries were observed in more than 60% of the specimens. They arise from the hypobranchial artery and are the main coronary arteries supplying the dogfish heart. These vessels give rise to the subepicardial network of ventricular coronary arteries, which invest the compact layer through short perforating branches, and to some perforating ventricular branches, which supply part of the trabeculated myocardium.

The other two vessels investigated were a pair of large intramyocardial ventricular arteries that run at the left and right sides of the atrioventricular (AV) junction. We called them the left and right intramyocardial ventricular branches (LIVB and RIVB, respectively).

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**FIGURE 1.** Diagram showing dogfish conus arteriosus (C), ventricle (V), and the coronary arteries studied. Panel A: Lateral view. Numbers show the areas defined for the intramyocardial branches, which are represented with dotted lines. Panel B: Transverse section, posterodorsal view. AV, atrioventricular canal; LIVB, RIVB, left and right intramyocardial ventricular branches, respectively; SCA, subepicardial conal arteries; VOT, ventricular outflow tract.
The LIVB arises from the left ventricular branch, a subepicardial artery that usually originates in the conoventricular groove from the left laterodorsal conal coronary artery. The left ventricular branch runs along the left AV groove. The RIVB has a more cranial origin, arising from a dorsal ventricular branch of the left laterodorsal conal coronary artery. The LIVB and RIVB produce a few small branches around the AV canal, and they split beyond the causal level of the AV junction to invest the spongy myocardium of the caudal ventricle. The LIVB-derived branches supply the left lateroventral area, and those derived from the RIVB invest the right laterodorsal part.

To establish whether there was a preferential localization of the lesions, six areas of the intramyocardial arteries were considered, from their cranial origin to their causal splitting. These areas are shown in Figure 1A.

The radial positions of the intramyocardial artery lesions were determined by the position of its medial axis. Twelve possible radial positions (1–12) were considered clockwise. Positions 3, 6, 9, and 12 were taken as right, ventral, left, and dorsal, respectively. Double lesions located in opposite sides of the artery wall were considered separately. Deviation of the observed radial position frequencies with respect to expected values, assuming random locations, were assessed by $x^2$ tests.

Three indexes were used to evaluate the extension of the coronary lesions. Incidence index was defined as the proportion of histological sections in which at least one lesion was present in the arteries studied. Lesion severity index (LSI) was defined according to Farrell et al.$^{15}$ and Saunders and Farrell$^{25}$ except that our range of lesion grades was from 1 to 5, so as to include the largest lesions occurring most of the lumen:

$$LSI = \frac{[(1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) + (5 \times n_5)]}{(n_1 + n_2 + n_3 + n_4 + n_5)}$$

where $n$ is the number of cross sections with a grade equivalent to the subscript. The proportion of affected individuals (PA) was defined as the number of specimens showing at least one coronary artery lesion in a given group (defined by sex, age, reproductive stage, etc.) divided by the number of specimens of this group.

Significant differences between groups were determined by Student's $t$ test or one-way analysis of variance (ANOVA). For the latter purpose, four length classes were arbitrarily defined: 270–400, 401–450, 451–500, and >500 mm.

**Results**

The lesions were characterized by breakage and disappearance of the inner elastic layer and intimal thickening containing cells and fibers (Figures 2 and 3).

Staining features suggested that the fibers were collagen. The cells observed under light microscopy showed morphological characteristics of smooth muscle cells with rounded nuclear profiles, probably arranged longitudinally to the vessel axis. The underlying media layer frequently appeared thinned and richer in fibers than the media on normal areas. Endothelial nuclei were usually observed over the lesions, but thrombi or aggregations of blood cells were never seen. Not even the larger thickenings showed a lesion core, foam cells, or extracellular lipids.

**Subepicardial Conal Coronary Arteries**

These vessels appeared relatively unaffected by lesions unrelated to branching points. Among 90 specimens in which the subepicardial arteries were sampled, only 13 specimens showed 16 lesions of this type (PA, 0.14). All these lesions were moderate and were composed of one or a few intimal nodules containing <10 nuclei in a transverse section (grade 1, Figure 2B).

However, all subepicardial coronary artery branchings (195 were observed) showed breakage, replication, or disappearance of the inner elastic layer and diffuse intimal thickening (Figures 2C–2F). These thickenings frequently extended 100–200 $\mu$m upstream of the branching point (range, 24–312 $\mu$m) but only 10–40 $\mu$m downstream (range, 8–80 $\mu$m). The vessel lumen was not significantly reduced.

Although an extensive study was not carried out, the larger subepicardial ventricular arteries showed similar intimal thickenings in their branching points. The incidence index of these lesions depended on the number of branchings in the sampled sections, and their severity was difficult to assess because of the variable position of the branches in relation to the section plane. For that reason, we decided not to carry out a statistical analysis.

**Intramyocardial Ventricular Branches**

The position of the intramyocardial ventricular branches, transverse to the section plane, allowed for an easy assessment of the lesions. These were not associated with branching points. The thickenings (Figures 3B–3F) were diffuse, always with complete disappearance of the inner elastic layer, from moderate to large, frequently located in opposite parts of the artery wall, thereby producing a crescent or slot-shaped lumen. In some instances, they occluded most of the lumen (Figure 3F).

We obtained incidence and severity indexes from each specimen studied (Table 1). The mean incidence found in the sample was 0.40. The mean LSI was 1.94. The PA in the whole sample was 0.91. Thus, only 9% of the specimens were free of lesions in the sampled sections of the intramyocardial arteries.

**Sex**

Differences in incidence and lesion severity indexes were statistically not significant between males and females (Table 1).

**Size**

Size was used as an estimate of the absolute age of the specimen, since an age/length relation is not available for Mediterranean dogfish populations. Figure 4 is a scatter-
plot showing the relation between total length and both incidence and severity indexes. Incidence index showed a positive but not significant correlation coefficient, whereas severity was significantly correlated (p<0.01) with total length (Table 2). The plot shows that an LSI >2.5 was not found in any of the 22 specimens smaller than 430 mm. Additionally, there were significant differences in LSI between size classes after a one-way ANOVA (F test, 2.96; p<0.05; Table 3).

Reproductive Stage

Dogfish have an extended reproductive cycle throughout the year, and we have found immature, mature, and spawning specimens in the same catch. The incidence of coronary lesions was not significantly related with reproductive stage (Table 1). Twenty-four immature specimens showed a mean incidence index similar to that of the whole sample (0.41). Lesions were found in all immature females. A significant difference in mean LSI was found between immature and spawning specimens for the whole sample (Table 1). In general, greater severities were found in mature specimens, whether they were spawning or not. However, total length was correlated with reproductive stage, possibly because spawning periods are longer in larger specimens. Mean total length of immature specimens was 396 mm, and mean total lengths of mature and spawning specimens were 466 and 497 mm, respectively. Therefore, it is important to distinguish between the effects caused by aging and reproductive stage. No significant differences in LSI were recorded between reproductive stages after a one-way ANOVA (Table 3).

Triglyceridemia and Cholesterolemia (Total and HDL-Related)

The serum lipid concentrations of the specimens studied were triacylglycerol, 1.14 mg·mL⁻¹ (SD, 0.49; n=104); total cholesterol, 0.96 mg·mL⁻¹ (SD, 0.29; n=105); and HDL cholesterol, 0.083 mg·mL⁻¹ (SD, 0.030; n=99). Detailed comparisons of the lipid concentrations between sexes and reproductive stages have been published elsewhere.²-six

Correlation coefficients showed no significant statistical relation between these parameters and both incidence and lesion severity indexes (Table 2).

Localization of Lesions in Intramyocardial Arteries

We have found significant differences in incidence depending on the localization of the lesions. LIVB showed a significantly higher incidence of lesions than RIVB (p<0.05). Mean incidence index in LIVB was 0.53 (SD, 0.39; n=80), whereas in RIVB, mean incidence index was 0.37 (SD, 0.26; n=104). However, the difference in mean LSI was not significant at the p<0.05 level of confidence.

Significant differences were also found between different areas of the intramyocardial arteries (Figure 5). No lesion was observed in area 6 of both arteries, near their distal branching point, and they were scarce in proximal areas 1 and 2. Area 6 was sampled from nine specimens, area 1 from 26, and area 2 from 31, although only three and five specimens showed LIVB in areas 1 and 2, respectively. This was because of the more caudal origin of this artery. Mean incidence indexes of areas 3, 4, and 5 were significantly larger than those of areas 1, 6, and for LIVB only, 2 (p<0.05). Mean LSI was not significantly different between areas, although area 3 showed the highest mean LSI in both intramyocardial arteries. The areas more markedly affected by the lesions show no pronounced curves, branchings, or taperings, although they go progressively deeper into the trabecular myocardium.

TABLE 1. Incidence and Severity Indexes and Proportion of Affected Individuals for the Lesions Found in the Intramyocardial Arteries of Dogfish

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incidence index</th>
<th>Lesion severity index</th>
<th>Proportion of affected individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>Immature male</td>
<td>0.30</td>
<td>0.15</td>
<td>9</td>
</tr>
<tr>
<td>Mature male</td>
<td>0.34</td>
<td>0.19</td>
<td>14</td>
</tr>
<tr>
<td>Spawning male</td>
<td>0.43</td>
<td>0.22</td>
<td>29</td>
</tr>
<tr>
<td>Total male</td>
<td>0.39</td>
<td>0.21</td>
<td>52</td>
</tr>
<tr>
<td>Immature female</td>
<td>0.48</td>
<td>0.23</td>
<td>15</td>
</tr>
<tr>
<td>Mature female</td>
<td>0.41</td>
<td>0.23</td>
<td>25</td>
</tr>
<tr>
<td>Spawning female</td>
<td>0.36</td>
<td>0.28</td>
<td>13</td>
</tr>
<tr>
<td>Total female</td>
<td>0.41</td>
<td>0.24</td>
<td>53</td>
</tr>
<tr>
<td>Immature M+F</td>
<td>0.41</td>
<td>0.22</td>
<td>24</td>
</tr>
<tr>
<td>Mature M+F</td>
<td>0.38</td>
<td>0.22</td>
<td>39</td>
</tr>
<tr>
<td>Spawning M+F</td>
<td>0.41</td>
<td>0.24</td>
<td>42</td>
</tr>
<tr>
<td>Total sample</td>
<td>0.40</td>
<td>0.23</td>
<td>105</td>
</tr>
</tbody>
</table>

Mean lesion severity index was significantly different between immature and spawning specimens.

*p<0.01.
The radial position of the lesions also showed important differences. Figure 6 shows a frequency diagram of the position of the larger lesions (grade ≥4). The medial axis of the lesion was preferentially oriented in the 2–8 direction in RTVB and in the 6–12 in LIVB. Lesions centered on lateral artery walls were rare or absent. The deviation of the observed frequencies from the expected, assuming random distribution of the radial positions, was significant for the RTVB ($\chi^2 = 36.7, p < 0.01, 11 df$).

**Discussion**

In this article, we describe coronary lesions in a shark species with a morphology similar, under light microscopy, to those described in several species of salmonid. Some authors have stressed the similarity between fish myointimal hyperplasia and the earliest stages of mammalian coronary arteriosclerosis.1–8

Our results showed two factors significantly associated with coronary arteriosclerosis in dogfish, namely, size (i.e., age) and a preferential localization of the lesions. LSI increases with total length of the fish, as shown by both the significant correlation coefficient between these variables and the ANOVA between size classes.

Regarding the distribution of the lesions, intimal thickening were present in every branching point of the main coronary conal arteries, although they were very scarce elsewhere along these vessels. This feature, which contrasts sharply with the severely affected main coronary artery of the salmonids, has been noted in five elasmobranch species other than *S. canicula*.16 We will discuss these findings below.

Coronary artery lesions were especially severe in definite areas of the right and left intramyocardial ventricular arteries, namely, at the level of the AV canal in the dorsal and ventral artery walls. In these areas, the mean incidence of the lesions was between 0.40 and 0.60, although the mean LSI was moderate, between 1.65 and 2.05. Notwithstanding, the largest lesions (grade 5, Figure 3F) were more severe than those reported in other fish. In salmonids, the intimal thickening can sometimes occlude the lumen of the main coronary artery by as much as 50%,25 although the average occlusion of the vessel lumen varied from 11.8% to 32.8% and the mean greatest occlusion ranged from 18.5% to 47.5% in a sample of 221 salmonids belonging to five species.27 In fish other than salmonids, incidence and severity of the lesions are even smaller. An incidence index of 0.05 has been estimated in vascular profiles of the ventricular coronary arteries of the mackerel (*Scomber scombrus*).10

Is there an etiopathological significance in the constant localization of the lesions? The anterior descending branch of the human left coronary artery is especially susceptible to atherosclerotic involvement.19,20 This has been attributed to a complex of physical, mechanical, and hemodynamic factors, including a more vigorous vibration, bending, and knuckling during myocardial contraction than other coronary arteries.21 Structural modifications, including intimal thickening, are associated with areas subjected to mechanical stress in mammals.22 A study of 100 young persons (1–20 years old) who had died of causes unrelated to the cardiovascular system showed a high incidence of intimal thickening with a peculiar localization, almost invariably involving the initial segment of the left anterior descending coronary artery.28 The early lesions, called musculoelastic intimal thickening by these authors, have the same histological features as those described in the dogfish, defined by focal or diffuse intimal proliferation of smooth muscle cells with a split and

**Table 2. Correlation Coefficient Matrix Between Some Quantitative Variables and Both Incidence and Lesion Severity Indexes**

<table>
<thead>
<tr>
<th>Total cholesterol</th>
<th>Total cholesterol</th>
<th>Triacylglycerol</th>
<th>HDL cholesterol</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>0.23*</td>
<td>0.04</td>
<td>-0.33†</td>
<td>-0.07</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td></td>
<td>0.33†</td>
<td></td>
<td>-0.08</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.33†</td>
<td></td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>0.05</td>
<td>-0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td>0.33†</td>
<td>0.01</td>
<td>-0.06</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

HDL: high density lipoprotein.

* $p < 0.05$.
† $p < 0.01$. 

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TABLE 3. Analysis of Variance of Both Incidence and Lesion Severity Indexes Between Size Classes and Reproductive Stages

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between size classes</td>
<td>3</td>
<td>0.148</td>
<td>0.049</td>
<td>0.972</td>
</tr>
<tr>
<td>Within size classes</td>
<td>104</td>
<td>5.136</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>5.285</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between reproductive stages</td>
<td>2</td>
<td>0.024</td>
<td>0.012</td>
<td>0.231</td>
</tr>
<tr>
<td>Within reproductive stages</td>
<td>102</td>
<td>5.261</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>5.285</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lesion severity index

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between size classes</td>
<td>3</td>
<td>4.163</td>
<td>1.388</td>
<td>2.955*</td>
</tr>
<tr>
<td>Within size classes</td>
<td>94</td>
<td>42.733</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>46.896</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between reproductive stages</td>
<td>2</td>
<td>1.849</td>
<td>0.925</td>
<td>1.880</td>
</tr>
<tr>
<td>Within reproductive stages</td>
<td>92</td>
<td>45.047</td>
<td>0.490</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>46.896</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only lesion severity index was significantly different between the size classes. *p<0.05.

fragmented internal elastic membrane and further deposition of collagen and elastin, which are rarely positive when stained for lipid. A gradual transition from musculoelastic lesions to raised fibrous plaque is postulated by these authors. Other studies on the distribution of atherosclerotic lesions in human coronary arteries have also suggested that hemodynamic factors are involved in the origin and development of the disease.29-31

The "response to vascular injury" hypothesis has recently been proposed to account for the lesion formation in the main coronary artery of the salmonids under the bulbus arteriosus, an elastic chamber formed from the dilated origin of the ventral aorta.16,17 According to these studies, mechanical deformation of the bulbus arteriosus during blood ejection from the ventricle produces mechanical stresses on the coronary artery, which lies on its surface. The more gentle deformation of the elasmobranch conus arteriosus, endowed with striated cardiac muscle, would produce a lower stress on the artery wall. Our results are consistent with this hypothesis. Coronary lesions in the dogfish are very rare in subepicardial conal coronary arteries, except in their branching points, but they are frequent in the areas of intramyocardial vessels that are presumably subjected to stress and vibrations derived from both the pulsatile blood flow between atrium and ventricle and the action of the AV valve leaflets. These leaflets are attached to the trabecular myocardium pierced by the LIVB and RIVB, as shown in Figure 6.

Thus, our observations suggest that local hemodynamic factors are primarily or secondarily involved in the pathogenesis of the intimal thickenings, perhaps through alterations in the endothelium. Altered endothelium could allow substances proceeding from the plasma or from the endothelium itself to stimulate smooth muscle cell migration and proliferation, although at present there is no evidence of such a mechanism.

It is important to note that the reproductive cycle, extended during most of the year, seemed to have no influence on the development of the lesions in the dogfish, although alteration in sex hormone status during maturation has been suggested as a causal factor of fish coronary arteriosclerosis. This factor probably has a larger significance in salmonids than in the dogfish because of the dramatic physiological changes during maturation and spawning undergone by the former. In fact, administration of human chorionic gonadotropin, estradiol, or testosterone—

\[ \text{FIGURE 5. Bar graphs showing mean incidence and severity indexes in the six longitudinal areas defined in the left and right intramyocardial ventricular branches (LIVB and RIVB, respectively). Mean incidence indexes of areas 3, 4, and 5 were significantly larger than those of areas 1, 6, and, for the LIVB only, 2 (p<0.05). Lesions were not found in area 6. Narrow lines show SEM.} \]
one has been demonstrated to promote coronary lesions in juvenile steelhead and rainbow trout.12

Lesion incidence in intramyocardial branches of immature dogfish is not significantly different from that of the whole sample. The larger mean LSI observed in spawning specimens compared with immature specimens should be attributed to the older age of the former. As a matter of fact, ANOVA showed a significant relation between LSI and size groups, as stated above, but not between LSI and reproductive stage groups. Maturation has been regarded as a secondary factor in the pathogenesis of the disease for the Atlantic salmon because, although it was associated with a significant increase in the incidence of lesions, this incidence is already high in immature fish.15

Although it has been proposed that lesions undergo regression after spawning,2-3,32 fish coronary arteriosclerosis has recently been regarded as a progressive condition that is related to growth rates17 and continues during recovery after spawning.25-33 Our results clearly agree with this viewpoint. We think that the reported decrease in the proportion of affected individuals from immature females (1.00) to mature plus spawning (0.84) is a result of the small number of immature specimens observed (n=15) rather than a true regression in the incidence of lesions. In fact, these differences were statistically not significant.

Studies on fish coronary arteriosclerosis have hitherto focused mainly on salmonids. However, three factors coinciding in these fish might complicate the pathophysiological interpretation of the disease.

First, many salmonids migrate to fresh water for spawning, and this migration has been related to an increase in coronary arteriosclerosis.2-3 Somewhat less severe forms of coronary arteriosclerosis have been reported in nonanadromous rainbow trout than in migrating steelhead trout.7 Dogfish stay in seawater during their entire lifetimes.

Second, total plasma cholesterol of salmonids is extremely high in comparison with mammals.34-35 Large levels of apo-B peptide-containing lipoprotein occur in concordance with the development of the lesions,13 although this finding cannot be viewed as a cause-and-effect relation, according to these authors. An association between a higher incidence of lesions and a dietary cholesterol supplement that caused an increase in the plasma low-density lipoprotein fraction has also been demonstrated.14-15 Dogfish have low levels of serum lipids,26,36,37 and we have shown that cholesterol and triacylglycerol contents in dogfish plasma are apparently not related to the incidence or severity of lesions.

Third, increasing environmental pollution of rivers has sometimes been suggested as a possible factor related to the origin of salmon arteriosclerosis.8,9 Exposure of dogfish to environmental poisons is low, since it inhabits a broad range of depths in Málaga Bay, where industrial chemical pollution is low because of the high seawater turnover through the Strait of Gibraltar.

Therefore, further experimental work could be derived from the study of an animal model with extensive spontaneous myointimal thickenings in definite locations, low concentrations of serum lipids, and that lacks a life stage in fresh water.

**FIGURE 6.** Diagram (left panel) and photograph (right panel) showing the most frequent orientations of the middle axis of the lesions with grade ≥4 in both intramyocardial ventricular branches. Dorsoventral orientations are more frequent in the lesions observed in the left intramyocardial ventricular branch (LIVB), whereas the lesions of the right intramyocardial ventricular branch (RIVB) are preferentially oriented in a right laterodorsal-left lateroventral direction, more or less parallel to the wall of the ventricular inflow tract (VIT). A, atrium; AVV, atroventricular valve (caudal leaflet); SV, sinus venosus; V, ventricle; VOT, ventricular outflow tract.
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