Coronary Disease in Spawning Steelhead Trout Salmo gairdnerii

Abstract. *Coronary degeneration was absent in young trout taken in fresh water and rare in immature fish at sea, but the incidence and severity were sharply greater in migrating fish and almost uniformly present in spawning fish. Several fish taken after they had reentered salt water after spawning had no lesions; lesions in fish taken during their second spawning migration were not cumulative. These facts suggest that the process is reversible.*

The Pacific salmons and steelhead trout (*Salmo gairdnerii*) are related anadromous fish that share a common habitat but have markedly different histories of spawning. Salmon die after spawning, but most steelhead survive spawning, many return to the sea, and some spawn a second or even a third time. Spawning salmon develop widespread vascular degeneration which frequently involves the coronary arteries (*1*). This fact led us to consider the prevalence, and possible reversal, of the degeneration in steelhead trout; the fact that steelhead survive spawning raised the question of whether vascular degenerative disease occurs in these fish, whereas ultimate return of some steelhead for repeated spawning suggested the possibility that these may have survived because they escaped development of pathological vascular changes. On the other hand, it was considered possible that vascular degeneration was ubiquitous among spawning steelhead as in salmon, but that the process regressed after spawning. We investigated these hypotheses in a systematic sample of 222 steelhead trout for all stages of their life cycle. Our results show that the incidence of coronary vascular degeneration approaches 100 percent during spawning and imply that lesions then regress.

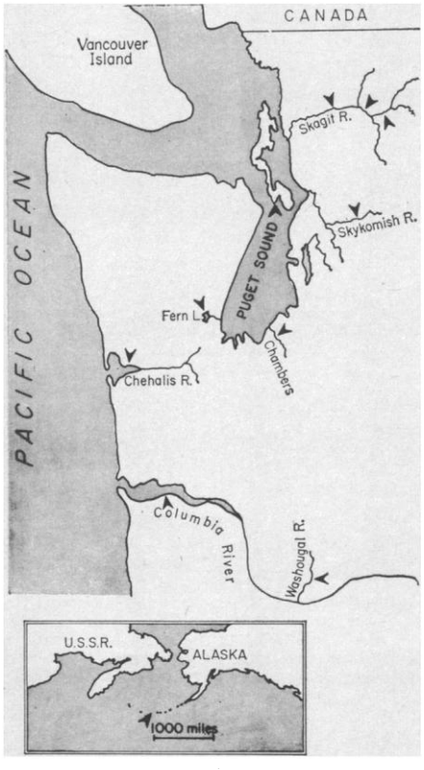

Fig. 1. Western Washington with collection sites (for steelhead trout) designated by arrows. (Insert) General regions in which fish taken on high seas were collected.

Hearts from the fish were systematically collected over a 4-year period (Table 1); collection sites are indicated in Fig. 1. Juvenile hearts were obtained from fingerlings which had not started their downstream migration. Sexually immature fish were collected in the North Pacific at longitude 160°W, latitude 54°N, an area in which salmon and steelhead mingle freely. Some of these fish had traveled a straight-line distance of about 3000 miles from their stream of origin (*2*). Returning migrants were taken with light tackle and artificial lures from Puget Sound and its freshwater tributaries during the seasonal steelhead run. Those taken in Puget Sound near the end of the saltwater phase of their migration were approaching sexual maturity. Fish taken from streams were at relatively advanced stages of sexual maturity. To insure that the sample included sexually mature fish, some were trapped on the spawning beds and held until they had spawned.

Vascular degeneration, indicated by the presence of fatty streaks, was not grossly evident in any of the specimens; hence observations were made by microscope. Hearts from freshly killed trout were preserved in 10-percent formalin and were taken to the laboratory for histological study. Scales were also removed from each fish and were examined for evidence of previous spawning. Hearts were sectioned transversely through the conus arteriosus, at a point where the coronary artery is a single trunk, and through the ventricle approximately midway between the base and the apex of the heart. Tissues were stained with hematoxylin and with elastic van Gieson before examination. Selected specimens were stained for fat with Sudan IV and for acid mucopolysaccharides with Alcian blue.

No evidence of abnormality was found in arteries of juvenile fish which had not migrated downstream into salt water. However, each of two specimens among a group of 30 taken on the high seas had one small area of splitting and reduplication of the internal elastic membrane (Fig. 2B). A few unidentified cells, probably smooth muscle cells, were present in the ground substance between layers of the intima. Colloidal iron stains showed that acid mucopolysaccharides were present; however, medial degeneration was absent, and continuity of the internal elastic membrane was not interrupted.

Coronary arteries from 12 of 20 migrating fish taken from salt water about 40 km from the entrance to spawning streams had evidence of vascular degeneration. In most, degeneration was limited to simple intimal splitting, but in some arteries the intimal tissues also appeared to be hyperplastic. Collections of hyperplastic cells involved much of the inner surface of such vessels, and cross sections of the lumen were extremely irregular (Fig. 2C). Of 27 migrants obtained from tidewater, 16 had intimal changes and intraluminal nodules of hyperplastic cells. In five of these vessels, the media underlying these nodules were replaced by fibrous connective tissue and amorphous material. Both the incidence and severity of vascular degeneration increased sharply after the fish began the freshwater phase of their migration. Thus, of 17 fish taken just inside the mouth

Table 1. Incidence of vascular lesions among steelhead trout taken at various collection sites. Numbers of fish known to have spawned previously are shown in parentheses.

Collection site	Fish collected (No.)	Fish with lesions	
		No.	Incidence (%)
Juveniles			
Fresh water (nonmigrants)	5	0	0
Sexually immature			
High seas, 160°W, 54°N	30 (4)	2 (1)	7
Spawning migrants			
Salt water, Puget Sound	20 (3)	12 (2)	60
Tidewater, Puget Sound	27 (4)	16 (2)	59
Fresh water, Lower River	17	14	82
Middle River	69	63	91
Spawning grounds	22 (4)	21 (4)	95
Traps	32 (1)	31 (1)	96

of the Columbia River, 14 had abnormal coronary vessels. Among 69 steelhead taken further upstream en route to the spawning beds, 63 had abnormal coronary vessels, and the degeneration occurred in 21 out of 22 in fish caught near the spawning beds. Of 33 hearts collected from steelhead trapped near the spawning beds and held in that area until sexually ripe, 32 had coronary vascular degeneration. Intimal changes and hyperplastic intraluminal nodules were the most common lesions, but the incidence of medial involvement also increased. In addition, eccentric involvement of the media with a process resembling arteriosclerosis was observed in 11 fish (Fig. 2D). Tests of these arteries for lipids with Sudan IV stain were negative, but Alcian blue stain confirmed the presence of acid mucopolysaccharides.

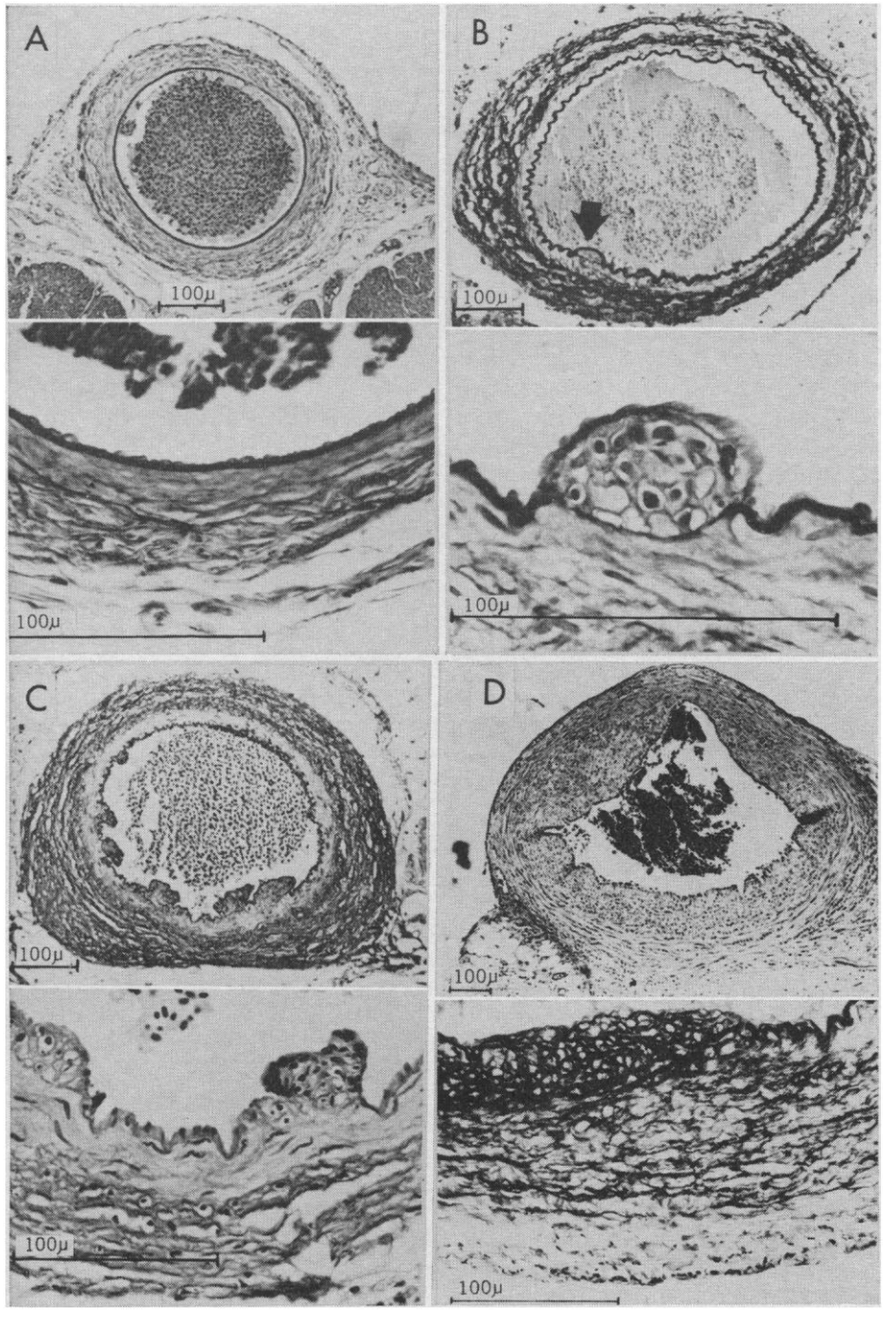

Fig. 2. Microscopic sections of coronary arteries from steelhead trout. (A) Normal coronary artery from sexually immature specimen taken on high seas. Internal elastic membrane is relatively thicker, but in other details the structure of the vessel approximates mammalian vessels (with van Gieson stain). (B) Early intimal lesion from fish taken on high seas. The internal elastic membrane is split and surrounds a focal accumulation of large cells of uncertain origin. The endothelial layer appears to be intact. (A and B, top) Van Gieson stain; (bottom) hematoxylin and eosin. (C) Coronary artery from freshwater migrant, with numerous intraluminal nodules that appear to represent focal areas of intimal hyperplasia (with hematoxylin and eosin stain). (D) Coronary artery from sexually ripe fish, with concentric intimal splitting, reduplication, and fibrosis. The Sudan stain was negative for fat. (C and D, top) Hematoxylin and eosin; (bottom) van Gieson stain.

The progressive increase in the incidence of vascular degeneration during the spawning migration suggests several causal relations, such as sexual maturation, chronological aging, and the adaptation from salt to fresh water. Sexual maturation as a possible factor in development of vascular changes was suggested earlier by Robertson *et al.* who showed that dramatic increases in corticosteroid concentrations occur in both salmon and steelhead during the spawning migration (*3*). Robertson's group suggested that an endocrinopathy akin to Cushing's disease may develop in anadromous species during spawning. In fish, the relation of vascular degeneration to aging is difficult to document. The chronological age of fish in this collection ranged from 1 to 7 years, but, with the exception of the nonmigrant juveniles, we could not predict the chronological age of steelhead solely on the basis of the histological appearance of the coronary vessels. For example, the coronary vessels of 3-year-old fish trapped and held until they had spawned were almost invariably abnormal, whereas chronologically older fish taken in salt water but at an earlier stage of sexual maturity had normal coronary vessels. Similarly, the incidence, severity, and progression of vascular degeneration were not related to the physical size of the fish. The lack of characteristic morphological changes in the coronary arteries of sexually immature fish of known advanced age, as well as the fact that degenerative changes were at least as common among small migrant fish as among large ones, indicate that the histological observed changes were related to some aspect of the spawning migration rather than to simple aging.

Sixteen fish in this collection had survived a previous spawning migration and were captured while en route to their second spawning. Three of these were tagged fish; precise information regarding their life histories was a matter of record, and 13 others were identified as previous spawners by examination of scales. Six of these 16 had normal coronary arteries; all were taken in salt water (Table 1). The incidence and severity of vascular lesions in the

remaining ten fish corresponded to the incidence and severity found in first spawners taken at the same sites. Thus, four fish taken on the high seas had spawned previously; the coronary arteries of three were normal, and the fourth had early intimal changes. Three fish taken from Puget Sound had spawned previously and were en route to their second spawning; typical lesions were present in two, and one was normal. Four fish taken from tidewater were identified as repeat spawners; two of these had typical intimal degenerative changes, and two were normal. Four fish taken from freshwater streams were repeat spawners; all were taken near spawning areas, and all had abnormal coronary arteries. The fish trapped on the spawning grounds included one repeat spawner with abnormal coronary arteries. Among these second-time spawners with abnormal coronary vessels, none had evidence of a cumulative process; that is, their lesions were indistinguishable from lesions in first-time spawners taken at the same location.

Thus, although the incidence of vascular degeneration approached 100 percent in fish at the time of spawning, some fish which were known to have spawned 1 to 2 years previously were found to be normal; and the degenerative process in second-time spawners resembled that of first-time spawners taken at the same stage of migration. These findings imply that the degenerative process regressed after spawning and that such reversal is a naturally occurring phenomenon.

We are not aware that reversal of coronary lesions is a normal event in the life cycle of any animal. Regression of human atherosclerotic lesions may occur in starvation, wasting disease, and malnutrition (*4*). Lesions induced by atherogenic diets may also regress after cessation of the diet or manipulation of hormone levels (*5*). These fish probably starve during the freshwater phase of their migration, when the lesions are in the stage of rapid development. The role of hormones in the degenerative process was suggested by Robertson *et al.* who showed that the quantity of 17 hydroxycorticosteroids was markedly increased during the spawning migration (*3*).

ROBERT L. VAN CITTERS
NOLAN W. WATSON
Department of Physiology and Biophysics and *Regional Primate Research Center, University of Washington School of Medicine, Seattle*

References and Notes

1. O. H. Robertson, B. C. Wexler, B. F. Miller, *Circulation Res.* **9**, 826 (1961); O. H. Robertson and B. C. Wexler, *Endocrinology* **66**, 222 (1960).
2. C. F. Pautzke and R. C. Meigs, *Washington State Department of Game Bulletin No. 3* (1940); R. W. Larson and J. M. Ward, *Trans-American Fisheries Soc.* **84**, 261 (1954).
3. O. H. Robertson and B. C. Wexler, *Endocrinology* **65**, 225 (1959); S. Hane and O. H. Robertson, *Proc. Nat. Acad. Sci. U.S.* **45**, 886 (1959); O. H. Robertson, M. A. Krupp, C. B. Favour, S. Hane, S. F. Thomas, *Endocrinology* **68**, 733 (1961).
4. V. D. Tzinzerling, M. A. Zakhar'evskaia, K. G. Volkova, in *Atherosclerosis*, A. L. Myasnikov, Ed. (National Institutes of Health, Bethesda, Md., 1962), transl. from the Russian, p. 72; S. L. Wilens, *Amer. J. Pathol.* **23**, 793 (1947); A. Strom and R A. Jensen, *Lancet* **260**, 126 (1947).
5. L. Horlick and L. N. Katz, *J. Lab. Clin. Med.* **34**, 1427 (1949); R. Pick, J. Stamler, S. Rodbard, L. N. Katz, *Circulation* **6**, 858 (1952).
6. Supported by grants from the American Heart Association, the Washington State Heart Association, and its Northeastern Chapter. We thank C. Millenbach, Washington State Department of Game, Olympia; A. Hartt, Fisheries Research Institute, University of Washington; C. Hunter, Fish and Wildlife Service, Bureau of Commercial Fisheries; Mrs. H. Pemberton, A. Tingley, W. Betschart, and R. Bogle for collecting specimens.

27 October 1967

Selective Phagocytosis: A New Concept in Protein Catabolism

Abstract. *The clearance of different metabolic products derived from two plasma proteins, prothrombin and fibrinogen, was studied with the aid of the isolated, perfused rat liver. Active thrombin and fibrin were rapidly cleared by the Kupffer cells. Inactive thrombin and a partially degraded fibrin molecule were also cleared but at much slower rates. This difference in clearance rates suggests the presence of a high degree of selectivity in the clearance of altered plasma proteins.*

Using the isolated, perfused rat liver for the study of phagocytosis (*1*), we have demonstrated that colloidal gold (Au^{198}), intact and disintegrated labeled rat platelets (*2*), and aggregated bovine serum albumin are cleared from heparinized rat blood at the same rate as from an artificial perfusate containing polyvinylpyrrolidone (PVP) but no heparin (*3*) (Fig. 1). Since PVP does not affect clearance by the reticuloendothelial system in the rat (*4*), one can study the clearance of materials known to be altered by blood from a perfusate containing PVP. Using the isolated, perfused rat liver technique, we have studied the fate of the metabolic products derived from two plasma proteins, fibrinogen and prothrombin.

The preparation of rat fibrinogen-Se^{75} (*5*) and of fibrinogen's metabolic products, fibrin and a product of plasmin-induced fibrin degradation (FDP) (Fig. 2B), has been described, as have techniques of liver perfusion, perfusate sampling, and the counting of the radioactivity of the perfusate, bile, and liver samples (*6*). Samples of albumin (*7*) and thrombin (*8*) were labeled with I^{131} by the method of McFarlane (*9*), to the extent that the iodination did not exceed the mean of ½ atom of I^{131} per protein molecule. The free I^{131} was removed by overnight dialysis of the protein solution in versene-treated dialysis bags against a continuous change of distilled water at 0°C (*10*). At the end of the dialysis, the supernatant fraction, after precipitation of the protein with trichloroacetic acid, contained less than 0.7 percent of the total radioactivity. Unlabeled thrombin was prepared from a commercially available bovine thrombin preparation (Parke, Davis Co.) by chromatography on an Amberlite XE64 column (*11*). To assess the effect of blockade of the reticuloendothelial system on protein clearance, we injected rats intravenously with colloidal carbon (*12*) (8 and 16 mg per 100 g of body weight) 2 hours before removal of the liver (see *13*).

Livers were initially perfused with 1 to 4 mg of bovine serum albumin-I^{131} added to the blood and the PVP perfusates. Radioactivity of these perfusates declined slightly during the first few minutes of the perfusion. This decline did not exceed 8 percent of the total activity. Subsequently there was no appreciable change in the perfusates' activities during a 2-hour perfusion. Hence the amount of bovine serum albumin-I^{131} promptly cleared is very small. This finding suggests that the labeling procedure causes little denaturation, for if denaturation were a significant problem clearance of radioactivity would probably be greater (*14*). This finding also confirms previous observations (*15*) that normal catabolism of plasma proteins by the liver takes place at a very slow rate.

Addition of 3.5 to 4 mg of solubilized (*6*) rat fibrin labeled with Se^{75} to whole blood perfusate resulted in its