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IN THE FRASER RIVER SYSTEM

BULLETIN XX

HISTOLOGICAL AND HEMATOLOGICAL CHANGES ACCOMPANYING SEXUAL MATURATION OF SOCKEYE SALMON IN THE FRASER RIVER SYSTEM

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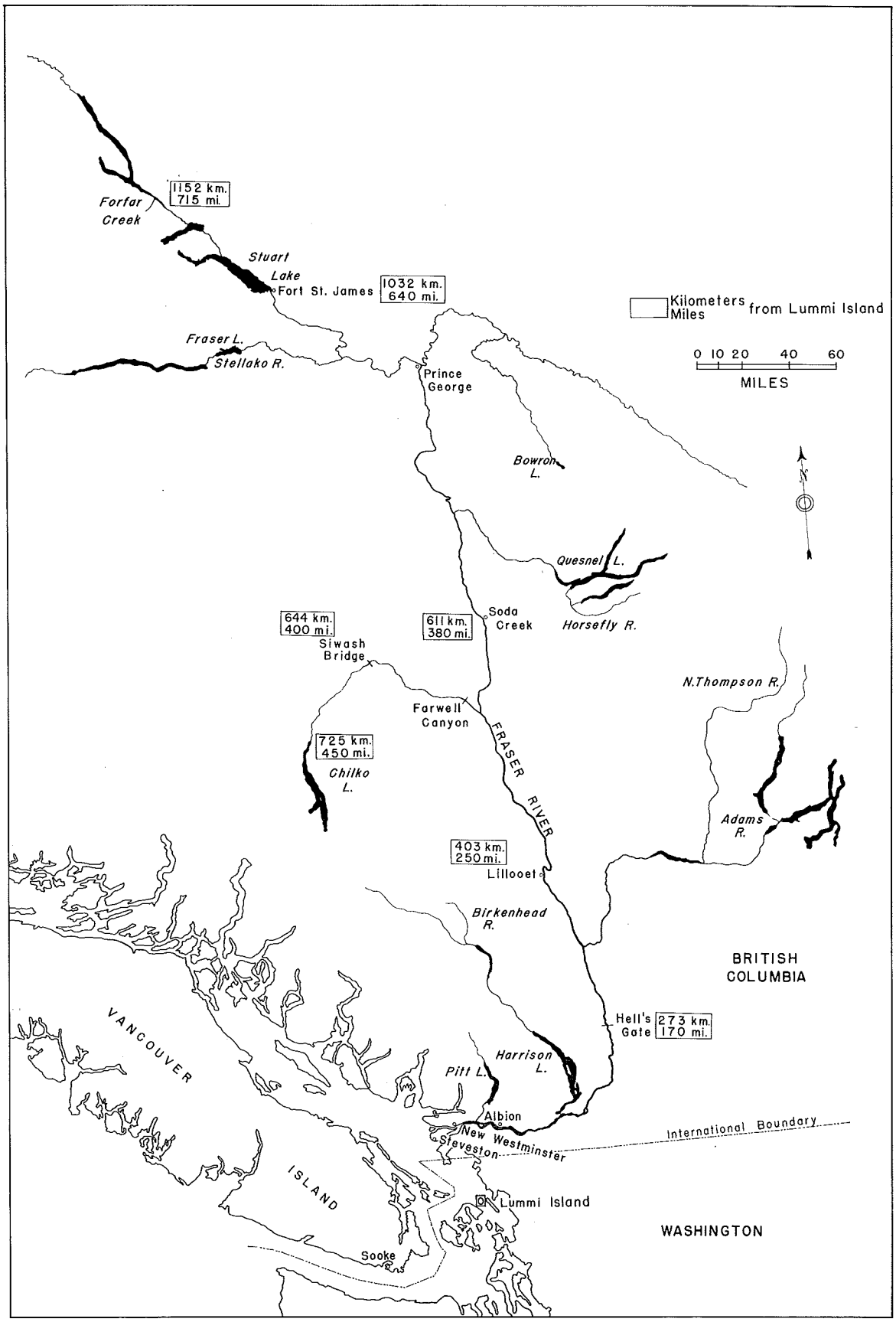

Map showing Fraser River spawning grounds with mileage calculated from Lummi Island, Washington.

ABSTRACT

A study of the changes occurring in the blood and tissues of adult sockeye salmon during their anadromous migration and on the spawning grounds was undertaken in the Fall of 1964. Progressive degenerative changes of the pituitary gland, adrenocortical tissue, kidney, liver and myocardium, and depletion of lymphocytes in the spleen and peripheral blood were found to accompany the sexual maturation and spawning of sockeye salmon of the Chilko River run. The cumulative effects of these changes on the physiology of the fish are considered responsible for the post-spawning death of sockeye salmon. The relationship between pre-spawning mortalities of Fraser River sockeye and the histological changes accompanying sexual maturation is discussed.

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INTRODUCTION

The anadromous migration and subsequent spawning of adult Pacific salmon (Genus *Oncorhynchus*) are the terminal events in the life cycle of the species; universal death follows the act of spawning. It is a matter of the utmost concern to the salmon industry that a variable percentage of sexually mature salmon, after surviving the arduous journey to the spawning grounds, does not live to complete this final act of the life cycle. In certain instances, mortalities among unspawned sockeye salmon *(Oncorhynchus nerka)* on the Fraser River spawning grounds have reached alarming proportions. Notable among these losses was the death of 90% of an estimated 1,002,000 fish escapement to Chilko River in 1963 (International Pacific Salmon Fisheries Commission, 1964). Other spawning escapements similarly have experienced severe pre-spawning mortalities in previous years. In 1961 a number of races suffered heavy losses, including the Bowron River run (60% mortality), the Horsefly River run (62% mortality) and the early Nadina run (86% mortality), (International Pacific Salmon Fisheries Commission, 1962).

In the summer of 1964, the International Pacific Salmon Fisheries Commission initiated a continuing program of investigations into the causes of pre-spawning mortalities among Fraser River sockeye. This program encompasses two major areas of research: (1) histology and histopathology, the subject of this report, and (2) disease and temperature, the text of other publications of the International Pacific Salmon Fisheries Commission. The histological study of migrating and spawning sockeye salmon described herein serves two major purposes.

The first objective of this study is to provide information basic to a recognition of intrinsic and/or extrinsic lethal conditions by defining the "normal" structural and functional condition of the various organs and tissues of the body, including blood, at this critical stage of the life cycle. Very striking degenerative changes in a number of tissues throughout the body are known to accompany sexual maturation and the ensuing post-spawning death of Pacific salmon (Robertson and Wexler, 1960). Thus the "normal" histology of spawning sockeye salmon embodies a series of progressive deteriorative processes which ultimately culminate in the death of the organism. Any pathological examination of dead or dying salmon must therefore take into account certain degenerative changes consistent with that particular stage of the life cycle. A histological study of migrating and spawning sockeye salmon, conducted during a year and on a population which

experienced no appreciable mortalities therefore provides scientists of the International Pacific Salmon Fisheries Commission with a reference of expected histological changes. This study will serve as a foundation for subsequent work and provide a basis by which future pathology can be intelligently measured and assessed.

The second objective of this study is to provide a histological comparison of the chronological segments of a migrating race of sockeye salmon. A relationship between arrival time on the spawning grounds and death of unspawned sockeye has been recognized for a number of years. In at least three spawning areas of the Fraser River system (Raft, Chilko and Horsefly Rivers) most losses have occurred primarily among the first arriving sockeye and heaviest losses have occurred when the entire run has arrived on the spawning grounds earlier than usual (International Pacific Salmon Fisheries Commission unpublished data). This project therefore attempts to determine, by a comparative study of the various segments of a run, if a histologic basis exists for the observed correlation between early timing and pre-spawning mortalities.

METHODS

A single race of fish, the Chilko River sockeye, were selected for the 1964 study. The Chilko sockeye, which can be identified in the fishery by their timing and scale characteristics (Henry, 1961), commence late in July a migration which involves a distance of approximately 450 miles from Lummi Island in Georgia Strait to the spawning grounds at the outlet of Chilko Lake (Frontispiece). The mean rate of migration for the Chilko sockeye is 21.5 miles a day and the consistency of chronological order is maintained during migration and spawning, although some mixing occurs on the spawning grounds (Killick, 1955). The fish arrive on the spawning grounds before they are ready to spawn and a period of approximately three weeks passes before actual spawning takes place. The chronological order is generally maintained in death, although considerable variation occurs in the time from spawning to death of individual fish.

A number of fish were obtained from the early and peak segments of the Chilko run at various points of the migration and at various stages of sexual maturity. Sampling stations, condition of fish, dates and numbers of fish examined are summarized in TABLE 1. In addition to those fish used for histological and hematological studies, a number of sockeye (females) were procured for the purpose of determining the relative stage of maturation of the various segments of the run at each of the sampling locations. Stage of maturation was determined by a comparison of ovary weight to total body weight and is expressed as per cent gonads (TABLE 2).

Sexually immature salmon were obtained by commercial reef net at Lummi Island, some 50 miles seaward from the mouth of the Fraser and thus very close to the beginning of their freshwater migration. Dip nets were used to procure fish near the termination of their upstream migration, at Siwash Bridge on the

TABLE 1—Sampling stations for the Chilko River sockeye run in 1964, showing the condition of the fish, dates of sampling and numbers of fish examined.

LOCATION	DISTANCE IN MILES	CONDITION OF FISH	DATES OF SAMPLING AND NUMBERS OF FISH EXAMINED (A) HISTOLOGICAL SAMPLES (B) HEMATOLOGICAL SAMPLES	
			Early Migrants	Peak Migrants
Lummi Island	0	Green, migration started	July 26 (A) 10 (B) 17	Aug. 6 (A) 10 (B) 18
Siwash Bridge	370	80% of journey completed	Aug. 18-19 (A) 10 (B) 20	Sept. 2-3 (A) 10 (B) 29
Chilko River	450	Ripe, pre-spawning	Sept. 11-12 (A) 10 (B) 20	Sept. 21-24 (A) 7 (B) 7
		Spawning	Sept. 16-24 (A) 10 (B) 15	
		Spawned-out	Sept. 15-25 (A) 10 (B) 21	
		Moribund, unspawned or partially spawned	Sept. 28 (A) 5 (B) 5	

Chilko River, 80 miles below the spawning grounds. Here the fish were more mature and beginning to take on the red external coloration of spawning sockeye. At the Chilko River spawning grounds located immediately below Chilko Lake pre-spawning, spawning and spawned-out fish were obtained by beach seine. The pre-spawning fish were sexually "ripe", the eggs moderately loose in the ovarian membranes and all fish sampled were bright red in coloration. Fish classified as spawning had expelled approximately 50% of the eggs in the case of the females or, in the case of the males, the milt was running freely with evidence of partial voidance of the testes. Fish were classified as spawned-out if the ovaries or sperm were completely expelled. Spawning and spawned-out sockeye could not be clearly identified according to their time of arrival. However, since the peak of spawning is known to have occurred between September 29 and October 3, it is assumed that the majority of spawning and spawned-out fish obtained before this date were from the early segment of the run. Each sample contained an approximately equal number of males and females, and no consideration of sex differences is made in this study.

TABLE 2—Gonad size of 1964 Chilko sockeye (females) expressed as per cent of total body weight. Numbers of fish sampled (in parentheses) and dates of sampling are included.

LOCATION	EARLY MIGRANTS	PEAK MIGRANTS	LATE MIGRANTS
Lummi Island	Mean (46): 3.5% Range: 1.8-5.3% July 21, 1964	Mean (71): 3.6% Range: 1.8-9.6% Aug. 2, 1964	Mean (79): 4.7% Range: 2.3-9.7% Aug. 13, 1964
Siwash Bridge	Mean (7): 10.6% Range: 5.8-13.2% Aug. 22, 1964	Mean (44): 11.7% Range: 8.4-21.3% Sept. 3-5, 1964	
Chilko River Pre-spawning	Mean (54): 12.7% Range: 9.3-16.1% Sept. 9-12, 1964		

A few unspawned or partially spawned sockeye were captured on the spawning grounds in a moribund state. These fish were found floating in shallow pools or eddies, obviously unable to complete the spawning act. No evidence of external injury was seen in any of these fish, and these moribund sockeye are included in the histological and hematological study.

Fish were stunned immediately after capture then bled by severing the caudal peduncle. A portion of blood was collected over anticoagulant for hematology. Heparin was employed as an anticoagulant for fish captured at Lummi Island; in all other samples EDTA (disodium ethylene diamine tetraacetic acid) was used. Erythrocytes were counted on the hemocytometer using Hendrick's solution (Hesser, 1960) as a diluting media. Blood smears were prepared for differential leukocyte counts, fixed in absolute methyl alcohol and stained with Leishman-Giemsa. Total leukocyte counts were determined by a ratio of leukocytes per 1000 erythrocytes on stained blood smears. Packed cell volumes were determined by centrifugation in Wintrobe hematocrit tubes.

Tissues obtained for study include pituitary gland, adrenal cortical tissue, pancreas, kidney, liver, spleen, heart and gills. Tissues were fixed in 10% formalin and preserved in 70% ethanol. Hematoxylin and eosin staining techniques were routinely employed. The acid fuchsin-aniline blue staining method was used for pituitary granule differentiation and Gomori's chromium hematoxylin-phloxine stain was used to differentiate pancreatic Islet cells. Liver, spleen, kidney and gill tissues of moribund fish were stained for bacteria by Lillie's method (crystal violet, Lugol's solution and safranin).

RESULTS

Gonad Size

In an effort to relate the histology and hematology of migrating and spawning Chilko sockeye to specific stages of sexual maturation, ovary weight and total body weight were determined on a number of female salmon at each sampling location. Both early and peak migrants were sampled where the chronological distinction could be made; in addition, sockeye of the late segment of the Chilko run were obtained at Lummi Island.

Gonad weight ranged from a low of 1.8% of body weight to a high of 9.7% among female sockeye sampled at Lummi Island, with mean values of 3.5%, 3.6% and 4.7%, respectively, for the early, peak and late segments of the run (TABLE 2). At Siwash Bridge, values ranged from 5.8% to 21.3% with means of 10.6% and 11.7% for the early and peak groups, respectively. Only the early migrants were sampled at the Chilko River spawning grounds prior to spawning. These fish had a mean gonad weight value of 12.7%, with a range of 9.3% to 16.1%.

A tendency was noted for later migrants to be slightly more mature in terms of relative gonad weight at any given sampling location, but little significance can be placed on this finding in light of the wide range of values encountered.

Blood

Red and white cell counts, hematocrit values and differential leukocyte counts for all categories of fish are presented in TABLE 3.* Both the range of values encountered and the average value for each group are given. Red cell counts and total leukocyte counts were not performed on migrants from the peak of the run at Lummi Island nor on early migrants at Siwash Bridge. Packed cell volumes were not determined for either early or peak migrants at Lummi Island nor for early migrants at Siwash Bridge.

ERYTHROCYTES

The highest average erythrocyte count was found in sockeye at the beginning of the spawning migration (1.23 million red cells in early migrants at Lummi Island). Values obtained from progressively more mature salmon indicate that a reduction in circulating erythrocytes occurred during the initial phase of the upstream migration and succeeding red cell counts remained slightly lower than in immature fish at sea. Immature red blood cells were frequently seen in all categories of fish. With the exception of moribund fish, mean packed cell volumes did not vary significantly from location to location.

Anemia was encountered in a few spawning, spawned-out and moribund fish. Two out of fifteen spawning sockeye (13%) and four out of twenty-one spawned-out sockeye (19%) evidenced a frank anemia. All moribund sockeye examined

* This work was carried out by Dr. Denise Colgrove.

TABLE 3—Cellular constituents of the blood of migrating and spawning sockeye salmon of the Chilko River run in 1964. Numbers of fish sampled are given in parentheses.

LOCATION AND CLASSIFICATION OF FISH		ERYTHROCYTES 10^6	HEMATOCRIT	LEUKOCYTES 10^3	DIFFERENTIAL LEUKOCYTE COUNT IN PER CENT			
					Band Neutrophils	Segmented Neutrophils	Lymphocytes	Macrophages
LUMMI ISLAND								
Early Migrants (17)	Av.	1.23	—	16.4	0.6	9.0	85.9	4.5
	Range	0.92-1.68	—	10.5-21.2	0-4	4-15	75-92	2-11
Peak Migrants (18)	Av.	—	—	—	0.8	8.0	86.8	4.4
	Range	—	—	—	0-3	3-15	78-92	1-8
SIWASH BRIDGE								
Early Migrants (20)	Av.	—	—	—	0.3	33.0	60.4	6.3
	Range	—	—	—	0-1	16-54	41-82	1-24
Peak Migrants (29)	Av.	1.08	44.0	17.3	0.2	23.0	66.8	10.0
	Range	0.80-1.44	35.5-50.0	10.2-29.2	0-2	8-44	51-88	0-32
CHILKO RIVER								
Early Pre-spawning (20)	Av.	1.13	42.0	23.8	0.7	33.0	53.3	13.0
	Range	0.82-1.32	36.5-48.5	12.0-43.5	0-6	22-56	10-70	1-32
Late Pre-Spawning (7)	Av.	1.09	43.0	16.7	0.5	29.6	45.3	24.6
	Range	0.88-1.44	32.5-57.5	8.0-27.0	0-2	5-49	15-77	12-61
Spawning (15)	Av.	1.14	45.0	16.3	1.7	32.3	32.4	33.6
	Range	0.34-1.64	15.5-63.0	11.5-23.0	0-5	10-60	15-64	12-60
Spawned-out (21)	Av.	1.17	46.0	18.0	4.8	46.6	28.1	20.5
	Range	0.26-1.80	8.0-68.0	10.5-27.5	0-16	11-84	6-50	0-44
Moribund (5)	Av.	0.16	5.5	13.8	1.0	19.0	35.0	45.0
	Range	0.05-0.32	2.5-12.5	8.5-17.0	0-4	6-42	26-59	28-64

were severely anemic, with an average red cell count of 0.16×10^6 and packed cell volume (hematocrit) of 5.5. Erythrocytes of moribund fish were primarily immature forms, often folded or distorted. Many cells were fractured in the preparation of blood films, indicating an increased fragility.

LEUKOCYTES

Total leukocyte counts were obtained by a ratio of white cells to 1000 red cells in the stained blood smear, hence these values serve as only an approximation of the number of leukocytes per cubic millimeter of blood. Thrombocytes were not enumerated. Highest white cell counts were found in early pre-spawning fish at Chilko River, giving a mean value of 23,800 leukocytes per cubic millimeter.

A definite relationship between leukocyte distribution and sexual maturation is evident. Differential leukocyte counts revealed a distinct shift in the relative proportions of neutrophils, lymphocytes and macrophages with successive stages of maturity (TABLE 3). In general, the proportion of neutrophils and macrophages increased from sea to spawning while the proportion of lymphocyte progressively decreased. The proportion of neutrophils, both immature (band) and mature (segmented) was highest in spawned-out fish. At sea, segmented neutrophils comprised about 9% of the white cells while these cells constituted approximately 47% of the leukocytes in spawned-out fish. Lymphocyte fractions declined from 86% of the white cells at Lummi Island to 28% in spawned-out fish. The proportion of macrophages increased approximately eight-fold from sea to maturity, constituting about 4% of the leukocytes at Lummi Island and 34% of the white cells in spawning sockeye. The predominant leukocyte type in moribund fish was the macrophage.

Total and differential leukocyte response in fish is not clearly understood at present, even though specific functions have been assigned each cell type. Hormonal influence on the leukocytes of mammals has long been recognized, and it is now an accepted fact that adrenal cortical secretion depresses the number of circulating lymphocytes and leads to an elevation in the number of circulating neutrophils. Elevated plasma levels of 17-hydroxycorticosteroids have been reported in spawning Pacific salmon (see discussion) and these hormones may be responsible for the changes observed in the circulating leukocytes of maturing sockeye. An increase in numbers of circulating macrophages has previously been observed in spawning salmon (Klontz, personal communication), an event apparently unrelated to the phagocytic properties of the cell.

The wide range of values obtained at any given sampling area raises some question as to the significance of any apparent differences in leukocyte distribution of early and peak migrants. A search of the literature reveals no information on which a diagnostic or prognostic interpretation can be based.

Histology

PITUITARY GLAND

The several areas or lobes of the pituitary gland are defined herein on a topographical basis, in accordance with the terminology employed by Robertson and Wexler (1962a). Thus four distinct regions of the gland are considered, namely the anterior, dorsal, ventral and neural lobes. The principal changes were found to occur in the dorsal lobe, hence this area will be described in some detail, referring to other areas of the pituitary gland less frequently.

At the beginning of the spawning migration, the ventral lobe of the pituitary was by far the largest region, comprising at least half of the gland (FIGURE 1). Small trabeculae of glial tissue, arising from the neural lobe, penetrated the ventral, and to a lesser extent, the dorsal lobe. Anterior lobe follicles were composed of brilliantly staining tall columnar cells (FIGURE 2).

The predominant cell type of the dorsal lobe of fish sampled at Lummi Island was the acidophil. A rather diverse staining affinity of these cells was observed and interpreted as reflecting a variation in the degree of granulation of the acidophils. While the cytoplasm of all these cells was clearly heterogenous in nature, well defined granules were readily discernible only in the more intensely stained cells. Basophils were generally far less numerous than acidophils, and likewise exhibited some variation in staining affinity. While chromophobic cells were rare, the markedly reduced staining of many acidophils and basophils suggested an undifferentiated or newly differentiated cell. No distinct differences in these characteristics were noted between fish of the early and peak segments of the run.

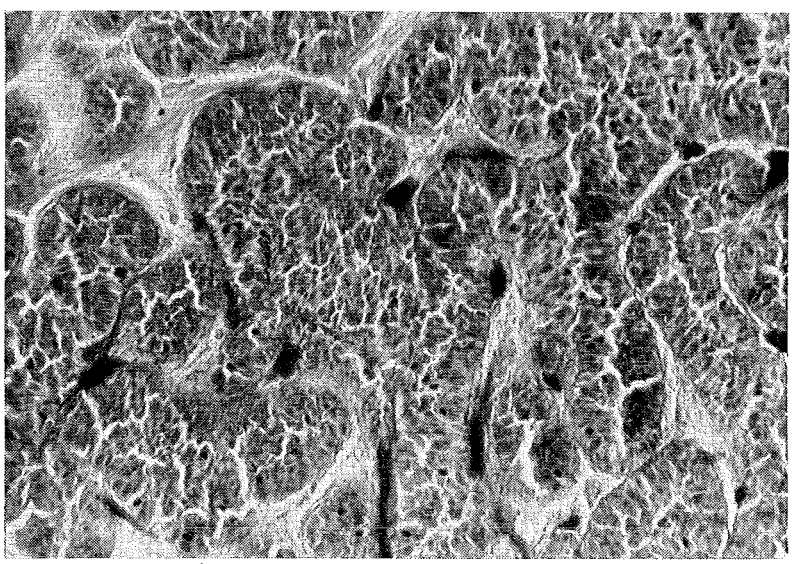

FIGURE 1—Pituitary gland, ventral lobe. Migrating sockeye salmon, Lummi Island. Acid fuchsin-analine blue. Normal ventral lobe. 125X

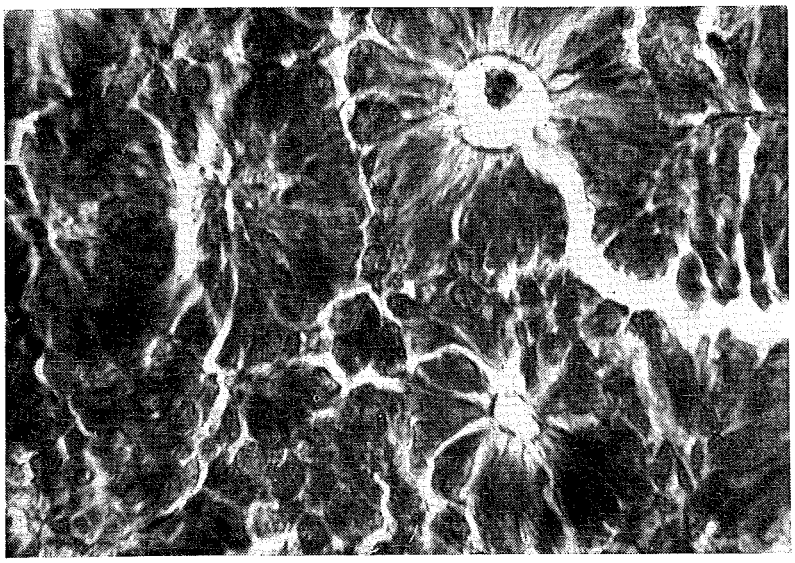

FIGURE 2—Pituitary gland, anterior lobe. Migrating sockeye salmon, Lummi Island. Acid fuchsin-aniline blue. Normal anterior lobe follicles. 450X

A great increase in numbers of basophils was observed in migrating sockeye at Siwash Bridge, acidophils and basophils appearing to be approximately equal in the dorsal lobe. The two cell types were more or less evenly dispersed throughout the major portion of the lobe, although areas primarily comprised of one cell type or the other were seen. Indistinctively acidophilic or basophilic cells were encountered infrequently, the majority of both cell types staining a uniform and fairly intense shade of color. Granules were often distinct, filling the entire cytoplasm.

Beginning degenerative changes, including vacuolization and loss of cytoplasm, were noted in the dorsal, anterior and ventral lobes of a few fish at Siwash Bridge. In the dorsal lobe, these changes occurred most frequently in the basophils although the acidophils were occasionally affected. Changes in the anterior lobe were limited primarily to the apical or luminal portion of the cells. Degenerative changes, when present, were focal in distribution. Increased amounts of glial tissue were observed in the ventral lobe. No differences were noted between fish of the early and peak segments of the run.

The dorsal lobe of sexually mature, pre-spawning sockeye at Chilko River had increased in size relative to the ventral lobe so that these two areas appeared approximately equal in size. The two major cell types of the dorsal lobe appeared equal in numbers and stained intensely. Chromophobes were encountered infrequently but partially degranulated cells, especially basophils, were not uncommon in the majority of fish. Evidence of degenerative changes, now including an occasional pyknotic (shrunken) nucleus, were encountered in all three lobes but tended to remain focal in distribution. A reduced affinity for staining was noted in

some cells of the anterior lobe, the apical portion of the cell being principally affected. Glial connective tissue was relatively abundant in the ventral lobe. No differences were observed between fish of the early and peak segments of the run.

Partial degranulation of basophils and, to a lesser extent, acidophils, was observed in the dorsal lobe of many spawning sockeye. Chromophobes were not abundant. Degenerative changes were frequently seen in all three lobes of spawning fish, often more pronounced in the anterior and ventral lobes than in the dorsal lobe. Cells of the anterior lobe tended to show a reduced affinity for staining, most evident in the apical portion of the cells. Vacuolization and loss of cytoplasm, when present, were similarly confined primarily to this portion of the cell. Increased amounts of glial tissue were noted in the ventral lobe, loosely arranged with large spaces between fibers.

In spawned-out sockeye, degenerative changes were generally severe and widespread throughout the pituitary (FIGURE 3). Pyknosis, loss of cytoplasm and absence of cells were noted in all three lobes. In the dorsal lobe, acidophilic and basophilic granules were often encountered free in the intercellular spaces, apparently released from degenerating cells. Cells of the ventral lobe were commonly isolated into discrete islands by thick septa of glial tissue. The dorsal lobe was similarly affected but to a lesser extent. Fibers of glial tissue were widely separated by vacuoles and spaces, forming a more or less reticular pattern instead of the normal parallel arrangement of fiber bundles. Vacuolization and loss of cytoplasm were pronounced in the anterior lobe.

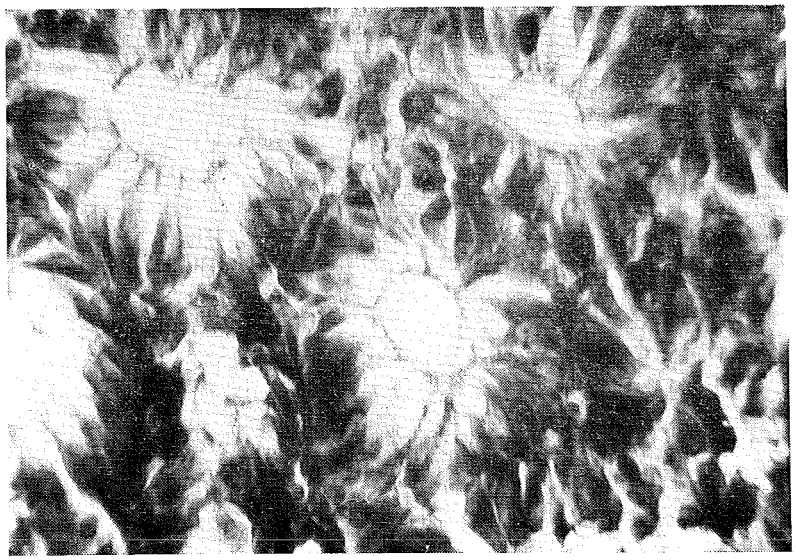

FIGURE 3—Pituitary gland, anterior lobe. Spawned-out sockeye salmon, Chilko River. Acid fuchsin-aniline blue. Vacuolization and loss of cytoplasm of cells of anterior lobe follicles. 450X

Moribund unspawned and partially spawned sockeye showed widespread cellular degeneration in all lobes of the pituitary and abundant, loosely arranged glial tissue throughout the ventral lobe (FIGURE 4). In general, pituitaries of moribund fish closely resembled those of spawned-out fish described above.

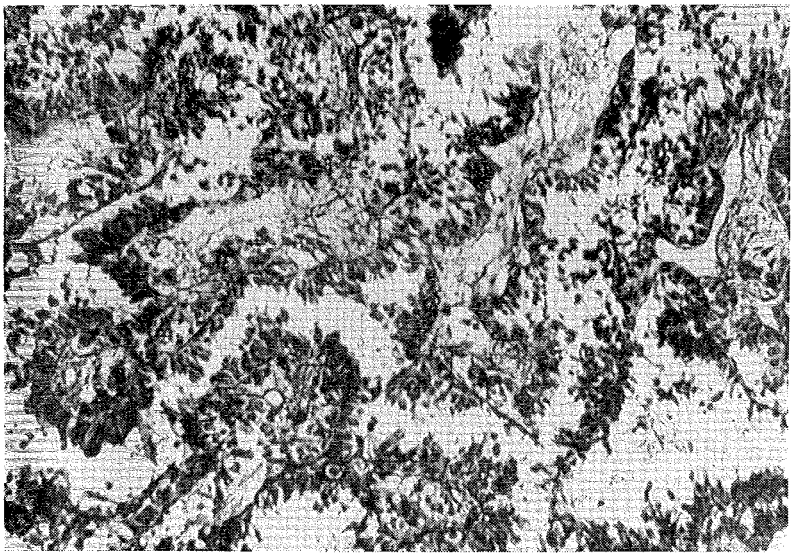

FIGURE 4—Pituitary gland, ventral lobe. Moribund sockeye salmon, Chilko River. Acid fuchsin-aniline blue. Extreme degeneration of ventral lobe; pyknosis, complete cytolysis, absence of cells and abundant, loosely arranged connective tissue. 125X

ADRENOCORTICAL TISSUE

Adrenal cortical cells examined from immature sockeye before entering fresh water (Lummi Island) occurred in small clusters throughout the "head" kidney (FIGURE 5). The individual cells were ovoid in shape and of fairly uniform size, averaging 10-12 microns in diameter. The cytoplasm was homogeneous to finely granular. Occasional cells with cloudy or frothy-appearing cytoplasm and indistinct cell outlines were seen, but these were infrequent within a section. The nuclei were oval in shape, approximately 6 microns in diameter with a distinctly visible network of chromatin. No histological differences were apparent between the adrenal cortical cells of fish from the early and peak segments of the run at Lummi Island.

Migrating fish sampled at Siwash Bridge exhibited a greater degree of cytoplasmic degeneration, usually focal in distribution, including a cloudy or "curdled" appearance and some shredding with loss of cell outline. Fragmented nuclei were infrequently observed, and the nuclei in general tended to stain darker with the chromatin material indistinctly visible. Cell size was essentially unchanged with nuclei approximately 6 microns in diameter and total cell diameters of 10-12 microns. There was some suggestion that adrenal cortical tissue was more

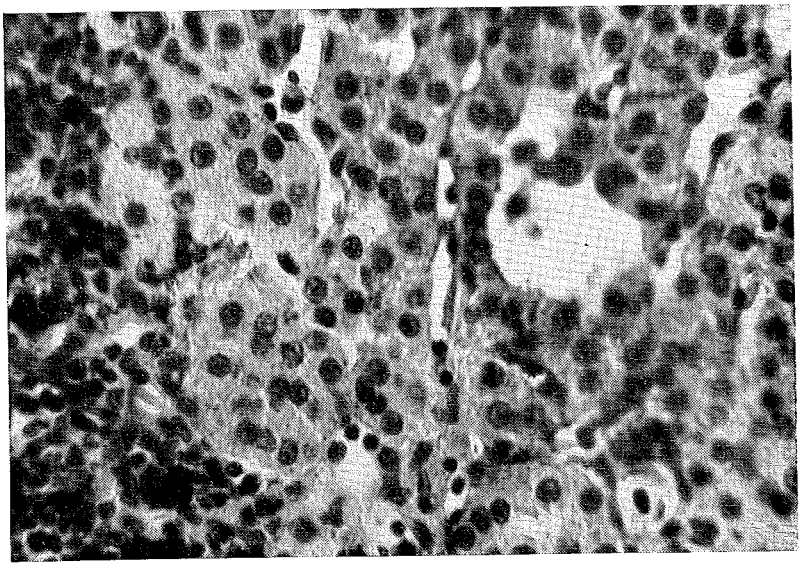

FIGURE 5—Adrenal cortical cells. Migrating sockeye salmon, Lummi Island. Normal tissue. H & E, 400X.

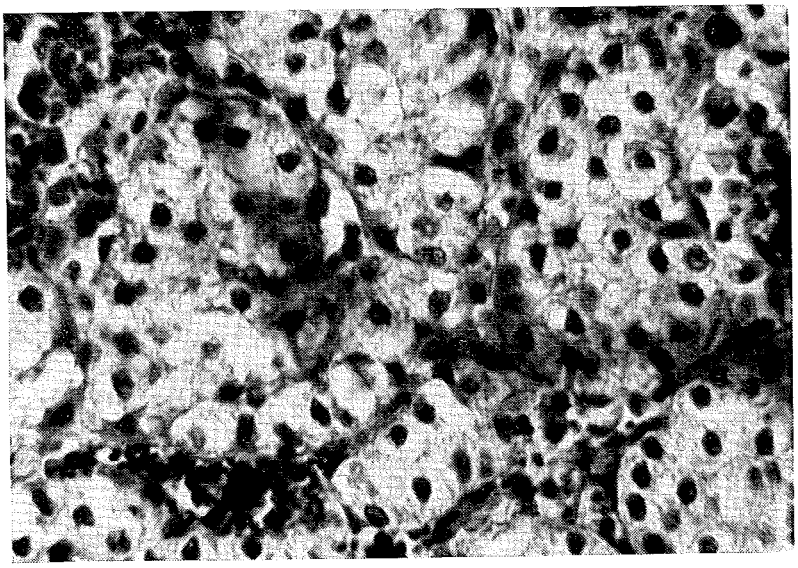

FIGURE 6—Adrenal cortical cells. Moribund sockeye salmon, Chilko River. Vacuolization, loss of cytoplasm and nuclear pyknosis. H & E, 400X.

abundant throughout the head kidney than in those fish sampled at Lummi Island, but this could not be determined quantitatively. No differences were observed between fish of the early and peak portions of the run.

Adrenal cortical tissue of sexually mature, pre-spawning sockeye exhibited little change over that of migrating fish sampled at Siwash Bridge. Cytoplasmic

changes still tended to be focal rather than generalized and cells with shredded, curdled cytoplasm were often surrounded by other cells showing no evidence of degenerative changes. Dark, homogeneous nuclei with no visible chromatin were commonly encountered. While a few slightly larger cells were noted (up to 16 microns in diameter), average cell size remained approximately 10-12 microns. Darkened nuclei showed little or no evidence of pyknosis. No differences were apparent between tissues of early and late pre-spawning fish sampled at Chilko River.

The majority of spawning and spawned-out fish obtained from Chilko River evidenced rather extensive degenerative changes, including nuclear pyknosis, shredding and vacuolization of cytoplasm, loss of cell outline and complete absence of cells. Again, areas of extreme degeneration were surrounded by apparently normal cells, essentially unchanged from cells examined in sexually immature fish obtained at Lummi Island.

Unspawned and partially spawned moribund fish from Chilko River showed extreme degeneration of adrenal cortical tissue (FIGURE 6). Loss of cytoplasm and absence of cells produced a web-like appearance in certain areas of the tissue examined. Nuclear pyknosis was widespread, and few areas of apparently normal cells were observed.

PANCREAS

Pancreata examined at the various stages of sexual maturity showed diverse quantities of fat, most abundant in immature fish and decreasing as fish approached spawning. Considerable variation was noted in size of the Islets of Langerhans (40 to 300 microns diameter) in individual fish and within groups of fish from specific sampling areas. No correlation was found between size of the Islets and stage of sexual maturity.

Cell types of the Islets were not always clearly distinguishable. In fish sampled at Lummi Island, alpha and beta cells appeared to be approximately equal in numbers. Granules were visible in alpha cells but extremely indistinct or absent in beta cells and in the acinar tissue. (It should be noted that granules of the beta cells are alcohol soluble and tissues had been preserved in 70% ethanol for periods up to one year prior to sectioning.)

Beta cells predominated in the Islets of migrating sockeye at Siwash Bridge. Brightly staining zymogenous granules were clearly visible in the acinar tissue. No differences were noted in pancreatic histology of sockeye from the early and peak segments of the run.

Pancreata of all fish examined on the spawning grounds, including pre-spawning, spawning, spawned-out and moribund samples were very similar histologically. The predominant cell type of the Islets was the beta cell, with pale staining cytoplasm but no discernible granules. A few alpha cells were present and, although some granulation was retained, the granules appeared less distinct

than those observed in immature fish. Some cells were present with clear, unstained cytoplasm, and though they are presumed to be degranulated cells their origin is not known. Pancreatic acini contained many distinct zymogenous granules. No evidence of degenerative changes was noted.

KIDNEYS

Kidneys of both early and peak migrants sampled at Lummi Island showed beginning degenerative changes of two types: vacuolization and loss of cytoplasm in the epithelial lining of a few isolated tubules and the accumulation of small, glassy, acidophilic hyalin-like droplets in the apical portion of tubular epithelial cells. Periodic Acid-Schiff staining techniques, although not specific for hyalin, were used to substantiate the character of these droplets. Neither condition was widespread, nor did they occur simultaneously within the same tubule. The majority of tubules were unaffected (FIGURE 7) and glomeruli appeared normal (FIGURE 8).

Kidneys of sockeye from both early and peak segments of the run at Siwash Bridge showed similar evidence of degeneration although these cellular changes were not obviously advanced from the tubular degeneration observed in Lummi Island fish (FIGURE 7). There was some suggestion of a thickening of Bowman's membrane in a few kidneys. Glomeruli (FIGURE 8) often appeared somewhat denser and larger than normal, with increased amounts of connective tissue causing a thickening of the walls of glomerular arterioles.

The kidneys of pre-spawning fish obtained from Chilko River appeared essentially the same as those of the migrating Siwash Bridge samples, and no

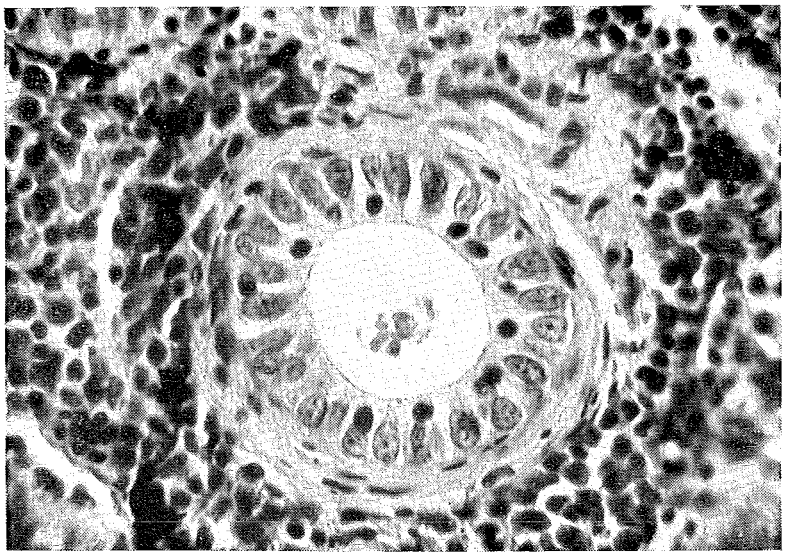

FIGURE 7—Kidney. Migrating sockeye salmon, Lummi Island. H & E. Normal kidney tubule. 400X

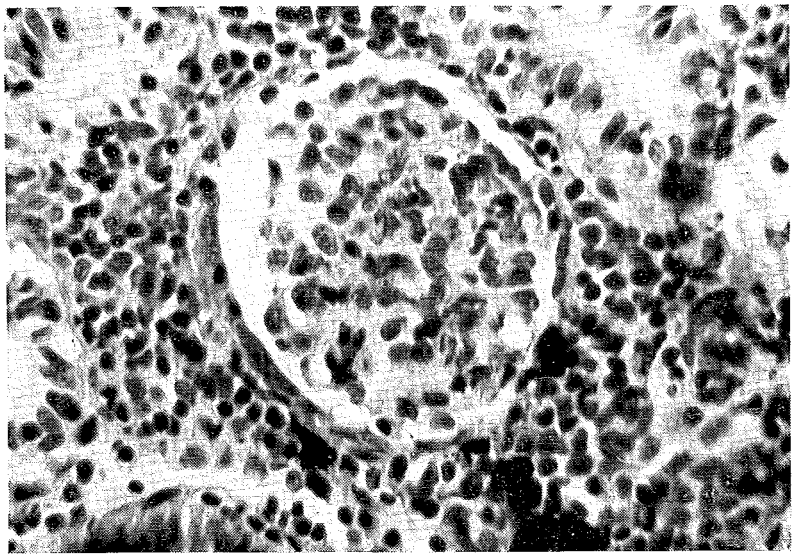

FIGURE 8—Kidney. Migrating sockeye salmon, Lummi Island. H & E. Normal glomerulus. 400X

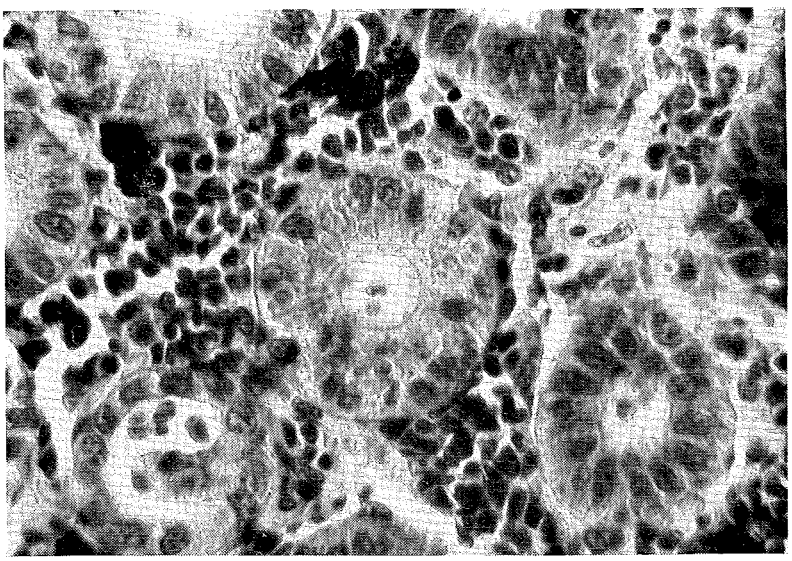

FIGURE 9—Kidney. Spawning sockeye, Chilko River. Tubular degeneration characterized by acidophilic cytoplasmic droplets. H & E, 400X.

differences were detected between tissues of early and late arrivals on the spawning grounds.

Spawning fish exhibited moderate to marked hyalin droplet degeneration of tubular epithelium (FIGURE 9). In some cases these droplets entirely filled every cell of a given tubule and a significant proportion of tubules within a section were

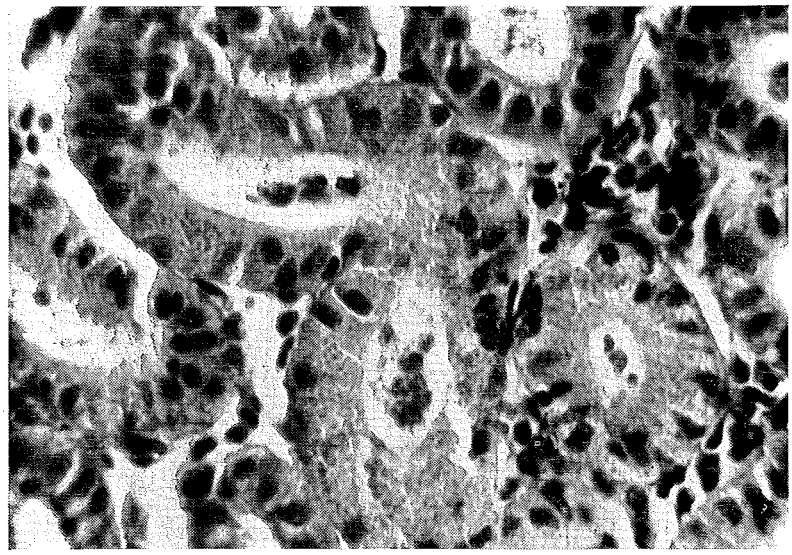

FIGURE 10—Kidney. Spawned-out sockeye, Chilko River. Cytoplasmic droplets and nuclear pyknosis in tubular epithelium. H & E, 400X.

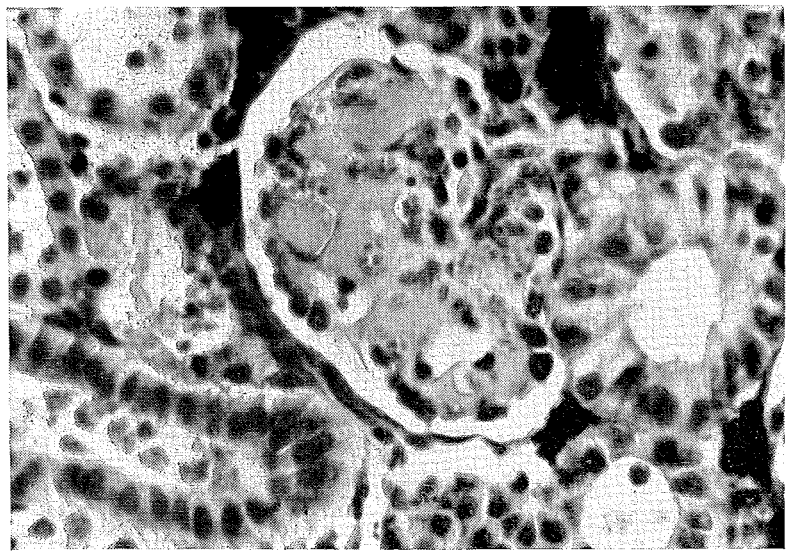

FIGURE 11—Kidney. Spawned-out sockeye salmon, Chilko River. H & E. Replacement of glomerular endothelium with homogeneous acidophilic hyalin-like material. 400X

thus affected. Vacuolization and loss of cytoplasm occurred infrequently. An increased thickening of the glomeruli was seen, and a hyalin-like material was now observed in the glomerular tufts. This hyalinization, while consistently present in at least one or more glomeruli of a section, varied considerably in the extent of

involvement. In some glomeruli only a trace of this material was evident while in others, all but a few capillary endothelial cells were completely obliterated by the material.

Spawned-out Chilko sockeye showed widespread hyalin droplet degeneration of tubules (FIGURE 10) and hyalinization of glomeruli varied from slight to marked (FIGURE 11). Moribund unspawned and partially spawned sockeye evidenced the same degree of degeneration observed in spawning and spawned-out fish. A hemosiderin-like pigment was present in kidneys examined from all sampling areas.

LIVER

Presumably due to improper or inadequate fixation, moderate to severe post-mortem deterioration, detectable both grossly and microscopically, was present in many of the liver samples studied and these changes often complicated the evaluation of ante-mortem cellular structure.

Sockeye from both the early and peak segments of the Chilko run sampled at Lummi Island showed moderate to heavy fat deposition in the liver. Cellular structure was considered normal.

At Siwash Bridge, fat deposits were greatly reduced or absent from livers of both early and peak migrating fish (FIGURE 12). No abnormalities were observed in areas unaffected by post-mortem changes.

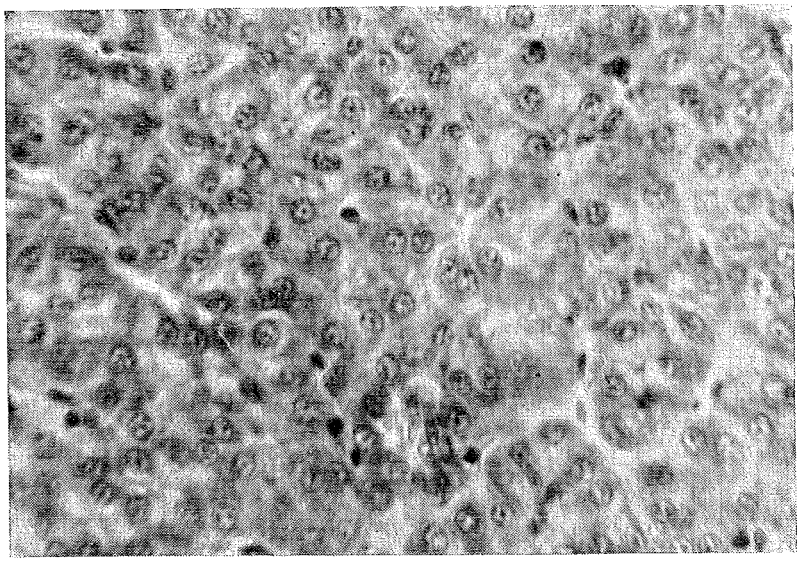

FIGURE 12—Liver. Migrating sockeye salmon, Siwash Bridge. H & E. Normal hepatic cells. 400X

Livers of most early pre-spawning sockeye taken from the Chilko River spawning grounds showed no detectable fat with hematoxylin and eosin staining techniques. Several of the late arriving sockeye, however, had slight amounts of fat remaining in the liver. Cytoplasm of hepatic cells tended to appear hazy and occasionally vacuolated.

Degenerative changes, generalized in distribution, were seen in the livers of spawning and spawned-out sockeye (FIGURE 13). The principal cellular change was a granular degeneration evidenced by a grainy cytoplasmic turbidity caused by minute, glassy acidophilic granules. In a few fish these granules approached 2.5 microns in diameter and cell outline was indistinct or invisible. A definite

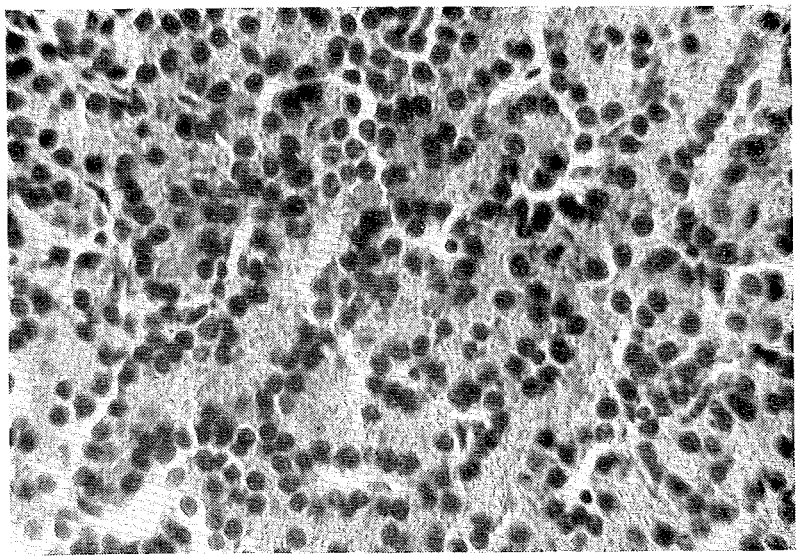

FIGURE 13—Liver. Spawned-out sockeye salmon, Chilko River. H & E. Dense, dark nuclei and granular degeneration of cytoplasm. 400X

similarity in size and character of these granules to the hyalin droplets observed in renal epithelium was noted. Nuclear changes ranged from abnormally coarse chromatin to dark, densely staining nuclei and pyknosis.

Moribund fish exhibited granular degeneration of the cystoplasm and dense, often pyknotic nuclei. These changes were far in advance of those encountered in normal, unspawned salmon, resembling the more severe degenerative changes seen in spawned-out fish.

SPLEEN

No abnormalities were noted in splenic tissue from sockeye of early and peak segments of the run at Lummi Island (FIGURE 14). A gradual depletion of lymphocytes and increase in connective tissue was observed as sexual maturation progressed. These changes were minimal or absent in fish obtained at Siwash

Bridge and only slightly more evident in pre-spawning sockeye sampled on the Chilko River spawning grounds. Hemosiderin was present in samples at all stages of sexual maturity. No differences were noted between migrants of the early and peak portions of the run.

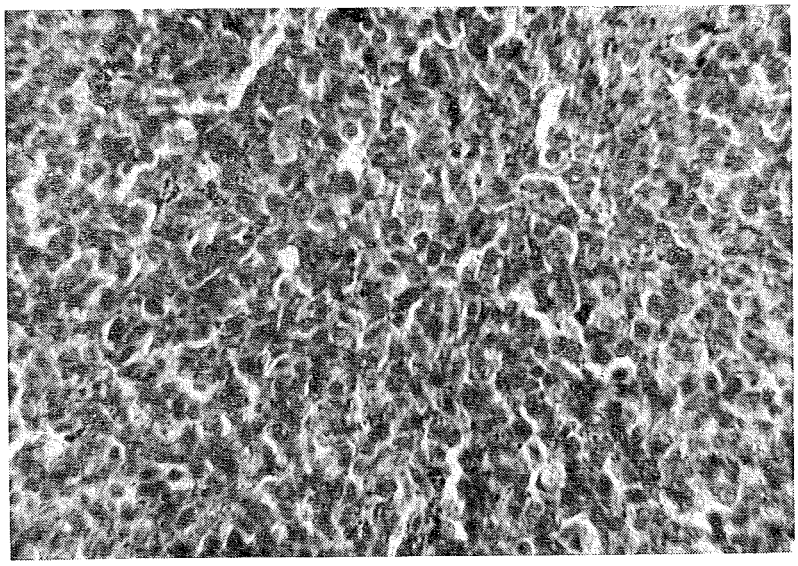

FIGURE 14—Spleen. Migrating sockeye salmon, Lummi Island. H & E. Normal abundance of lymphocytic tissue. 400X

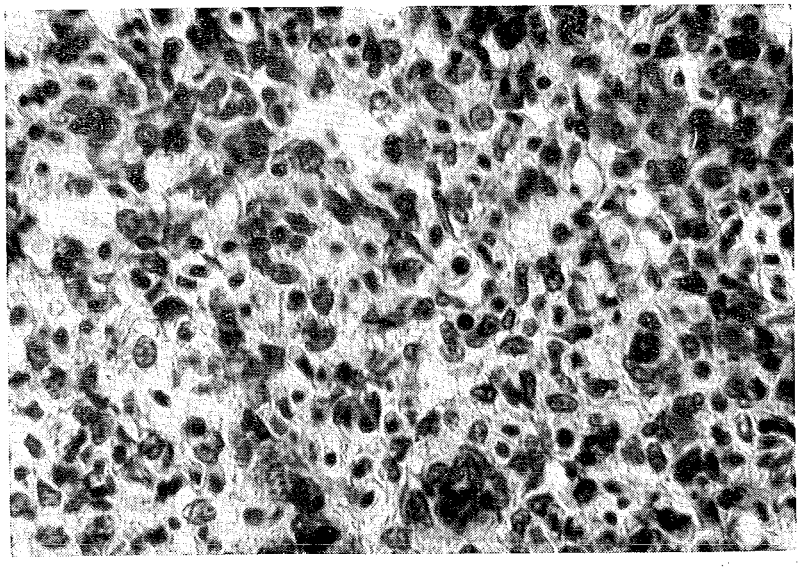

FIGURE 15—Spleen. Moribund sockeye salmon, Chilko River. H & E. Depletion of lymphocytes. Section composed primarily of connective tissue and red blood cells. 400X

As compared to Lummi Island samples, spawning and spawned-out sockeye spleens consistently showed a moderate increase in connective tissue and a decrease in the number of lymphocytes. Moribund, unspawned sockeye also exhibited these changes, (FIGURE 15) the diminution of lymphocytes being extremely marked in one fish. Focal necrosis was seen in two of the spleens, and a third fish showed a generalized splenic necrosis.

HEART

Myocardia of sockeye from early and peak portions of the run at Lummi Island appeared essentially normal. Areas of edema were observed in several hearts, presumably the result of the brief but violent struggle of the fish prior to death. Small edematous areas were similarly found in myocardia of occasional fish at almost every stage of sexual maturity.

At Siwash Bridge, myocardia of approximately fifty per cent of the salmon examined appeared normal (FIGURE 16) but the remainder showed evidence of initial degenerative changes. These changes consisted of a loss of fibrils from the central portion of a few muscle fibers, so that in cross section the affected fibers appeared hollow, with the remaining fibrils oriented around the periphery of the fiber. The condition was not generalized or pronounced at this stage of migration. Rarely did more than one or two fibers in an isolated fiber bundle exhibit this change, and only a small percentage of fiber bundles were thus involved. No difference was noted in the extent or severity of myocardial degeneration between migrants of the early and peak segments of the run.

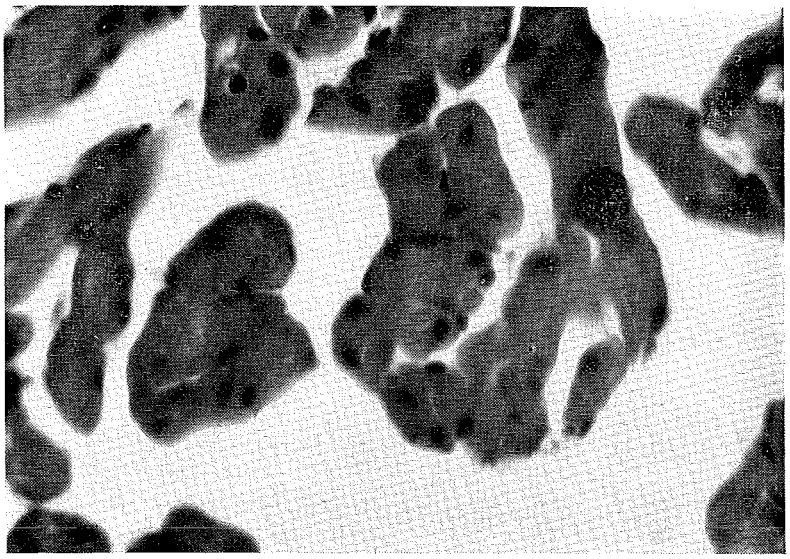

FIGURE 16—Heart. Migrating sockeye salmon, Siwash Bridge. H & E. Normal cardiac muscle fibers in cross section. 450X

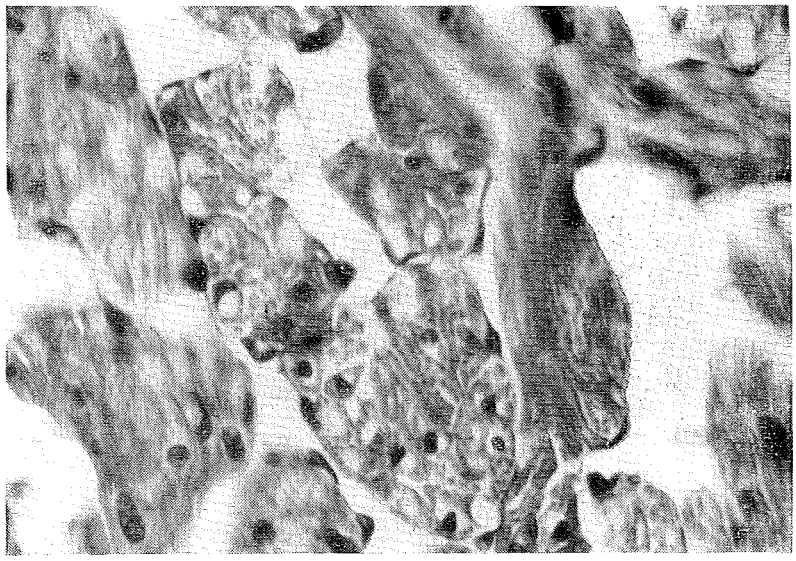

FIGURE 17—Heart. Moribund sockeye salmon, Chilko River. H & E. Cross section of muscle fibers showing loss of fibrils. 450X

Approximately 70% of both early and late pre-spawning sockeye at Chilko River exhibited a loss of muscle fibrils. In general the condition did not appear to have advanced from the stage observed in migrating sockeye at Siwash Bridge, although it was observed in a larger proportion of the fish. In longitudinal section, affected fibers appeared vacuolated and contained scattered granules, but nuclei were generally unaltered. Adjacent, unaffected fibers appeared normal in every respect.

Spawning and spawned-out sockeye had essentially the same incidence of myocardial degeneration as pre-spawning fish. In most cases evidence of degeneration was slight, and only one fish (spawning) showed a pronounced and generalized loss of fibrils. All moribund fish examined showed a moderate loss of myofibrils (FIGURE 17).

GILLS

Gills were examined primarily for condition of respiratory epithelia. No consistent abnormalities were found in any group of fish examined. Several fish showed a detachment of a few microns of respiratory epithelia from the underlying supporting tissue, but it is difficult to say with certainty that this was not an artifact.

Particular attention was paid to gills of moribund fish because of the incidence of columnaris disease on the Chilko River spawning grounds (Wood, 1965). A bacteriological stain (Lillie's method) was therefore employed in addition to routine staining techniques, and while gram negative rods, suggestive of *Chondrococcus columnaris* were present, no evidence of gill damage was seen either grossly or microscopically.

DISCUSSION

The post-spawning death of Pacific salmon has been the subject of investigation by several authors. While a multiplicity of factors are undoubtedly involved, accumulated evidence implicates certain endocrinological disorders as being causally related to the lethal process. Robertson *et al.* (1961) report a significant increase in plasma levels of 17-hydroxycorticosteroids in spawning chinook salmon *(O. tshawytscha)*, and Schmidt and Idler (1962) found similarly elevated levels of circulating adrenal cortical hormones in spawning sockeye salmon of the Chilko run. The effects of hyperadrenocorticism are widespread and deleterious, and entirely comparable histological changes were found to occur in hydrocortisone-treated rainbow trout and spawning Pacific salmon (Robertson *et al.*, 1963). These changes included degeneration of the pituitary gland, adrenal cortical tissue, stomach, liver, kidney and cardiovascular system, atrophy and degeneration of the thyroid gland, and depletion of lymphocytes in the spleen and thymus.

Robertson *et al.* (1961) state that the elevated levels of circulating adrenocorticosteroids observed in spawning salmon are the result of a hyperplasia (and hyperfunction) of the adrenocortical tissue. This hyperplasia was described as accompanying the sexual maturation and spawning of three species of Pacific salmon (Robertson and Wexler, 1959). In contrast to this finding, no histological evidence of a hypertrophy or hyperplasia of the adrenal cortical cells was found in Chilko sockeye. Average cell size remained the same from sea to spawning and there was no distinct indication of an increase in adrenocortical tissue within the head kidney. It must be borne in mind, however, that the histological findings described in fish sampled at Lummi Island do not necessarily represent the state of the various tissues at the beginning of the spawning migration. The spawning migration actually commences at some undetermined time and at an undetermined distance relative to the passage of fish through the Georgia Strait, and gonadal development is relatively advanced by the time sockeye reach Lummi Island. Adrenocortical tissue of sockeye may already be in a state of hyperfunction at this stage of the spawning migration. Furthermore, abnormally high levels of adrenocorticosteroids may result from a decreased excretion as well as an increased production of the hormones. Idler and Truscott (1962) state that impaired metabolism and clearance of adrenocorticosteroids result in high levels of these hormones in the blood of mature and spawning sockeye salmon. Certainly, evidence of impaired renal function was present in spawning Chilko sockeye, although the effects of the observed kidney degeneration on the clearance of adrenal cortical hormones cannot be determined by histology alone.

Progressive degenerative changes of the pituitary gland, adrenal cortical tissue, kidney, liver, and myocardium, and depletion of lymphocytes in the spleen and peripheral blood were found to accompany the sexual maturation and spawning of sockeye salmon of the Chilko River run. These changes resemble, in part, the manifestations of hyperadrenocorticism. For the purposes of this paper, however, the significance of these changes lies in the effect rather than the cause of this degeneration. Cellular degeneration obviously reflects an altered or reduced

cellular function. Depending on the extent of involvement within a tissue and the reserves of that tissue, function at the organ level may in turn be altered or reduced. Histology alone does not necessarily define the exact nature of a functional alteration resulting from structural changes, nor the extent to which function has been reduced, except where a generalized condition of necrosis throughout a tissue indicates a complete absence of function. It is difficult if not impossible to incriminate lesions of a single tissue or organ as being responsible for post-spawning death when virtually every vital organ in the body is undergoing degeneration. Rather, death would appear to result from the cumulative effects of several or perhaps all degenerative processes occurring in spawning salmon. If a true hyperadrenocorticism exists, changes in fluid and electrolyte balance and effects on metabolism resulting from high levels of mineralocorticoids and glucocorticoids must also contribute significantly to the death of Pacific salmon.

Post-spawning death, then, is a complex physiological phenomenon involving a multiplicity of factors. The processes ultimately responsible for post-spawning death of sockeye salmon are initiated early in the sexual development of the fish, as evidenced by cellular changes commencing early in the spawning migration. Initial degenerative changes are detectable in some organs long before sockeye reach the spawning grounds. The pathogenesis of post-spawning death does not begin with the spawning act; no pathological condition, detectable by microscopic anatomy, suddenly arises in spent salmon that was not present to some degree in pre-spawning fish. The intimate relationship between the tissue degeneration of spawning salmon and the process of sexual maturation has been clearly illustrated by Robertson and Wexler (1962b) and suggests a common initiatory mechanism for both phenomena. These authors demonstrated that early castration of kokanee salmon *(O. nerka kennerlyi)* inhibited the degenerative changes normally occurring in these fish when sexually mature at four years of age, and prolonged life span by several years.

The time, relative to spawning, at which these lethal processes terminate is subject to individual variation as demonstrated by the variation in survival time following the spawning act. The time from spawning to death may range from a few days to several weeks (Killick, 1955). Since a morbid condition exists in advance of the reproductive act, it is reasonable to expect that the deteriorative processes responsible for universal post-spawning death of sockeye salmon may, in some cases, progress to a degee of severity incompatible with life prior to spawning, and result in the premature death of the fish.

Moribund, unspawned fish examined at Chilko River showed degenerative changes comparable to those observed in spent fish and notably advanced from those changes characteristic of unspawned salmon. No evidence of disease was observed in these fish with the exception of anemia. Bacteriological staining of kidney, spleen and liver sections disclosed no organisms. While this anemia undoubtedly contributed to the moribundity of these fish, the underlying cause of anemia is not known. Anemia has not been typically associated with pre-spawning mortalities of Fraser River sockeye in the past, nor is it a consistent finding in

spent fish although it may well be a terminal event in many cases. On the basis of histological evidence, it may be concluded that these fish died from the same pathology universally responsible for post-spawning death of sockeye salmon.

Loss of a few unspawned salmon may therefore be expected to arise from the histopathology accompanying sexual maturation. This is not meant to imply that mortalities of the magnitude encountered in 1961 and 1963 can be attributed to "natural" causes. Perhaps little inference can be made on past losses from the results of a survey conducted on a sockeye run which spawned successfully. But from the standpoint of management and protection of future runs, certain implications become apparent.

It is important to recognize that, on the basis of histological changes, a rather delicate balance exists between time of spawning and time of death in sockeye salmon. Such ill-defined factors as individual resistance and biological variations can account for a natural loss of some fish prior to spawning, but it may also be concluded that in all sockeye this phase of the life cycle represents a period of minimal body resistance. External stress factors, normally tolerated by immature fish, could well prove lethal to spawners. Certainly, adverse environmental conditions or unnatural delay of spawning could be expected to increase the incidence of pre-spawning mortalities arising from the histopathology accompanying sexual maturation.

Historical data indicate a predisposition to pre-spawning mortalities among the early arrivals to the spawning grounds of the Fraser River system. If innate histopathology were responsible for these losses, one might expect a comparative study of migrants from the early and peak segments of the run to reveal more rapid or severe degeneration among fish of the early segment. However, entirely comparable histological changes were found to accompany the sexual maturation of both early and peak segments of the Chilko sockeye run. If a physiological predisposition to unspawned mortalities exists among early migrants, other avenues of approach must be utilized to define the factors responsible. However, on the basis of histological evidence, it appears most probable that the higher incidence of unspawned mortalities among early arrivals would be due to unfavorable environmental factors operating during the early period of the run.

A similar histologic survey, conducted on the Early Stuart sockeye run in the summer of 1965 is currently nearing completion. Results obtained to date confirm the findings of the Chilko investigation. In general, histological changes similar to those described in Chilko River sockeye were found to accompany the sexual maturation and spawning of sockeye of the Early Stuart race, and no differences were detected in the histology of fish of the early and peak segments of the run (Colgrove, 1966).

SUMMARY

In recent years, various spawning escapements of Fraser River sockeye have suffered pre-spawning mortalities on the spawning grounds ranging from a negligible portion of the run to losses as high as 90% of the escapement. Historical data indicate that most losses have occurred primarily among the first arriving sockeye and heaviest losses have occurred when the entire run has arrived on the spawning grounds earlier than usual. In order to obtain a better understanding of this complex and critical phase of the life cycle, a study of the changes occurring in the tissues and blood of adult sockeye salmon during their anadromous migration and on the spawning grounds was initiated in the Fall of 1964. The investigation serves in part as a comparative study of the various chronological segments of a spawning migration.

The Chilko River race of sockeye was selected for this investigation. Sexually immature salmon were captured approximately 50 miles seaward from the mouth of the Fraser River shortly before the beginning of the freshwater phase of the spawning migration. Migrating sockeye were obtained near the termination of their upstream migration at a location 80 miles below the spawning ground. At the Chilko River spawning ground, located approximately 400 miles from the mouth of the Fraser River, pre-spawning, spawning, spawned-out and moribund-unspawned sockeye were sampled. Fish from both the early and peak segments of the run were sampled where possible.

Stage of maturity of fish sampled at each location was determined by obtaining gonad and total body weights of a number of female sockeye. Average gonad weights ranged from approximately 3.5% of body weight in sexually immature sockeye sampled at sea to 12.7% of body weight in pre-spawning fish on the spawning grounds.

The following hematological and histological changes were found to accompany sexual maturation and spawning of sockeye salmon.

1. A comparison of red cell counts obtained at the various locations indicates that a slight reduction in numbers of circulating erythrocytes occurred during the initial phase of the upstream migration. Anemia was encountered in 13% of the spawning sockeye sampled, in 19% of the spawned-out fish and in 100% of the moribund-unspawned salmon obtained on the spawning grounds.

2. Differential leukocyte counts revealed a distinct shift in the relative proportions of neutrophils, lymphocytes and macrophages with successive stages of maturity. The percentage of neutrophils and macrophages increased from sea to spawning while the proportion of lymphocytes progressively decreased.

3. Changes observed in the pituitary glands of migrating and spawning sockeye included an increase in numbers of basophils and an increase in the relative size of the dorsal lobe as sexual maturation progressed, degranulation of basophils and acidophils in sexually mature and spawned-out fish, and increased amounts of loosely arranged connective tissue in the ventral lobe of mature and spawned-out fish. Progressive degenerative changes occurred in the dorsal, anterior and ventral lobes.

4. A progressive degeneration of adrenocortical tissue occurred during the migration and spawning of sockeye salmon, beginning initially with cytoplasmic changes in a few scattered cells and progressing to widespread cellular degeneration, including loss of cytoplasm and cell outline, nuclear pyknosis and absence of cells.

5. Pancreatic histology revealed an increase in relative numbers of beta cells in the Islets of Langerhans as sexual maturation progressed. The Islets varied in size, and no correlation between Islet size and sexual development was noted. Zymogenous granules, indistinct in sockeye sampled at sea, were clearly visible in the acinar tissue of sexually mature fish.

6. Mild tubular degeneration was observed in the kidneys of migrating sockeye salmon, consisting of vacuolization of cytoplasm and the accumulation of glassy, acidophilic hyalin droplets in the epithelium of a few isolated tubules. Hyalin droplet degeneration of tubules was widespread in spawning and spawned-out fish, and glomerular changes observed included a thickening of Bowman's membrane and of the glomerular tuft and a hyalinization of the glomeruli.

7. Hepatic fat stores were depleted during the spawning migration. In spawning and spawned-out salmon a granular degeneration of the cytoplasm of hepatic cells was observed, and nucleii appeared dense and often pyknotic.

8. A gradual diminution of lymphocytes and increase in connective tissue occurred in spleens of sockeye salmon as sexual maturation progressed.

9. A loss of myofibrils from the central portion of cardiac muscle fibers was observed in approximately half the migrating sockeye sampled below the spawning grounds and in about 70% of the fish sampled on the spawning grounds. The condition tended to be localized and restricted to a relatively few fibers.

10. No consistent abnormalities were detected in the gills of any group of fish examined.

The cumulative effects of these changes on the physiology of the fish are considered responsible for the post-spawning death of sockeye salmon. Moribund, unspawned fish examined on the spawning grounds showed a degree of degeneration throughout the body comparable to that observed in spent fish and notably advanced from the stage of degeneration characteristic of unspawned salmon. It was concluded that these fish died from the same pathology universally responsible for the post-spawning death of sockeye salmon.

Entirely comparable histological changes were found to accompany the sexual maturation of both the early and the peak segments of the Chilko sockeye run. Other factors must influence the higher incidence of unspawned mortalities among early arrivals on the spawning grounds.

It is important to recognize, from the standpoint of management, that the factors responsible for post-spawning death of sockeye salmon are initiated long before the reproductive act is culminated and that spawning sockeye cannot tolerate adverse environmental conditions or unnatural delay of spawning.

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