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Foreword

Most scientists who are not engaged in the field of diseases of fishes still believe that fish pathology covers only a very insignificant area of comparative pathology. After Hofer published the first book on diseases of fishes in Munich in 1904, the growth of fish pathology was slow until the end of World War II. However, Marianne Plehn and W. Schäperclaus did make great contributions in this interim period.

During the last 20 years progress was as fast as that in other fields of biology and medicine. Conferences on diseases of fishes in America started in 1953 when the first symposium was sponsored by the American Fisheries Society. Since then, symposia have been held more often and on a larger scale. There were four symposia in the United States on various aspects of diseases of fishes, and one symposium on hepatoma of rainbow trout. Research on hepatoma is an excellent example of how a well-organized effort can solve a difficult problem in a very short time.

There have been three symposia in Europe, sponsored by the International Office of Epizootics and organized by Dr. P. Ghittino. Another symposium sponsored by the Fisheries Society of the British Isles and the London Zoological Society was organized by Lionel Mawdesley-Thomas and held in London in 1971. The last symposium was organized jointly by the United Nations Food and Agriculture Organization and the IOE and held in Amsterdam in 1972. There were also numerous fish disease conferences held in the Soviet Union, where there are many outstanding fish disease specialists, of whom Dogiel and Bauer are probably the most widely known. Fish disease symposia are also being held in Japan. Unfortunately, the language barrier and the lack of adequate translations stand in the way of full utilization of this information in the western world.

This present symposium was different because its organizers represent medical research. Utilization of fishes for research in comparative pathology was begun by Schlumberger and Lucké, whose monograph on tumors of cold-blooded vertebrates is a milestone in comparative pathology. Nigrelli, Scarpelli, Dawe, Ashley, Wood, Dunbar, Reichenbach-Klinke, and lately Mawdesley-Thomas, Roberts, Mulcahy, Yasutake, Herman, and others have greatly contributed to comparative pathology by research on fishes. This symposium was the first international conference on contributions of fish pathology to comparative pathology and therefore is a milestone which will be long remembered.

S. F. Snieszko

August 9, 1972

Preface

The nine universities that make up Universities Associated for Research and Education in Pathology, Inc., take great pride in this timely and pioneering symposium on Fish Pathology.

In a very real sense the Registry of Comparative Pathology, which cosponsored this symposium, was developed because for many decades there has been a strong interest in comparative pathology at the Armed Forces Institute of Pathology and particularly in the Veterinary Pathology Division. At first this interest was fostered by the Committee on Pathology of the National Academy of Science-National Research Council, which I had the privilege of chairing. Now it is encouraged by the nine universities who joined together in the late 1960s to find ways and means of sustaining the American Registry of Pathology.

This has been a very rewarding effort in many ways and this symposium was a good example of the opportunities that present themselves for those who are interested in the broad interspecies approach to the study of disease.

On behalf of the nine universities I represent and on behalf of the Advisory Committee of the Registry of Comparative Pathology, which has representatives from three additional universities, I want to pay tribute to and to express our appreciation to the University of Wisconsin Sea Grant Program for joining hands with the Registry in sponsoring this symposium.

I want to pay special tribute to and to thank Drs. George Migaki and William E. Ribelin, who worked so hard in developing the program and making the arrangements. Also, thanks are due to Colonel R. W. Morrissey, Lt. Colonel F. R. Robinson, and Lt. Colonel P. K. Hildebrandt for the hospitality of the Armed Forces Institute of Pathology and the Walter Reed Army Institute of Research for the outstanding facilities that these Institutes made available for this conference.

I want to pay tribute to and to thank the Division of Research Resources, National Institutes of Health, United States Department of Health, Education, and Welfare, whose material support has made this Registry and this symposium possible. And last, but not least, I want to express our appreciation to the Armed

Forces Institute of Pathology and its leadership on the Registry for their support and help not only here and now but over the years as the Registry of Comparative Pathology has had its birth and early development.

R. W. Wissler

August 7, 1972

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THE Pathology of Fishes

EDITED BY

William E. Ribelin
George Migaki

The University of Wisconsin Press

1

Comparative Fish Histology

LAURENCE M. ASHLEY

Comparative fish histology is of increasing importance today, as is comparative fish pathology. While the literature on this subject is still fragmentary and scattered, a considerable number of papers are available. An atlas of salmonid histology now in preparation by this author and two others (Joseph H. Wales and William T. Yasutake) promises useful supplementation of textbooks and courses in comparative vertebrate histology. In his excellent textbook of comparative histology (1), Warren Andrew remarked, "In the true sense each animal is a microcosm made up of marvels which lie beyond the field of vision of the naked eye." This is particularly true with fishes, among which specific variations worthy of histological comparison are legion. Common names of but a few fishes suggest interesting differences, e.g., seahorse, scorpionfish, boxfish, hatchetfish, porcupinefish, ribbonfish, climbing perch, fanged viperfish, four-eyed fish, anglerfish, lanternfish, and lungfish. All have differences in their morpho-histology.

THE INTEGUMENT

The skin may be naked or scaly. Dermal scales of several types may be present, depending on the species. Scales are enclosed in dermal scale pockets and are arranged in an imbricated pattern. The epidermis may be thin or thick, slightly keratinized and with an abundance of mucoid cells. An extensive lateral line system of canals, nerves, and neuromasts relays impulses induced by external stimuli via cranial nerves 9 and 10 to the brain. Taste buds occur on the lips, oral valves, and oral and pharyngeal mucosa of most fish, and in addition on the skin and barbels of catfishes and certain others. Several special cell types may occur in the epidermis. Melanophores and other pigment cells may be seen below the epidermis and below the dermis, which typically contains both vascular and fibrous layers.

DIGESTIVE SYSTEM

Some species have membranous and bony devices which permit extraordinary protrusion of the lips for feeding. Others have fused jaws and must suck food into the mouth through slightly protruded lips (e.g., seahorses and pipefishes). Inside the mouth are upper and lower oral valves formed as U-shaped folds of oral mucosa to regulate ingress and egress of respiratory water. The slightly movable tongue may contain teeth on its upper surface. Heavy grasping and smaller teeth are set on bone in the jaws. The pharynx is perforated on each side by four pairs of gill slits, the first gills being modified as pseudobranch glands (described below). The caudal end of the pharynx often contains pharyngeal teeth which may aid in macerating food before it reaches the stomach. The very short esophagus typically has longitudinal folds of mucosa containing mucous cells but no discrete cardiac glands. The gastric mucosa contains tall columnar cells which are apically mucoid, but the mucus is different from that in the esophagus. A stomach is absent from Cyprinidae (e.g., carp, goldfish), Cyprinodontidae (e.g., mummichog), Poeciliidae (e.g., molly, guppy, mosquitofish), Scaridae (e.g., parrotfishes), and others, but is well developed in most fishes, wherein there are gastric pits and fundic glands but few pyloric glands, depending on species. An oblique muscle coat is variable in its development or absence. Brunner's duodenal glands are not found in fishes. The muscularis mucosae, usually very thin and difficult to demonstrate, varies with the species. In carp, skeletal muscle runs almost the entire length of the intestine, but in trout skeletal muscle is replaced by smooth muscle in the anterior stomach. A gizzard, devoid of gastric glands, occurs in the gizzard shad and related species. The intestine usually shows little differentiation into regions, and its mucosa exhibits short, rather wide villi. The hindgut is not enlarged into a colon as in mammals, but it does have many mucous cells in the mucosa. Spiral valves are common in some species and prominent folds of mucosa occur in others. The intestine is of various lengths in different species, while in one, the stoneroller sucker (*Campostoma anomalum*), the intestine is neatly wound around the air bladder in a series of coils. The submucosa differs from that of mammals in having a stratum compactum or dense fibroelastic layer next to the muscularis mucosae and a granular layer of mast cells or tissue eosinophils predominating. These are more obvious in certain species. There are no true glands in the intestine of carp and goldfish.

The number of caeca varies with the species from 0 to 100 or more. These are finger-like diverticula at or just beyond the pyloric sphincter. Their mucosa is longitudinally folded and goblet cells are common. Caeca are believed to aid in food digestion. The various coats or layers are similar to those in the intestine but the muscularis mucosae may be absent.

The pancreas is usually diffuse, scattered in the mesentery or fascia of the caeca, and fat is often interspersed among pancreatic acini. Pancreatic islets will be mentioned later. The acinar tissue of the pancreas is quite typical of that of higher vertebrates. In the channel catfish and certain other species, a hepato-pancreas occurs and is represented by a layer of pancreatic acini around each portal vein or hepatic triad. There are no islets in the hepatic pancreas. Some species have a splenopancreas with pancreatic acini similarly distributed in the spleen. The pancreas of lungfish lies within the intestinal wall between the serosa and muscularis.

The liver is generally less well organized into lobules than it is in higher vertebrates. Portal triads often have their hepatic arterioles removed some distance from the associated vein and bile duct. Central veins are rather irregularly dispersed and the muralia (two-cell plates) of hepatocytes are often irregularly arrayed about the central veins. Thus, a zonal pattern is less distinct than in mammals. Sinusoids are often collapsed and hepatocytes are often filled with glycogen, giving a very vacuolated, pale appearance in hematoxylin and eosin (H&E) sections.

Kupffer cells are seldom seen clearly but bile canaliculi occur regularly and can be seen in well-fixed and stained H&E sections.

CIRCULATORY SYSTEM

Cardiac muscle in fishes resembles that of other vertebrates histologically, but the atrial and ventricular walls are relatively thinner and papillary muscles are poorly developed. The bulbus arteriosus is strongly elastic. Arteries and veins are thinner-walled, in keeping with a smaller blood volume per gram of tissue in fish as compared with terrestrial vertebrates. Arteries thus have poor smooth muscle coats as compared to their fibrous externas. The media and adventitia seem to blend. Lymphatic ducts and sinuses collect lymph from all parts of the body. Paired jugular and lateral lymph vessels return the lymph from head and trunk respectively, where the lymph and blood vessels unite at or near the common cardinal vein. Dorsal and ventral longitudinal lymphatics may also occur. Cyclostomes differ from higher fishes in having several somewhat diffuse connections between lymph and blood vessels, and are therefore said to have a hemolymph system. Small flattened lymph hearts with valves and contractile muscle fibers occur in the tail of true eels (*Anguilla*) and trout (*Salmo*). Deep lymphatics collect the lymph from viscera (2).

SPLEEN

The spleen is much different morphologically from that in mammals but is similar cytologically in having a reticular stroma, white pulp and red pulp, and a network of blood vessels. It may be more active hematopoietically than spleens of higher vertebrates. Blood volume in the spleen is decreased as fish exercise. Trout in "dash" swimming (as to capture prey or to escape) use white skeletal muscle which has only one-third to one-fourth as many blood capillaries as the red muscle which is used continually in respiration, balance, and similar continuing activities.

The spleen is a hematopoietic organ which may also function in blood cell destruction. It lies next to the stomach in bony fishes and in ganoids. The red blood corpuscles are ovoid and nucleated in all vertebrates except mammals. Nucleated thrombocytes occur in fishes. There are no platelets. In fish, unlike mammals, lymphocytes are the most common type of white blood cell in the circulating blood, neutrophils come next, then eosinophils, monocytes, and basophils, the last three of which are uncommon in circulating blood. Plasma cells are seldom seen and rarely if ever in the circulation. Macrophages are common in the connective tissues, where they appear to originate. Red blood cells and white blood cells may mature in the circulating blood, where immature forms can often be seen. Lungfishes have two types of eosinophils and two types of basophils. The African lungfish has red blood cells up to 45 microns in length.

RESPIRATION

Because fishes breathe mainly by means of internal gills, these organs are of particular interest to comparative histologists. Bony fishes have five pairs of embryonic gill arches, the first of which becomes modified during development into a pseudobranch gland, thus leaving branchial arches 2 to 5 for the functional gills. Each gill filament contains an afferent and an efferent branch from the corresponding branchial artery. Each filament also contains a rod of hyaline cartilage between the afferent and efferent filamentar vessels. Gill lamellae are roughly semicircular, attached basally and partially overlapping one another in series along either side of the filament. In longitudinal section lamellae look like villi—a view that is deceiving to the uninitiated. Lamellae are covered with simple squamous epithelium on either side. Between these two thin layers are alternating pillar (pilaster) cells and blood capillaries in the form of a capillary plexus. Blood enters each plexus from the afferent and exits via the efferent filamentar artery. Respiratory gases, ammonia products, and various mineral nutrients enter or leave the respiratory blood stream by passing first through a doubled plasma membrane of a pillar cell, then through two roughly parallel plasma membranes of a squamous epithelial cell. Electron micrographs would probably reveal minute pores in these delicate membranes. A little work has been done along this line. At the bases of gill lamellae may be found special chloride cells and albuminous cells for secretion of these substances.

In lungfishes, the air bladder contains both alveolar "sacs" and capillary plexuses similar to amphibian lungs. Teleost gas bladders are generally only for hydrostatic functions and contain a "red" gland and sometimes a special "oval" structure, both of which are concerned with hydrostatic functions. In physostomatous fishes a patent (open) pneumatic duct connects the gas bladder to the anterior end of the esophagus or to the intestine, depending on the species.

EXCRETORY SYSTEM

While the degenerate parasitic hagfish has a functional pronephros, other fish have a functional mesonephros containing well-developed glomeruli with nephron tubules and a collecting duct system. There is no distinct cortex and medulla as there is in higher vertebrates. The nephron of teleosts consists of the glomerulus with Bowman's capsule, a short neck piece, a first and second portion of the proximal convoluted tubule, the distal convoluted tubule, which is followed by the collecting tubules which drain into the mesonephric duct. The mesonephric duct dilates into a sinus-like bladder and empties via a short duct through the urogenital sinus. The nephron of teleosts lacks a loop of Henle. Juxtaglomerular bodies have been described for many species. Some fish have aglomerular mesonephric kidneys and no functioning pronephric kidney. Lungfish *Ceratodus* sp. has short caudally-placed kidneys firmly attached to the ovary or testes.

REPRODUCTIVE SYSTEM

Ovaries and testes in young fishes are slender cords of delicate stroma and many gonia or developing sex cells. The ova develop from a delicate germinal epithelium and come to lie in "skeins" enveloped by a thin-walled capsule which ruptures when the eggs ripen. The eggs, now free in the peritoneal cavity, exit normally via genital pores beside or within a genital papilla. Pacific salmon spermatogonia develop in clusters of cysts which burst when the gonia have become spermatids or spermatozoa. After spawning, cells of testicular ducts and of ovarian tissues of Pacific salmon become necrotic. Vital signs deteriorate rapidly and the fish dies. Steelhead and sea-run cutthroat trout may survive two or three such spawning runs before showing signs of morbidity.

NERVOUS SYSTEM

The microscopic structure of the central nervous system of fishes is similar to that of mammals but morphologically much simpler. The retina of the eye contains more cones than rods in most teleost fishes but various species differ in this regard. The lens is spherical and fish are nearsighted. Ganglion cells are similar to those of higher vertebrates and most nerve endings are of the simple type. There are few specialized or encapsulated endings in teleosts. Unique giant neurons (Mauthner cells, Fig. 1.1) number from two dozen in cyclostomes to only

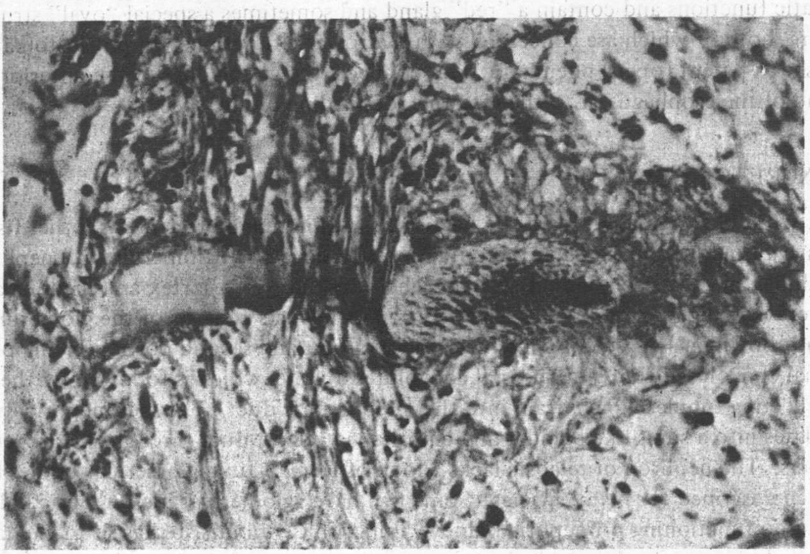

Fig. 1.1. Section through a Mauthner's giant neuron from the brain of a lake trout (*Salvelinus namaycush*). (Courtesy of A. H. Walsh.) Hematoxylin and eosin (H&E); ×500.

two in teleosts; they occur near the cranial nerve roots in the medulla, and their giant axons (Fig. 1.2) course along the spinal cord. These carry high-velocity impulses similar to those carried by annelid giant nerve cells. An interesting crista with a delicately attached otolith occurs in each ampulla of the semicircular canals (Fig. 1.3) and a typical macula is present in the utriculus and sacculus. The laminations of otoliths are sometimes used to determine age. The meninges of teleosts are much simplified and exist as only one or two membranes instead of three. Elasmobranchs and cyclostomes have only one, the meninx primitiva. A mucoid parameningeal membrane may enclose the primitive meninx. The primitive paleothalamus lacks the higher coordination centers of most vertebrates.

SPECIAL SENSE ORGANS

The distribution of taste buds was noted above. Fish appear to utilize both taste and smell in relation to their homing instincts. In the eel, salmon, and steelhead, smell is well developed by a highly folded olfactory mucosa whose neuroepithelial cells sample the water passing through the nasal pits or capsules, most of which have anterior and posterior openings. Both chemical and vibratory sense may be mediated via the lateral line system of neuromasts, pits, and canals, and its cephalic branches (Figs. 1.4, 1.5). Further auditory sense is mediated via the eighth cranial nerve to the small legena, but the inner ear is mostly concerned with equilibratory sense originating with slight movements of otoliths over the