ANATOMICAL AND HISTOLOGICAL STUDIES ON SELECTED ORGANS OF GOLDEN SHINER *NOTEMIGONUS CRYSOLEUCAS* (MITCHILL)

By

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Diploma

Hong Kong Baptist College

Hong Kong

1973

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1976

ANATOMICAL AND HISTOLOGICAL STUDIES ON

SELECTED ORGANS OF GOLDEN SHINER

NOTEMIGONUS CRYSOLEUCAS (MITCHILL)

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ACKNOWLEDGEMENTS

It is with pleasure I express my sincere appreciation to my adviser, Dr. Milton R. Curd, for his suggestions, patience and consel in directing this research. Special thanks are given to Dr. Helen E. Jordan for her encouragement and advice. Dr. Jordan gave freely her time and equipment. Appreciation is also extended to Dr. Bryan P. Glass for his serving on the advisory committee; to Mr. Henry Hong-nin Chau for his technical assistance; to Mr. Conrad Kleinholz who graciously provided domestic medicated fish food; to Mr. Fred Lawson for preparing the photomicrographs; to Dr. George V. Odell for reviewing the manuscript.

Laboratory space, fish-holding facilities and tissue-preparation media were furnished by the Oklahoma Cooperative Fishery Unit. Fish were provided by the Department of Zoology of Oklahoma State University.

I am most grateful to my parents, Mr. Yue Wing Kee and Mrs. Yue-Wong Choi Kum, for their understanding, patience, encouragement and support. Without all these, my graduate study in the United States of America would have been impossible.

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CHAPTER I

INTRODUCTION

Information on fish histology is fragmentary. However, for easier and more accurate evaluation and understanding of development, histomorphology, histochemistry and histopathology of fishes, knowledge of the normal histology is necessary. Up to the present, most studies have been on the digestive tracts of those bony fishes which have direct and/or indirect impact on our economy and environment. These fishes are either food fish, game fish, or forage fish.

One of the popular bait fishes, the golden shiner (Notemigonus crysoleucas, Cyprinidae) is studied in this research. Like fathead minnows, the golden shiner is cultured extensively and intensively as a a freshwater bait minnow in the United States, but its reproduction in pond culture has been drastically reduced by the prevalence of an ovarian microsporidian parasite (Pleistophora ovariae) (Summerfelt 1964; Summerfelt & Warner 1970a, 1970b). Hypotheses on both the method of invasion of this parasite and the route of migration via the blood stream have been postulated (Kudo 1924; Amlacher 1970; Van Duijn 1973). Any studies on the host-parasite relationship require our understanding the normal histology of the host and the life cycle of the parasite. The morphology of both normal and parasite-infected shiner ovaries has been reported by Warner (1972), the anatomy and histology of other organs of this cyprinid have otherwise never been described.

Previous workers have stated that while there are variations and modifications in the digestive tracts of the fishes, the basic pattern of the tract is simple and the essential parts include a mouth, pharynx, esophagus, stomach, intestine and anus.

Although teeth in the buccal and pharyngeal cavities of most fishes function in grasping, tearing and mechanically grinding prey, fishes lack structures comparable to the salivary glands of higher vertebrates. The mucus from unicellular glands distributed throughout the stratified squamous epithelium lining the mouth constitutes the only secretion in the buccal cavity to lubricate food. Its role in digestion is yet to be determined.

There are different degrees of reduction in the stomach and sometimes gastric glands are entirely lacking. However predaceous fishes have typical stomachs. In the stomachless species, for example the cyprinids which are herbivorous and/or omnivorous fishes, the intestine is usually long and coiled. An intestinal bulb is formed in the anterior most part of the intestinal tract. In general, the intestine is a simple tube whose diameter is about the same along its length and it is little differentitated into distinct regions. Grossly, the layers in the intestinal wall are equivalent in all teleost species and the fundamental histology remains uniform at all levels of the tract; but quantitative differences in the number of certain cell types and their distribution are noticeable. Al-Hussaini (1949) stated that the functional morphology of the alimentary tract of fishes is possibly related to the differences in their feeding habits. For example, golden shiners have mixed food habits; they feed on zooplankton, insects and their larvae and sometimes algae (Miller & Robison 1973). Consequently, the

intestine is expected to be of intermediate length. Complete accounts on the normal histology of the intestine for the members of the Cyprinidae have been prepared for the stoneroller (Campostoma anomalum), carp (Cyprinus carpio), goldfish (Carassius auratus) and Lake Sevan Khramulya (Varicorhinus capoeta sevanti). Accessory digestive glands such as the liver and the pancreas and hemopoietic organs have also been studied in goldfish and carp. Work on other species of the family is scanty or wanting.

Regardless of the little efforts directed towards the study of normal histology, reports on the pathological conditions in pisces have increased substantially in the last decade. Such information is available for some minnows as well (Chavin & Young 1970; Tafanelli 1972; Warner 1972). A brief report on the effects of Chondrococcus columnaris on golden shiners is included in this paper.

In order to supplement our knowledge on the anatomy and histology of the golden shiner, the normal histology of selected organs and general internal anatomy of this species are described using light microscopy and photomicrographs. The organs considered are the midgut, liver, pancreas, kidney and spleen.

CHAPTER II

LITERATURE REVIEW

Among the members of the family Cyprinidae, histological observations have been made on some species. Curry (1939) described the anatomy and histology of the digestive tract of carp. Carp are stomachless and their intestinal folds are arranged to form a meshwork. The intestine shows no internal indications of divisions into the conventional regions of a vertebrate intestinal tract. The epithelial cells covering the folds are simple columnar cells having a striated top-plate. Goblet cells are tall and numerous. They originate as epithelial cells which during development differentiate into the mucous cells. The mucosa is not accompanied by an underlying basement membrane; rather it is supported by the richly vascularized submucosa. Here the fibers are coarse and loosely interwoven. This layer is usually thin except for that region which is near the vent. The muscularis includes both the thick circular layer of smooth muscle and the thin longitudinal layer on its outside. The serosa is a single flattened peritoneal cell layer to which is attached a dense coat of subserosal connective tissue.

In studying the digestive tract of the stoneroller (Campostoma anomalum, Cyprinidae), Rogick (1931) found that the intestine of this herbivore is very lengthy and coiled around the air bladder. A true stomach is absent and is replaced by an enlargement, the intestinal bulb. The intestinal wall is composed of mucosa, submucosa, muscularis and

serosa. Prominent secondary branches also extend from the intestinal folds. The cellular elements and arrangement of both the primary and the secondary intestinal folds are the same as those present in the bulbar region. McVay and Kaan (1940) studied the intestinal tract of goldfish, another popular bait minnow. They described it as resembling closely that of the stoneroller.

In still another stomachless cyprinid, the Lake Sevan Khramulya (Varicorhinus capoeta sevangi, Cyrpinidae) which feeds on periphyton and detritus, the intestinal mucosa is very much folded and the lamina propria poorly developed. The histology of its intestinal tract is similar to other fishes with similar feeding habits (Verigina 1969). Since a true stomach is absent in all the cyprinids studied so far, it is generally believed that all the cyprinids lack a stomach and true intestinal glands (Ashley 1975).

Several studies have dealt with the comparative histology of the digestive tracts of non-cyprinid fishes. Gastric glands are described in many fishes with stomachs, for instance, the cod (Gadus morhua, Gadidae) has well developed glands in the stomach, the numerous pyloric ceca and the intestine. The histology of its intestine is typical of teleost but there is no muscularis mucosa and no distinct lamina propria (Bishop & Odense 1966).

Dawes (1929) indicated that there is no differentiation between the gastric glands in the "cardiac" and the "pyloric" ends of the stomach of plaice (Pleuronectes platessa, Pleuronectidae). The mucosa of its stomach, pyloric ceca and intestine consists of the columnar epithelial cells, mucus-producing cells and granular cells. On the other hand, Pasha (1964a & b) found the corpus and the pylorus are well differentiated in

the herbivorous blue bream (Tilapia mossambica, Cichlidae) and in the omnivorous catfish (Mystus gulio, Siluridae) in that no gastric glands are in the pyloric region. The histology of the intestines of these fishes is typical of teleost . But in blue bream the lamina propria merges with the submucosa and the musculature of the intestinal tract is uniform and almost the same throughout its length. Whereas, in catfish both the pylorus and the rectum have a thicker musculature than the other parts of the intestine.

Blake (1930; 1936) discovered that both the sea bass (Centropristes striatus, Serranidae), a predaceous fish, and the sea robin (Prionotus carolinus, Triglidae), a bottom-feeding fish, possess stomachs. The arrangement of layers and tissues of their stomachs follow the general structure and composition of other carnivorous fish. In the sea bass, the gastric glands are deep, tubular, and regularly as well as radially arranged. Pyloric ceca are found as appendages on the digestive tube at the region of pylorus. The same author also documented a stratum granulosum which consists of large, irregular and nucleated cells with heavily granulated cytoplasm in the intestinal wall of the sea robin.

Bucke (1971) found that a pouch-like stomach is also present in pike (Esox lucius, Esocidae), another voraciously predaceous fish. Its esophagus and intestine are short. In addition to the stratum granulosum, the lamina propria of the intestine is further strengthened by the stratum compactum.

In general, the liver of fishes is elongate and less well organized into lobes than it is in higher vertebrates (Ashley 1975). It is composed of a mass of hepatic cells which are polygonal with centrally located nuclei and nucleoli. Blood vessels are usually seen throughout

the gland. The hepatic arterioles in portal triads are often removed some distance from the associated vein and bile duct, and central veins are so irregularly dispersed that a zonal pattern is indistinct. Sinusoids are often collapsed. Being filled with glycogen, hepatocytes appear very vacuolated and pale in hematoxylin and eosin (H & E) sections. Kupffer cells are seldom seen clearly (Ashley 1975).

Several workers have documented the histology of liver of a few freshwater fishes. Curry (1939) reported that carp liver has a very large triangular right lobe, but a very small left lobe. Hepatic lobules can also be demonstrated. The hepatic cells are polygonal and have a spherical nucleus among the reticular cytoplasm (Smallwood & Derrickson 1933; Curry 1939). Chavin and Young (1970) described the liver in small goldfish to be a diffuse unlobulated organ. The hepatic cells are large, polyhedral and densely granulated with abundant glycogen. The small round nuclei tend to be centrally oriented. Bucke (1971) indicated that pike also has an unlobulated liver in which the hepatic arteries and portal veins are often not associated with one another.

Grosso (1950) wrote a brief historical account on previous works that confirmed the presence of and described the histology of fish pancreas and islet epithelial cells in both freshwater and marine species. Bucke (1971) found that the pike pancreas is compact but only few islets of Langerhans were observed. Exocrine pancreatic alveolar cells in pike are circular and prominent. The surrounding adipose tissue is usually peripheral; sometimes, it penetrates the pancreatic tissue. However, the cod has a diffuse pancreas. Islets of Langerhans are found in the pancreatic tissue along the bile duct and at the apex of the gall bladder. Among the cyprinids, both carp and goldfish have a diffuse

pancreas. In carp, masses of pancreatic tissue are scattered among the intestinal loops. The pancreatic duct proceeds along the right side of the intestine and enters at the papilla. In the proximal two-thirds of the columnar pancreatic cells, there are many large dark staining granules whereas the nucleus is located in the clear distal third. Characteristic of carp pancreas, islets of Langerhans which are present as small irregular masses of clear cells without ducts are scattered throughout the organ. The capillaries supplying these masses are large and resemble sinusoids (Smallwood & Derrickson 1933; Curry 1939). On the other hand, in goldfish the islets appear as discrete, rounded and encapsulated nodules aggregated around the cystic duct, common bile duct and gall bladder. Another cell type, the delta cell, is also recognized in the goldfish (Chavin & Young 1970). Bowie (1924) studied the islets of Langerhans in gray snapper (Neomaenis griseus, Lutjanidae). By noting the staining reactions, identifying characteristic cytological elements and the position of cells within the islets, he was able to differentiate between zymogen cells and the three tinctorically distinct endocrine cells, the alpha, beta and gamma cells.

The spleen in fishes is small, and species specific both in form and in location. It usually lies next to the position of the stomach and is elongated and coated with a fine capsule of connective tissue. The capsule encloses a reticular stroma of splenic parenchyma which consists of the splenic pulp and the blood-filled anastomosing splenic sinuses (Ashley 1975). Although it functions to destroy red blood cells, it is potentially hemopoietic, particularly under stress of certain pathological conditions (Cowdry & Finerty 1960). In their studies of the formation of blood cells in teleost fishes, Jordan and Speidel (1924)

and Catton (1951) gave a brief description of the spleen in flounder, tautog, scup, minnow, buffalofish, trout, goldfish, English brown trout, common roach and perch. According to these investigators, the spleen is constituted essentially of a mass of intermingled, closely packed lymphocytes, erythrocytes and transition stages between the two. Few blood vessels, especially arterioles, are widely scattered and enveloped by an irregular collection of lymphocytes forming the spleen corpuscles. Smallwood and Derrickson (1933) and Curry (1939) stated that in carp spleen corpuscles do exist. The central core of lymphoid tissue of the spleen is surrounded by a region of pulp. The whole organ is encapsulated and completely hidden between the liver on the left, the pancreas on the right and the intestine below.

Morphologically, the opisthonephroi, the adult fish kidneys, follow the basic structure of vertebrate kidneys (Hilderbrand 1974). The kidney is composed of endocrine, hematopoietic and excretory tissues (Hickman & Trump 1969; Bendele & Klontz 1975). Important as it is in osmo-regulation, salt balance, excretion and as the primary hemopoietic organ, the fish kidney exhibits a simple organization. Cortex or medulla are not discernible (Andrew & Hickman 1974; Hilderbrand 1974). The intertubular renal tissue resemble the splenic lymphomyeloid tissue in that it contains relatively few red blood cells (Jordan & Speidel 1924).

Edwards (1928; 1929; 1933; 1935), Edwards and Schmitter (1933), Graffin (1937) and Jaffee (1956) contributed much to our outstanding of the anatomy, morphology and cytology of vertebrate kidneys, both aglomerular and glomerular. They documented that the nephron of the glomerular kidney of goldfish consists of a renal corpuscle with a neck segment, whose cells are ciliated, a bisegmental proximal convolution

in which the brush border of cells in the first segment is more prominent and taller than that of cells in the second segment, a ciliated or non-ciliated intermediate segment and a distal convolution. Epithelial cells of normal nephrons exhibit filamentous mitochondria. Nash (1931) discovered that the kidneys of normal freshwater fish contain numerous glomeruli; the larger the fish, the more the glomeruli. Larger glomeruli also have more branchings in their tuft of capillaries. Furthermore, literature on the physiology of fish kidneys is readily available and which sometimes includes brief descriptions of the histology of this organ; for example, in goldfish (Jaffee 1956; Jaffee 1958; Chavin & Young 1970), and in toadfish and in sculpin (Defrise 1932).

Other than protozoic and helminthic infections, bacteria, viruses, toxins and neoplasms are the major causes of rapid and phenomenal fish kills in ponds, lakes and hatcheries. The general inflammatory response elicited in fishes by bacteria can be either non-proliferative or proliferative (Van Duijn 1973; Wolke 1975). Non-proliferative response tends to be acute, vascular, non-granulomatous and characterized by necrosis. Bacteria eliciting this response often produce dermal ulcers. They also tend to destroy elements of the hematopoietic system. Proliferative response tends to be more chronic, granulomatous and characterized by hypertrophy and hyperplasia of epithelial and other tissue elements.

Chondrococcus columnaris (Myxobacteriales), a Gram-negative pathogen to many species of fishes, has an extensive geographic distribution. It causes endemic columnaris disease which is acute, systemic and characterized by large areas of epidermal necrosis, dermal ulceration and gill destruction (Borg 1960; Ajmal & Hobbs 1967; Pacha & Ordal 1970; Fujihara & Hungate 1971). It is a non-proliferative motile slime bac-

terium measuring 0.6 microns in diameter by 6 microns in length. It tends to form short, column-like masses on the edges of tissues. Sometimes fruiting bodies and microcysts are produced. This bacterium is readily isolated from lesions.

The gross lesions of C. columnaris are confined to the skin and gills but may vary as to the species of fish involved. It produces lighter-pigmented, smooth-edged "saddle" lesions between the dorsal fin and the caudal fin and which often extend down either side toward the lateral line. Similar lesions which may occur anywhere on the body and become confluent often originate on the caudal fin and progress forward. In scaled fish fins may also be affected. Hemorrhagic patches at the base of fins and about the mouth and ulcerations of the head are found in English roach and perch (Ajmal & Hobbs 1967). When gills are involved, the characteristic signs are congestion, whitened discolored areas, complete loss of gill filaments and acute necrosis.

CHAPTER III

MATERIALS AND METHODS

Source and Maintenance of Fish

Golden shiners, Notemigonus crysoleucas, obtained from a fish farm in Fordyce, Arkansas on March 14, 1975 were placed in indoor holding tanks which were pre-cleaned and disinfected with chlorox. A continuous flow of fresh dechlorinated, agitated tap water was maintained in each tank. The room temperature was adjusted around 20-21 ^{o}C and a photoperiod of 12-14 hours was provided. The fish were fed commercial minnow meal each morning. Debris and excreta deposited in the tanks were removed either every day or on alternate days.

An incidental outbreak of columnaris disease occurred in some of the fish in late March, 1975. Typical symptoms of columnaris disease were observed. Fish kill occurred within a week of the development of the symptoms. Upon autopsy, petechiae were found on the liver and intestine. Scrapings from liver, intestinal mucosa and discolored areas were smeared and stained. Long, slender and Gram-negative bacteria which aggregated to form columns were present. Similar symptoms elicited by C. columnaris in other fishes were observed by some workers; thus a columnaris disease was suspected (Davis 1921-1922; Borg 1960; Ajmal & Hobbs 1967; Snieszko 1969; Amlacher 1970; Pacha & Ordal 1970; Fujihara & Hungate 1971; Bullock, Conroy & Snieszko 1971).

The shiners were given a salt bath to remove possible fungal or-

ganisms and bacteria on the skin. A few days after the salt bath, 1% potassium permanganate solution, a therapeutic agent that is effective against infection by parasitic copepod, fish lice and ordinary fungal and bacterial diseases in fishes, was applied for 4-6 hours and then diluted (Van Duijn 1973). Several days later, individual fish were treated in an oxytetracycline-neomycin bath (concentration at two ounces per gallon) for 15 minutes. Following treatment mortality decreased. Those fish that survived began to swim actively and to feed voraciously. Markedly emaciated fish were removed. In order to assure the more healthy fish a more complete recovery from the infection, domestic medicated fish food that consisted of chips of liver and oxytetracycline was substituted for the commercial fish food for three weeks. Then the regular minnow meal was resumed. Oxytetracycline was employed because it was the only antibiotic that was available at that time.

Sampling and Tissue Preservation

Sampling was carried out from June through November, 1975. At each sampling, ten to fifteen fish were killed by severing the isthmus, the ventral aorta and then across the nape. A total of eighty fish was preserved. Sex was determined by examining a saline smear of a portion of the fresh gonads. A strip of muscle was incised in the abdomen of the ventro-lateral body wall of the killed fish to facilitate quick penetration of 10% phosphate-buffered formalin. The preserved fish were dried in a paper towel, weighed and their total length measured. The peritoneal cavity was cut open to expose the viscera, the gross anatomy of which was studied under a binocular microscope before a part of the intestine at the midgut level, the right lobe of the liver, the spleen

and the kidney were removed. The fixed organs were post-treated in Susa fixative overnight, dehydrated with 2-propanol, cleared in xylene, infiltrated and embedded in paraplast, using standard methods (Humason 1967).

Staining and Examination of Sections

Hematoxylin and eosin are routinely utilized for histologic and pathologic studies. They are preferred for their ubiquitous application, permanency with reasonable differentiation of nuclei and cytoplasm, relative stability and reproducible results. Giemsa's stains are applied to differentiate and demonstrate cell types, morphological details of splenic or lymph node tissue, inclusion bodies, mast cell granules, blood cells, bacteria, rickettisae, other tissue components and protozoan parasites. On the other hand, Mallory's trichrome stain is suitable for demonstrating nuclei and connective tissue components (Lillie 1965; Thompson 1966; Humason 1967). All three staining techniques were used in this study for their respective characteristic staining properties in order to facilitate identification and description of the normal histology.

Three slides, each with 6-12 serial sections cut at seven microns, were prepared from each organ of individual fish. One slide was stained with Delafield's or Harris' hematoxylin and eosin, one with Giemsa's stain, and the other with Mallory's trichrome stain. The procedures of sectioning, staining, clearing and mounting with permount followed routine laboratory methods (Lillie 1965; Thompson 1966; Humason 1967). These sections were examined for their gross appearance and histological structures. By taking advantage of the incidental outbreak of the

columnaris disease, pathological conditions in the organs concerned in this study were recorded from sick shiners and included in the description of the normal tissue of the corresponding organs.

CHAPTER III

OBSERVATIONS

The range of total length and that of total body weight were 79 mm to 102 mm and 4.07 g to 9.07 g; while the average total length and the average total body weight of preserved shiners were 90.40 mm and 6.01 g, respectively. When the fish were aged with respect to their sizes, they were all assigned to age group 1 and were regarded as small-sized fish (Summerfelt & Warner 1970a). The ratio of males to females was found to be 1:9 (8:72). The anatomy and the histology of the selected organs examined in this study were observed to be identical in both the male and the female shiners; thus, the general description reported herein is relevant to both sexes.

Intestine

Gross Morphology

After the esophagus emerges through the transverse septum, that part of the digestive tube is enlarged slightly into a bulb before it assumes a tubular form (Figure 5). The intestine then veers to the right side of the peritoneal cavity, extends caudally throughout the length of the coelom to the posterior limit of the base of the pelvic fins, bends sinistrally upwards and forwards and straightens cephalad along the median line of the body but slightly ventral to the anterior gut. Anteriorly, it recurves in the region of the septum, doubles

sharply back on itself into a loop on the lateral side of the left anterior half of the peritoneal cavity and eventually approaches the anus which is just posterior to the pelvic fins (Figures 2 & 4). Based on this manner of coiling, the intestine was arbitrarily divided into three regions, viz. the foregut, the midgut and the hindgut (Figure 4). The foregut is of a wider diameter than either the midgut or the hindgut. The intestine, when uncoiled, is longer than the body cavity and equals the length from the transverse septum to the tip of the ventral lobe of the caudal fin.

Histological Structures

The midgut is supported by reticulate fibers of the mesenteries supporting adipose tissues and the acinar cells of the diffuse pancreas (Figure 10). The intestinal wall consists of the mucosa, the submucosa, the muscular layers and the serosa (Figure 7). In longitudinal sections, the mucosal epithelium is thrown into folds which cross bridge with one another and form a meshwork. As observed in transverse sections, these villiform mucosal epithelial folds project deep into the intestinal lumen.

The tall epithelial cells lining the mucosa are of the simple columnar type. They have a low ciliated lumenal border and indistinct lateral boundaries. The oval to elliptical nucleus lies in the basal half and sometimes at the base of the cell. It contains an acentrally-placed nucleolus which is not too easily recognized among the coarse vesicular nuclear chromatin materials. The cytoplasm is homogeneous. The only discrete glands in the midgut are the mucous cells which are dispersed throughout the epithelium. Occasionally small lymphocytes

which possess compact spherical and more deeply stained nuclei are scattered interstitially among the epithelial columnar cells. The mucosal epithelium rests on a thin basement membrane which is often obscured by lymphocytes and the fibroblastic tissues of the lamina propria.

A muscularis mucosae was not observed. Numerous capillaries are scattered in among the dense fibroblastic tissue of the submucosa close to the muscle layers and numerous eosinophils are found in some of the preparations. The muscularis is thin with the inner circular layer of smooth muscle almost two to three times as thick as the outer longitudinal layer. A bundle of loose connective tissue containing numerous capillaries and ganglia separates the muscle coat from the serosa which is a thin layer of areolar tissue and simple squamous epithelium.

Liver and Pancreas

Gross Morphology

The main bulk of the liver is situated in the right antero-lateral part of the peritoneal cavity, accommodating some folds of the intestine in deep grooves. It consists of three lobes, viz., the left lobe which traces the intestinal loop (Figure 2), the dorsal lobe which extends dorso-laterally touching the ventral side of the right gonad above and the anterior part of the foregut below, and the right lobe on the right side of the peritoneal cavity (Figure 3). Both the dorsal and the right lobes are large and somewhat triangular whereas the left lobe is small and elongate. The gall bladder is inclosed in the right lobe and the ductus choledochus enters the right side of the intestine just posterior to the intestinal bulb (Figures 3 & 5). Pancreatic elements are sur-

rounded by adipose tissue that makes it difficult to recognize the gland macroscopically in preserved material; however, being held by the mesentery in contact with the intestine and embedded in the adipose tissue surrounding the intestinal tract (Figure 11), it is spread around the gall bladder, dorsal to the anterior fold of the intestinal tract and extends between the fore- and the mid- gut. Consequently part of it is well surrounded by the liver lobes and part of it is contained within the spleen. There are areas of pancreas scattered among the parenchyma of the liver (Figure 8). The pancreatic and the bile ducts join just before entering the intestinal bulb.

Histological Structures

The liver is unlobulated. Hepatic acini, masses of hepatic parenchymal cells which assume no definite size or zonal organization, are intercepted by ramifying hepatic sinusoids. The liver cells in the central areas of the lobes are organized into linear cords two-cell wide around branches of portal and hepatic veins (Figure 9). This relative regularity of structure and arrangement is lost in the outer regions of the lobes. The polyhedral hepatic cells have well-defined cell membranes. The nucleus of these cells is large and round with a smooth surface, vesicular, with scattered chromatin clumps and a centrally placed nucleolus. It is usually slightly displaced to one side of the cell. Most cells have a single nucleus but binucleate cells are found occasionally. The cytoplasm is variously granulated and vacuolated (Figure 9). No lymphatics or lymph tissues or hemopoietic tissues can be differentiated. Fine bile canaliculi are not easily distinguishable and their distribution is not associated with or related to the position

of the portal vessels.

Groups of pancreatic acini underlined by the basement membrane are attached to the intestinal tract and the liver by means of connective tissue or mesenteries enveloping capillaries and small vessels. They also extend around and along the vessels supplying the liver lobes. Pancreatic acinar cells are pyramidal and often arranged around portal veins in the liver. Although the pancreatic and the hepatic cells are closely associated, there is a complete differentiation of the two types of cells. The large vesicular nuclei of the pancreatic exocrine cells lie basally. The acinar cells which surround lumens of blood vessels are arranged in single rows. Their nuclei are found on the periphery of the rosette. Each nucleus possesses an inconspicuous, acentrally located nucleolus. The apical region of the acinar cells are packed with numerous spherical "droplets", the secretory zymogen granules, which are refractile and stained pale pink (H & E), pale blue (Giemsa's), or orange (Mallory's) (Figure 12). Islets of Langerhans have not been distinguished in the preparations made in this study.

In the sections of the sick fish, there were groups of necrotic cells present in these compound organs which were not stained by either hematoxylin and eosin or Giemsa's stain and/or Mallory's trichrome, but appeared in various shades of yellow, green brown and gray. These necrotic cells had lost their nuclei or their nuclei had become pyknotic. Small and large refractile, yellow to brown granules sometimes were present in their cytoplasm which was homogeneous and apparently devoid of any fatty substance or glycogen. Their cell membrane disintegrated and the outlines of individual cells no longer remained intact. Occasionally, single, small focal granulomas which were often located near

a blood vessel or on the periphery of the liver lobe, were circumscribed and delineated from adjacent normal liver cells by a thin layer of fibrous connective tissue (Figure 10).

Kidney

Gross Morphology

The opisthonephric kidneys fuse to form an elongated organ lying along the entire dorsal body wall above the peritoneal cavity. The membranous capsule enveloping the kidney is heavily pigmented.

Histological Structures

The intertubular space is packed with red blood cells, lymphocytes and mast cells. Numerous capillaries and larger blood vessels are seen throughout the organ and these are usually congested with blood cells. Embedded in the hemopoietic tissues are the nephrons. The spherical renal corpuscle of a nephron includes the glomerulus confined within the space surrounded by a thin epithelial Bowman's capsule (Figure 13). The renal corpuscle is connected by a short neck segment to the proximal convoluted tubule, the wall of which is lined by a single row of pyramidal cells. Each pyramidal cell bears a high and prominent ciliated lumenal border (Figure 14). The lateral cell membranes are indistinct but are interdigitated with those of the adjoining cells. The oval and vesicular nucleus is variably positioned while the nucleolus it contains is located acentrally and is not easily discernible. The granular cytoplasm is stained pink with H & E, blue with Giemsa and red with Mallory. Except that the cuboidal cells of the distal convoluted segment have much lower ciliated borders and a basal nucleus, the other

cellular characteristics are similar to those observed in the cells of the proximal convoluted tubules.

The collecting tubules are characterized by columnar cells whose vesicular nuclei occupy the basal halves of the cells. The nucleoli are somewhat centrally placed and the cytoplasm is granulated.

In fish affected by the columnaris disease, islands of brown to gray necrotic cells (H & E) were observed in intertubular tissue. Focal granulomas which were similar to the splenic focal granulomas and circumscribed with fibrous connective tissue were also found. Pronounced glomerular degeneration, atrophy, macrophage infiltration and vacuolar displacement of normal renal tissue were observed in the kidneys of two shiners.

Spleen

Gross Morphology

The elongated and dorso-ventrally flattened spleen is positioned dorsal to the foregut. In essence, it is completely inclosed within the groove between the foregut and the intestinal loop while the left liver lobe overhangs it dorso-laterally (Figure 5).

Histological Structures

The splenic substance is compact and encapsulated by simple squamous epithelium. Dispersed among the red pulp are poorly defined areas of white pulp which contain chiefly lymphocytes and macrophages. Trabeculae or loose intrasplenic connective tissues which are composed of large nuclei of fibroblasts and faintly pink collagenous fibers (H & E) are obvious in some sections only. Both large vessels and capillaries ramify

throughout the splenic parenchyma. These vessels form the major supporting tissue of the whole organ. Spleen corpuscles which are the small vessels surrounded by lymphocytes are also present (Figure 15). Pancreatic acini frequently appear embedded in the mesenteries supporting the spleen or even in the spleen proper along the splenic blood vessels.

Pathological conditions due to the columnaris disease were observed in affected shiners. Upon autopsy, petechiae, discoloration, nodulation and splenomegaly were discovered. Microscopically, extensive focal granulomas were observed. Necrotic cells were either gray to brown (H & E), or pale yellow to bluish green (Giemsa), or gray (Mallory). Macrophage infiltration and vacuolar displacement of the nodular necrotic areas were profound. Macrophages aggregated to form focal granulomas which were deposited with refractile pigments, appearing yellowish brown in H & E, yellowish green in Giemsa's and dark green to dull brown in Mallory's. These pigments were probably hemosiderin, the breakdown product of hemoglobin. These focal lesions were also mottled with small red rod-like "granules" (in both H & E and Giemsa) which might be the bacterial pathogens or cellular debris. Regeneration in the form of fibrosis was noted in the affected areas.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Cyprinids are generally considered to be stomachless. The absence of a true stomach suggests a similarity in the digestive tract among the members of this group of fish. Although diversity in terminology presents difficulties in an exact comparison of the histological structures with the detailed descriptions of the histology of the intestinal tract in other fishes, the foregoing account furnishes a basis for comparing the intestine of the golden shiner with that of other cyprinids and non-cyprinids. The anatomy of the intestine of golden shiner (Notemigonus crysoleucas, Cyprinidae) is found to resemble that of stoneroller (Campostoma anomalum, Cyprinidae), goldfish (Carassius auratus, Cyprinidae), carp (Cyprinus carpio, Cyprinidae) and mummichog (Fundulus heteroclitus, Cyprinodontidae) in that all these species are lacking a stomach and an intestinal bulb is formed in the first portion of the intestinal tract just beyond the transverse septum. Since both the bile and the pancreatic ducts enter the intestinal bulb, the enlarged region of the gut, it can be designated the duodenum as in the case of mummichog (Babkin & Bowie 1928).

The intestine of golden shiners is coiled but separate from the air bladder. It is as a whole about equal to the total length of the fish. In contrast to the extreme coiling of the intestine of stoneroller and the intermediate condition in goldfish in which the intestine

is typically twice the body length, golden shiner obviously has a short intestine as does mummichog (Babkin & Bowie 1928; Rogick 1931; McVay & Kaan 1940).

Pyloric ceca which have been observed in the sea bass (Centropristes striatus, Serranidae) and the sea robin (Prionotus carolinus, Triglidae) by Blake (1930 & 1936) were not observed. Yet, the surface area available for digestion and absorption in golden shiner is enhanced because the mucosal folds exhibit a meshwork as reported in carp by Smallwood & Smallwood (1931). The fundamental histology of the midgut is in general similar to that found in other herbivorous teleost fishes. However its intestinal mucosa lacks a stratum granulosum and a stratum compactum. The stratum granulosum has been observed in sea bass and sea robin (Blake 1930; Blake 1936), while both the stratum granulosum and the stratum compactum have been described in pike (Esox lucius, Esocidae) (Bucke 1971). As is characteristic of several teleost species other than the cyprinids, no muscularis mucosa was found (Blake 1930; Blake 1936; Wier & Churchill 1945; Bucke 1971). The only glands that are present in the intestine are the unicellular mucous glands. They are dispersed throughout the midgut, giving one the impression that they are more numerous in the region of the hindgut.

Like that of carp, the liver of golden shiner has a small left lobe, but the dorsal and the right lobes are large and triangular. The liver resembles that of carp, topminnow guppy (Poecilia reticulata, Peociliidae) and three-spine stickleback (Gasterosteus aculeatus, Gasterosteidae) in that the arrangement of the hepatic "triads" is lost. The wall of the smaller vessels radiating from larger blood vessels to separate adjacent sinusoids in the central mass of the liver lobes are two cells thick but

are of irregular thickness on the periphery (Smallwood & Derrickson 1933; Curry 1939; Hale 1965). Furthermore, hepatic structure in golden shiner is not as regular as described in gizzard shad (Dorosoma cepedianum, Clupeidae) and pike (Wier & Churchill 1945; Bucke 1971).

The shiners possess a diffuse pancreas which also exists in other minnows, for example carp and goldfish (Smallwood & Derrickson 1933; Curry 1939; Chavin & Young 1970). This gland is so scattered among the mesenteries supporting the various viscera that it is difficult to locate macroscopically; whereas it is readily observable as a compact structure in pike and as a racemose gland in gizzard shad (Wier & Churchill 1945; Bucke 1971). This situation, as well as the loss of some parts of the gland during the preparations of histological sections, contribute to the failure of locating the various pancreatic ducts or ductlets communicating between distinct groups of pancreatic acinic cells dispersed among the intestinal loops. In spite of this, intrahepatic acinar cells are observed because they follow the course of the portal vessels throughout the liver. The same condition is described in other teleost species (Kristal 1946; Hale 1965). Some of the pancreatic elements are also found intrasplenically when they penetrate the spleen along with the large splenic vessels.

Besides the close resemblance in the structure of the pancreas among the minnows, islets of Langerhans have not been distinguished or located in golden shiner. However the histology and the histochemistry of the islets present in several cyprinids, viz., tench, European goldfish and common carp have been studied by Khalilov (1968). Upon treatment with different fixatives and stains, the various endocrine cell types and their cellular characteristics are differentiated. The same endocrine

cell types are found to prevail in both gray snapper (Neomaenis griseus, Lutjanidae) and guppy (Lebistes reticulatus, Poeciliidae) (Bowie 1924; Grosso 1950).

The normal histology of kidney has been described in only a few fish species. For those species that are documented, most of them are approached from the physiological point of view and usually under the manipulation of some forms of chemical compound (Edwards 1929; Defrise 1932; Edwards 1933 & 1935; Edwards & Schmitter 1933; Chavin 1956; Jaffee 1956 & 1958; Chavin & Young 1970). The kidneys of golden shiners have the generalized glomerular morphology usual in fish. The different parts that constitute a glomerular nephron include the renal corpuscle, the neck segment, the proximal convolution, the intermediate segment and the distal convolution. The histological and cytological details are comparable to those for goldfish kidney (Edwards & Schmitter 1933; Chavin & Young 1970).

Other than the kidney, the spleen is another active hemopoietic organ in fishes. It exists in all groups of teleosts with almost no deviation from the fundamental structure which is simply a reticular stroma overlaid with a parenchyma of intermingled erythrocytes, definite and differentiating lymphocytes, a dense network of blood vessels and their branches. The morphology of the spleen has been studied in flounder (Paralichthys dentatus, Pleuronectidae), tautog (Tautoga onita, Labridae), scup (Stenotomus chrysops, Sparidae), topminnow (Fundulus heteroclitus, Cyprinodontidae), buffalofish (Ictiobus bubalus, Catostomidae), trout (Salmo shasta, Salmonidae), goldfish (Carassius auratus, Cyprinidae), English brown trout (Salmon trutta, Salmonidae), common roach (Rutilus rutilus, Cyprinidae) and perch (Perca fluviatilis, Perci-

dae) (Jordan & Speidel 1924; Catton 1951). The histology of the spleen of golden shiner agrees to that described for these species.

Lesions produced in golden shiners by Chondrococcus columnaris were observed mainly in the liver, spleen, kidney and pancreas. They were either nodular necroses or focal granulomas. The gross lesions included hemorrhagic patches at the base of fins and about the mouth, the typical "saddle" lesions, flabby skin, sloughing scales, petechiae in the liver lobes and splenomegaly. Similar lesions have been reported in other fishes (Borg 1960; Ajmal & Hobbs 1967; Pacha & Ordal 1970; Fujihara & Hungate 1971).

Many investigators agree that water quality is a major factor in columnaris outbreaks. The disease is associated with high water temperature in water polluted with organic matters, especially during summer months, usually in excess of 18 °C (64.4 °F) in both field and laboratory studies (Pacha & Ordal 1970; Snieszko 1973). In hard water with increased organic matters, the bacteria can persist for longer periods of time but their survival time is shortened if the water pH is reduced to 6.0. Ordinarily excessive supply of fish food and its accumulation will generate a comparable situation which encourages the thriving of the bacteria. Furthermore, strain virulence of C. columnaris is related to water temperature and route of infection. Pacha and Ordal (1970) found that highly virulent strains are able to produce disease at lower water temperatures than those strains of low virulence and produce disease readily by contact. Thus fluctuations in water temperature, crowding and other stress conditions like water qualities contribute to a rapid establishment of the infection by C. columnaris in a fish population. The development of the columnaris disease in this laboratory was probably

due to the variation in temperature over the weekends of the early period of holding during which the air-conditioners were turned off to save electricity in the period of energy crisis. The temperature varied between 78 °F (25 °C) and 84.5 °F (28.5 °C). The stress condition was aggravated with the accumulation of bottom wastes; therefore daily removal of wastes was carried out after the incidence and is recommended as a regular clean-up procedure in indoor holding tanks.

CHAPTER VI

SUMMARY

This study with routine histological techniques on the midgut, liver, pancreas, spleen and kidney of golden shiner (Notemigonus crysoleucas, Mitchill) exposes several points of anatomical and histological interest on the above-mentioned organs of the fish.

1. The digestive tract of golden shiner resembles that of other cyprinids such as carp, goldfish and stoneroller.
2. The anterior part of the intestinal tract just beyond the transverse septum is enlarged into a bulb. Both the bile and the pancreatic ducts empty into the posterior part of this intestinal bulb.
3. The intestinal folds adjoin to form a meshwork like that in carp.
4. The columnar epithelial cells of the intestine have ciliated lumenal borders and there are no intestinal glands in the midgut except the mucous cells.
5. No triad arrangement of the hepatic blood vessels is observed.
6. The shiner pancreas is diffuse and disseminated in the mesenteries around the intestinal tract among the liver parenchyma and the spleen proper. It belongs to the hepato-pancreatic type.
7. In the sections of pancreas examined, the islets of Langerhans were not observed.
8. Spleen corpuscles are present.

9. A nephron of the glomerular kidney consists of a short neck, proximal convolution, intermediate segment and a distal convolution.

10. Chondrococcus columnaris elicited nodular necrosis and focal granuloma with the deposition of pigments in the liver, pancreas, spleen and kidney.

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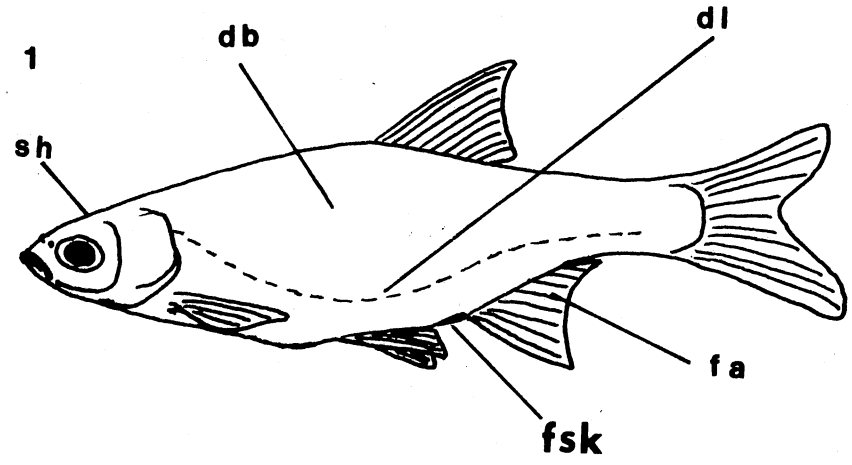
APPENDIX

Figure 1. External morphology of the golden shiner showing striking characteristics of this fish species. sh: small head; db: deep body; dl: decurved lateral line; fsk: fleshy scaleless keel; fa: falcate anal fin.

Figure 2. Lateral view (left) to demonstrate the relative positions of the viscera. mg: midgut; hg: hindgut; ll: left liver lobe; lg: left gonad; v: vent.

Figure 3. Lateral view (right) to show the relative positions of the organs in the peritoneal cavity. dl: position of dorsal liver lobe (dotted line); rl: position of right liver lobe (dotted line); ab: air bladder; rg: right gonad; v: vent; gb: gall bladder; fg: foregut.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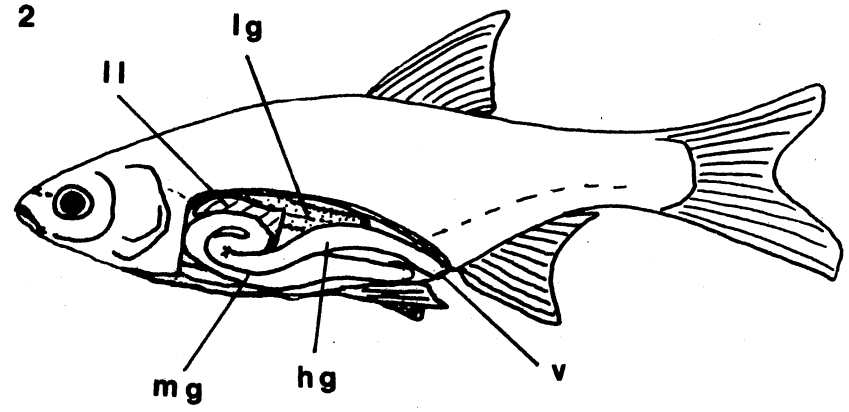
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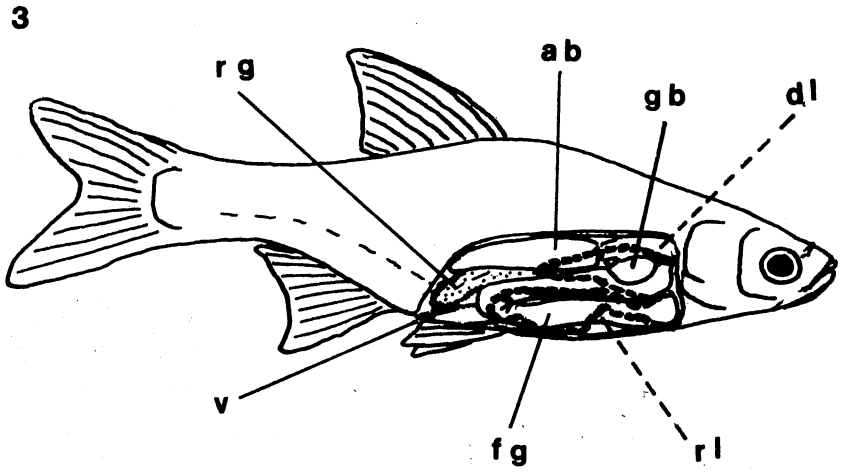
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Figure 4. Ventral view to illustrate the intestinal coiling (2X). fg: foregut; mg: midgut; il: intestinal loop (midgut); hg: hindgut; v: vent; ab: air bladder; lg: left gonad; ok: opisthonephric kidneys; ll: left liver lobe; rl: right liver lobe.

Figure 5. An isolated intesinal tract of the golden shiner. ib: intestinal bulb; gb: gall bladder; bd: bile duct; fg: foregut; il: intestinal loop; hg: hindgut; v: vent; sp: spleen; at: adipose tissue embedding diffuse pancreas; pd: pancreatic duct.

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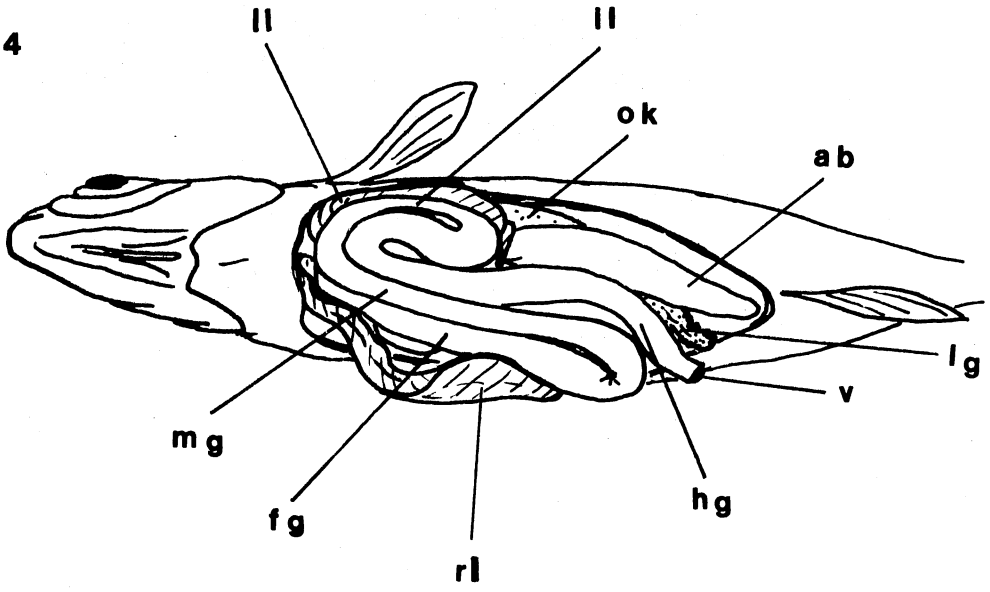

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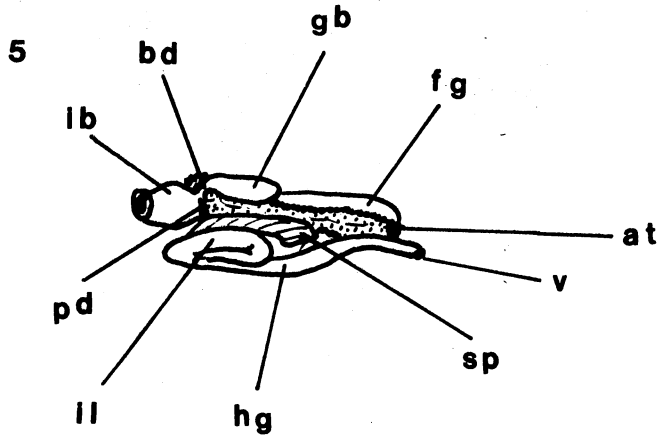


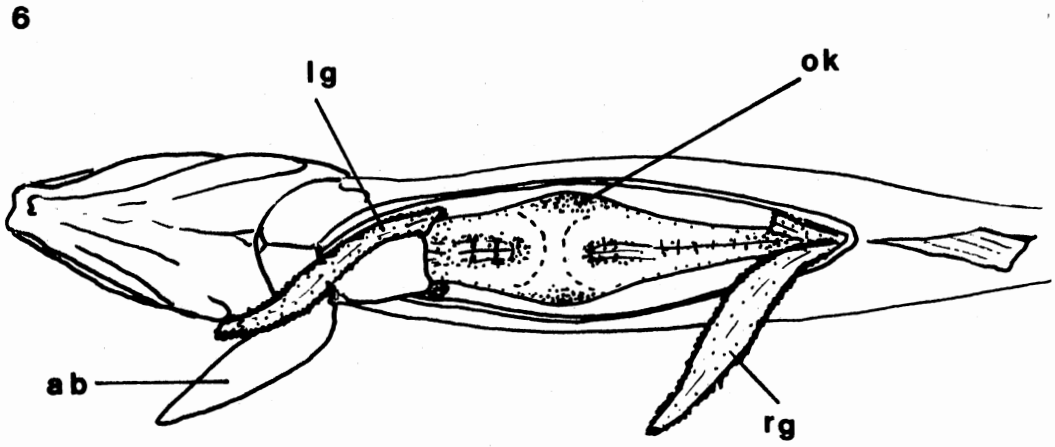


Figure 6. The shiner opisthonephroi with the viscera removed (2X). ok: opisthonephric kidneys; lg: left gonad; rg: right gonad; ab: air bladder.

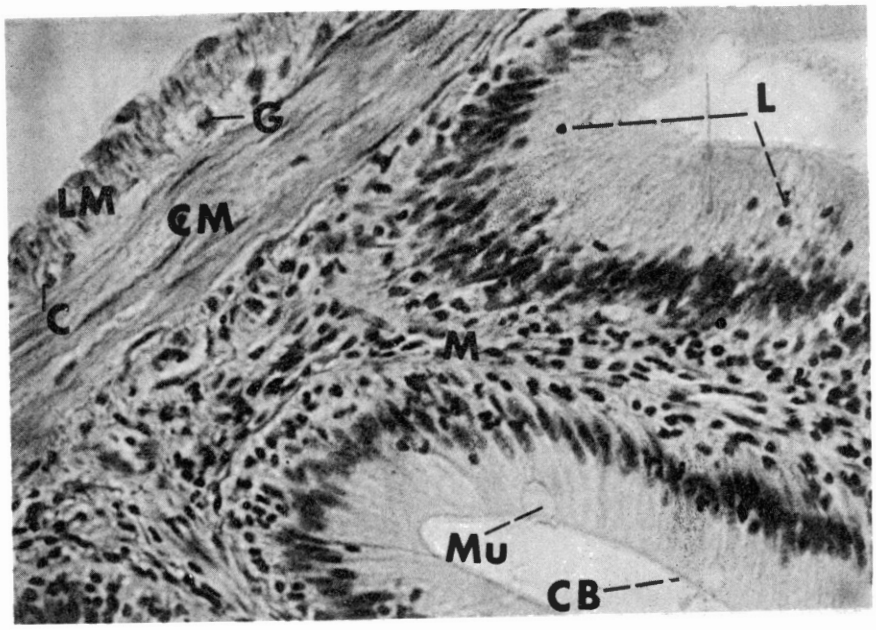


Figure 7. Cross section of the midgut displaying the fundamental architecture of this portion of the intestinal tract of the golden shiner (480X). M: mucosa; CB: ciliated lumenal border; Mu: mucous cells; L: lymphocytes; LM: longitudinal muscle; CM: circular muscle; G: ganglion; C: capillary.

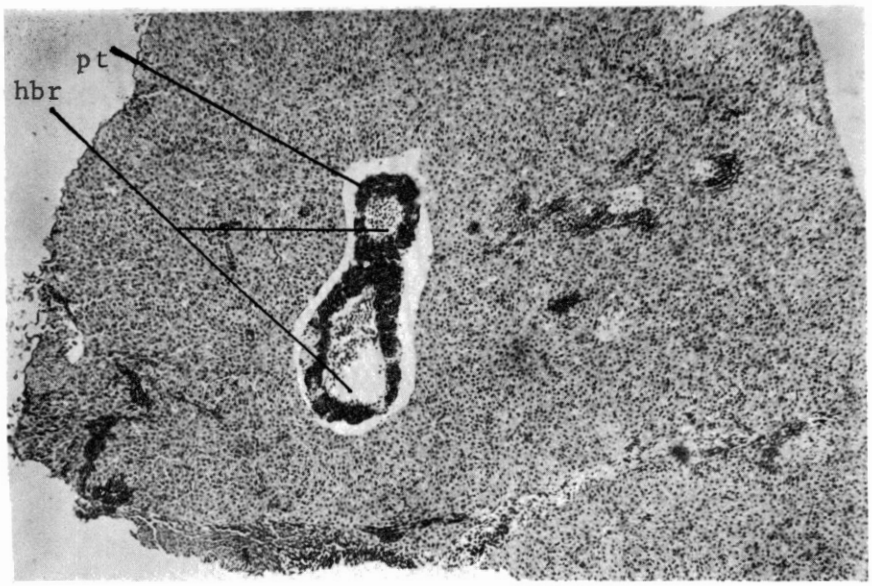
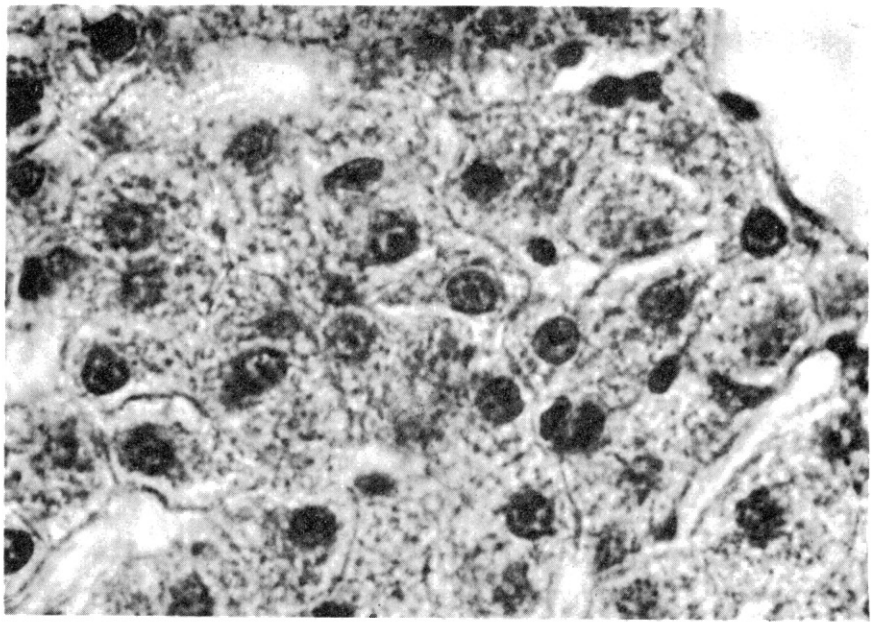


Figure 8. Longitudinal section of the liver penetrated with pancreatic tissues (75.6X). hbr:hepatic blood vessel; pt: pancreatic tissue.

Figure 9. Normal hepatic parenchyma cells (1200X).

Figure 10. Focal granuloma in the liver of the golden shiner affected by Chondrococcus columnaris. The lesion is infiltrated by macrophages (1200X).
fg: focal granuloma; m: macrophages.



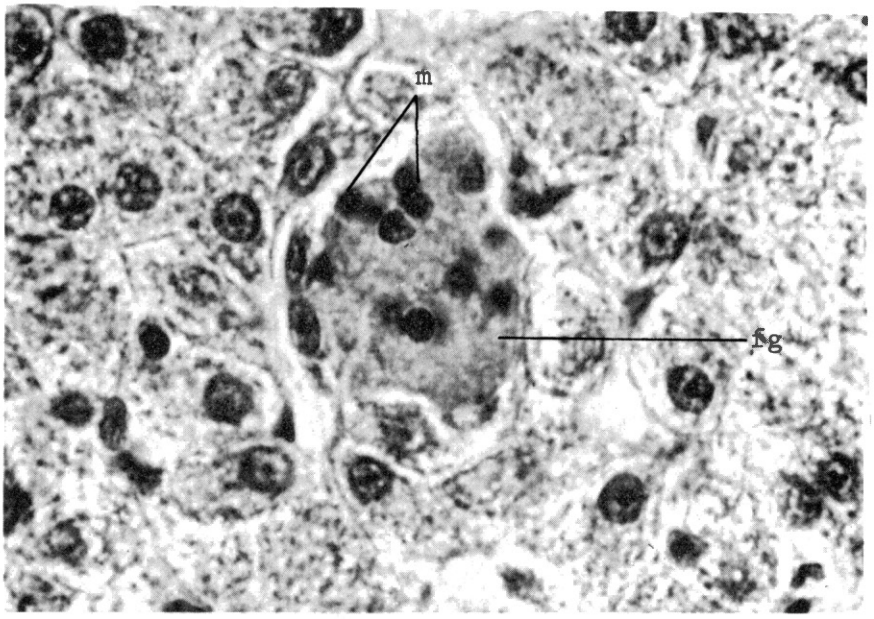

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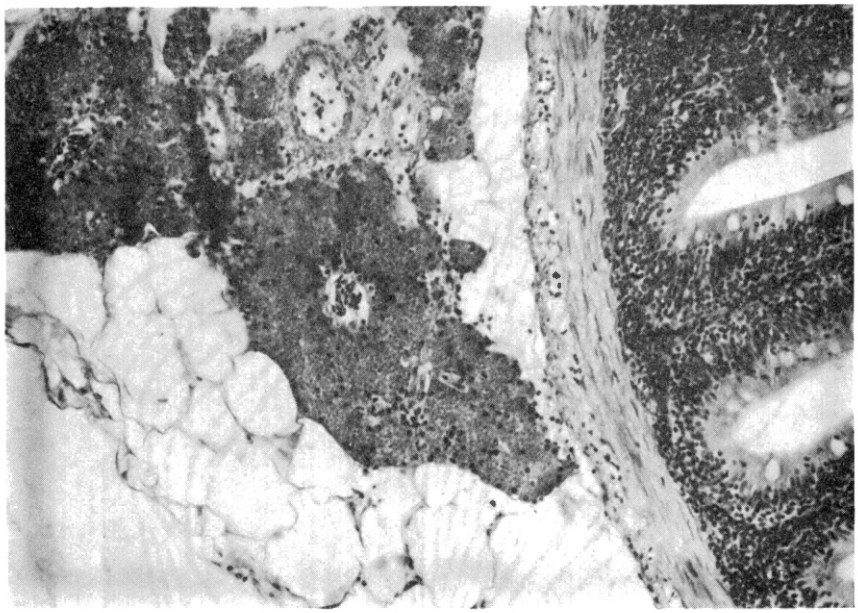
Figure 11. A portion of the diffuse pancreas embedded in the adipose tissues around the intestine (midgut) (192X).

Figure 12. Pancreatic cells. A: normal acinic cells; B: pigmented necrotic cells (1200X).



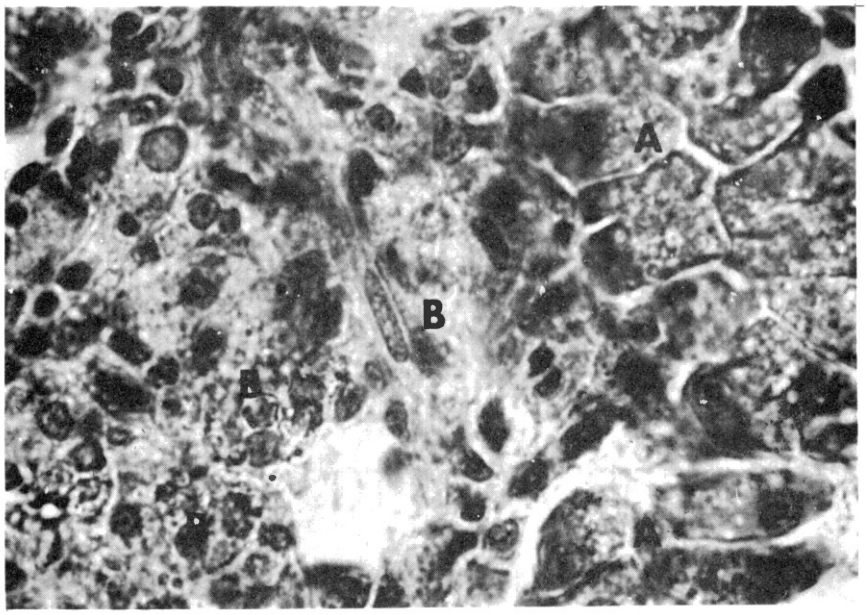

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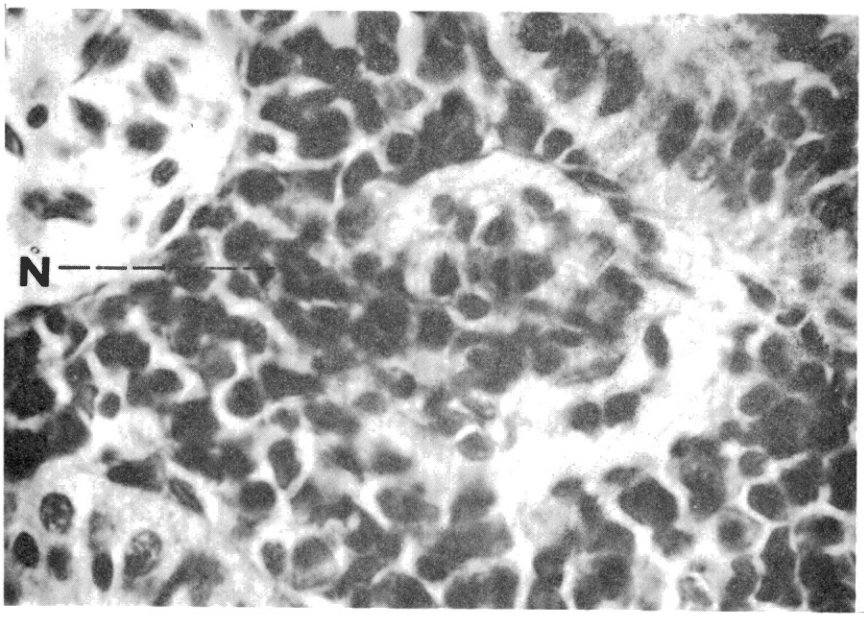
Figure 13. A glomerulus and its neck (N) (1200X).

Figure 14. Cross section of a proximal convoluted tubule. A: vesicular nucleus and an inconspicuous nucleolus; B: interdigitating lateral membrane of adjacent cells; C: ciliated lumenal border. (1200X).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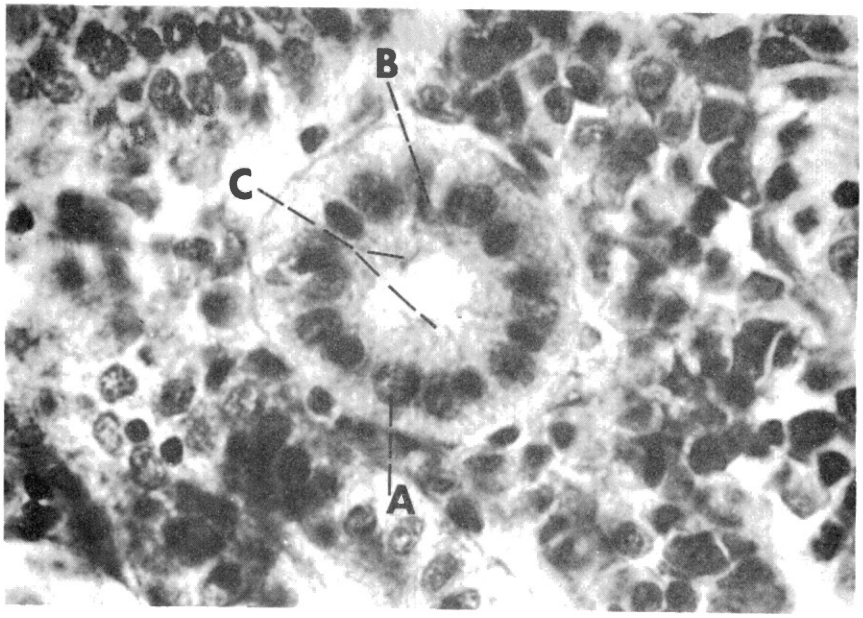
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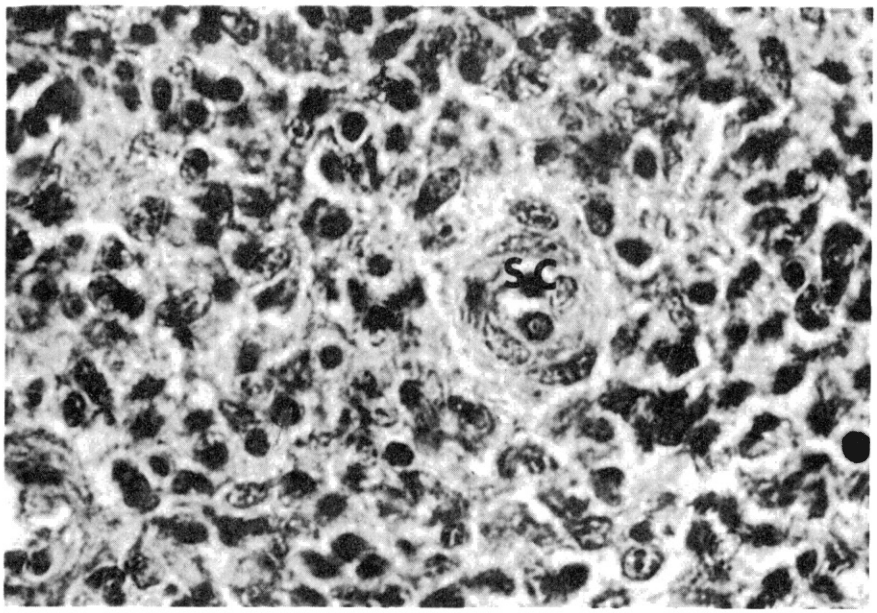

Figure 15. A spleen corpuscle (SC). (1200X).

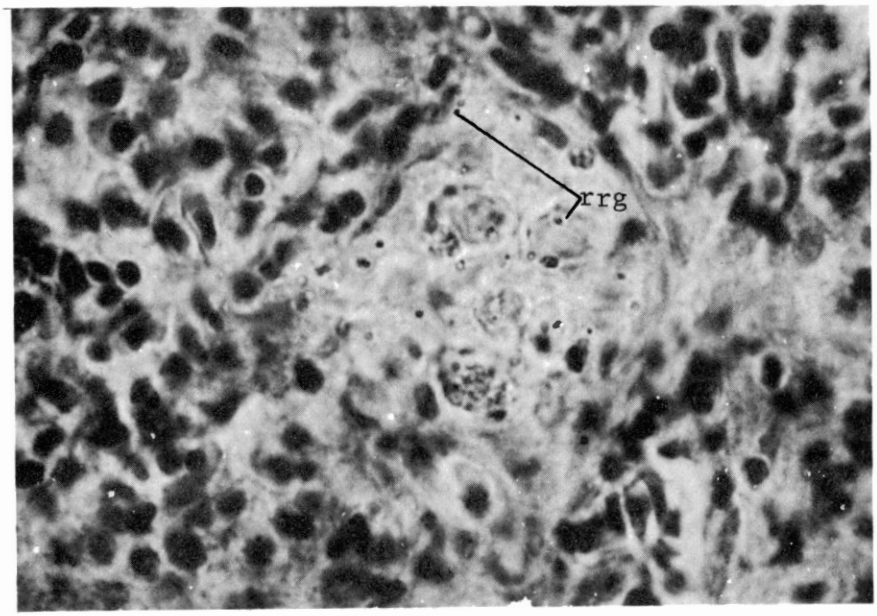

Figure 16. Heavily pigmented necrotic splenic parenchyma cells (480X). rrg: red rod-like "granules".

VITA

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Master of Science

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