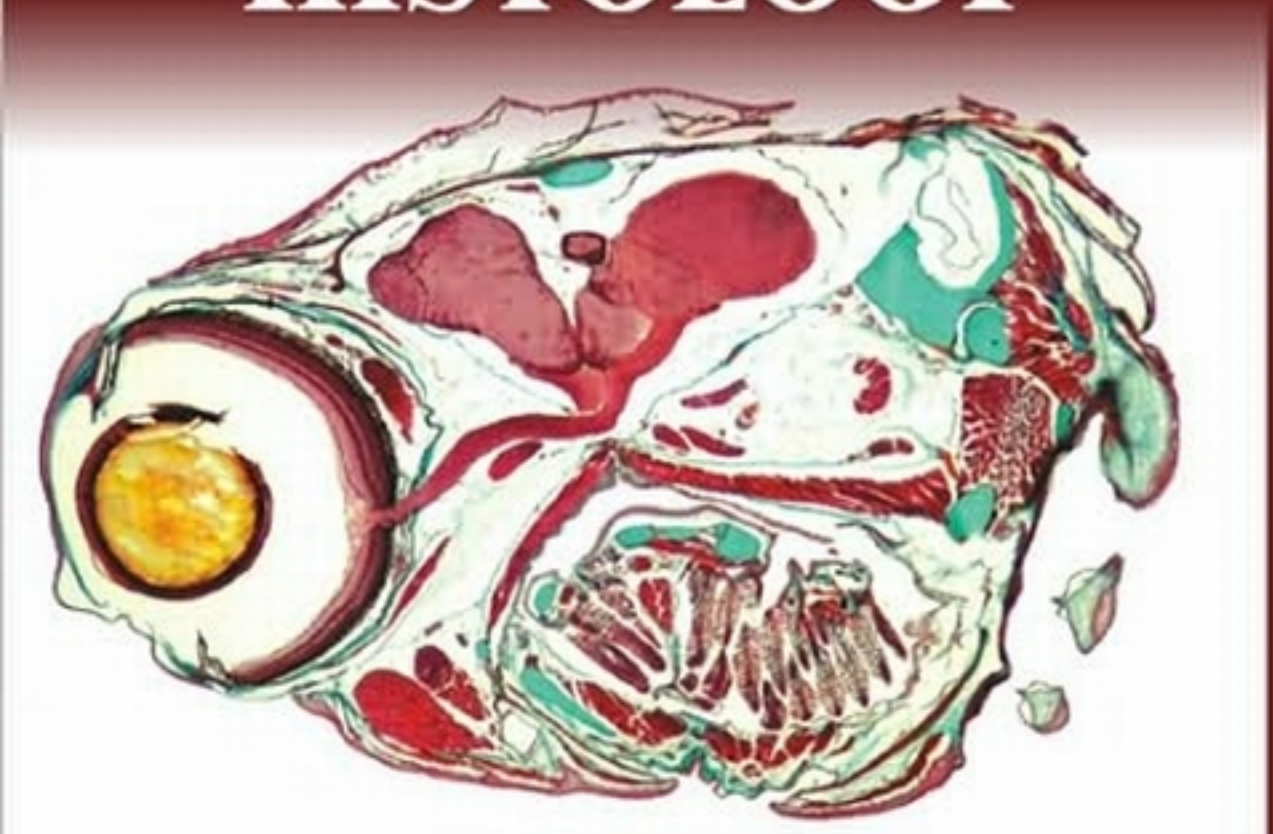
Atlas of FISH HISTOLOGY



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Preface

Hystology is the discipline of biology that involves the microscopic examination of thin (5-7 μm) stained tissue sections in order to study their structure and correlate it with function.

Histology can detect signs of disease not easily recognized on gross examination and can therefore be of interest in fish health supervision.

Fish constitute nearly 60% of all vertebrate species and are economically of major importance. Owing to overfishing, aquaculture now accounts for more or less 45% of the world fish-meal consumption. Fish health in fisheries is therefore an important concern and as it is not always possible to diagnose fish disease purely on the basis of behaviour or physical changes. Further investigations and tests are often crucial to arrive at a definite diagnosis.

The reporting of normal histology of fish tissues and organs serves as a foundation upon which to gather and build our ichthyopathology knowledge base.

The samples were fixed in Bouin's fluid and embedded in paraffin. The sections were stained with Hematoxylin and Eosin (H-E), and mainly by Masson's trichrome. The Periodic Acid-Schiff (PAS) reaction was used for staining polysaccharide in combination with hematoxylin and orange-G. Lectins as histochemical reagents enable more accurate characterization of sugar sequences in oligosaccharide units of glycoconjugates at the light microscope level. Such localization is shown in some preparations.

Our aim has been to present a general reference guide providing an extensive set of histological images of fishes (about 40 species). Although several studies treat histological aspects in relation to pathology, no recent synthesis on the normal histology of fish is available. We therefore believe this atlas will be a main contribution to this field.

This atlas is designed for use by students and researchers, biologists, ichthyologists, fish farmers, veterinarians working in fisheries and, of course, by comparative histologists who want to learn more about the fish world.

Initially, this work was intended to be exhaustive and bilingual English and French. Due to financial considerations, we had to severely cut the introductive texts and captions, to withdraw dozens of images and to eliminate the French text. An exhaustive electronic version will be available in French at the end of this year.

All photomicrographs are original. Light microscopy has been used exclusively and illustrated with color photomicrographs. Tissue and organ samples chosen to illustrate this work have been selected from reared food fish, as well as from species in the aquarium and in the wild.

In addition to the caption and the magnification, each iconographic document refers to the scientific name of the fish and the stain applied. The names of the fishes whose sections were used to illustrate the different chapters are listed in Appendix 1.

Note: whenever possible the tissues were removed from fishes born in captivity or destined to consumption. Certain african specimen were collected by Professor Max Poll (1908-1991), Head of the Fish Section of the Tervueren Museum (Belgium), who participated in field expeditions in Central Africa.

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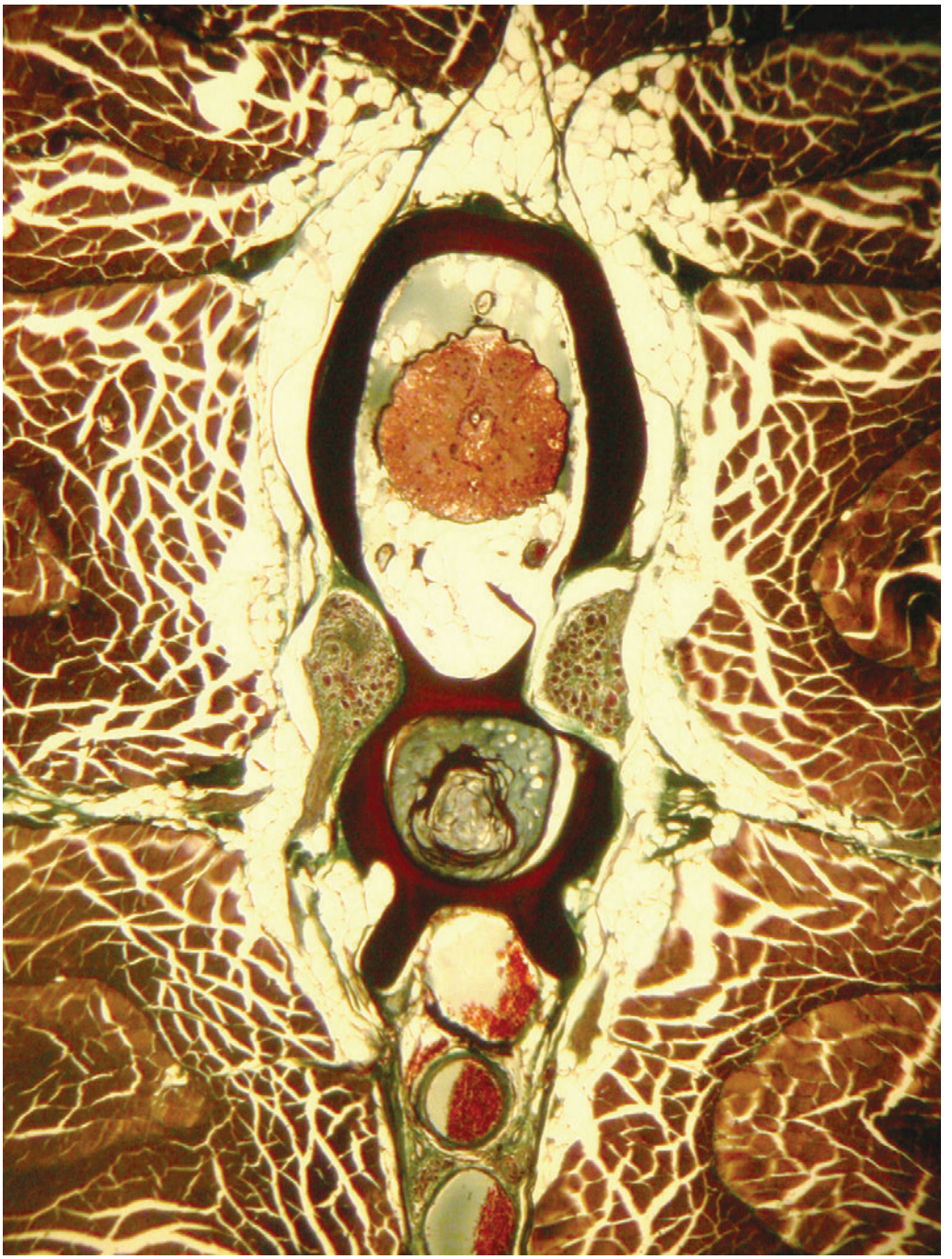

Anguilla anguilla : Transverse section through the posterior region of a young specimen. Spinal cord and vertebra.
(see Chapter 12, Fig. : 12.22)

Acknowledgements

For their help, advice and/or support throughout months of dissections, cutting, staining, photographing, interpreting, writing, editing and correcting of the present atlas, we would like to sincerely thank the following persons:

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E. Parmentier, PhD, Université de Liège (Belgium) for giving us permission to use his micrograph (Fig. 3.11);

Georges Lenglet, PhD, Head of the Vertebrate Section 1, Institut Royal des Sciences Naturelles de Belgique (Belgium); Pr. Christian Michel, PhD, Université de Liège (Belgium); Pr. Louis De Vos, PhD, Université Libre de Bruxelles (Belgium) for reading and correcting the original French texts;
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Isabelle Coppée, Franck's beloved wife, and their three daughters for having borne his white nights and absence while finalizing this book.

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Abbreviations used in captions.

For the magnification :

LM : low magnification, 8 - 40x

MM : medium magnification, 100 - 250x

HM : high magnification, 400 - 640x

IM : immersion, 1000x

For the colorations :

^{3}H-TdR : tritiated thymidine method

α−X Ab : anti-X antibody (X = protein)

AB : alcian blue

AUR : aurantia

AZ : Heidenhain's azan

FR-HB : Feulgen Rosenbeck reaction - Heidenhain blue

H : hematoxylin

H-E : hematoxylin – eosin

H-Pb : Mac Conaill's lead hematoxylin

LEC x : lectin (x = used lectin)

MT : Masson's trichrome stain (with either light green or acetic anilin blue solution)

OR : Orange-G

PAS : the Periodic Acid Schiff reaction

TB : toluidine blue

TI-Ag : silver impregnation according to Tinel

WR : Wright's stain

Only after the last tree has been cut down,
Only after the last river has been poisoned,
Only after the last fish has been caught,
Only then will you find that money cannot be eaten.
Cree proverb (Indians of Canada).

Introduction to histotechniques and fish gross anatomy

Various steps are involved in producing a stained histological section using the paraffin procedure.

Tissue processing

Immediately after death or euthanasia, the tissue or organ is cut into small pieces (preferably inferior to $^1/_2$ cm^3). These pieces are placed into a fixative such as 10% neutral buffered formalin or Bouin's liquid, which, ideally preserves normal morphology and facilitates further processing.

Four to ten cm long fish have the abdomen slit with a scalpel, the intestine detached at the vent, and the internal organs pulled out slightly for optimal fixative penetration.

Larger fish (>10 cm) will require on-site excision of 0.3 cm sections. Do not fix whole fish.

Once the specimen has been fixed (24-48 hours), it is dehydrated using a series of graded water / alcohol mixtures up to 100% alcohol. Next, the specimen is placed into a solvent such as toluene, benzene or xylene (or butanol), which is miscible with both 100% alcohol and the embedding agent. This intermediate step, called clearing, is essential before infiltrating the dehydrated tissue with hot paraffin wax (58-60°C) because alcohol and paraffin do not mix. During infiltration, hot melted paraffin completely replaces the clearing agent. When infiltration is complete, the specimen is transferred to an embedding mold of fresh paraffin, which is allowed to harden at room temperature.

Sectioning

The block of paraffin is then secured to the microtome and oriented appropriately with respect to the knife. With each revolution of the microtome handle, the specimen moves to the blade and a section of the desired thickness (5-7 μm) is cut. The sections are then placed onto glass microscope slides.

Routine staining of paraffin sections

The basic procedure includes getting rid of the paraffin in the sections (deparaffinization) and rehydratation of the specimen so that biological stains or dyes may be used. This is essentially a reversal of the above mentioned dehydratation steps. Staining times will vary with thickness of section, age of some stains...

Most examination of histological sections occur with bright-field microscopy. Cell and tissue components are similar optically; studying them is difficult without enhancing their optical properties through stains.

Various stains are available to the histologist. Hematoxylin and eosin (H-E) is a frequently used combination of stains. Hematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm, lysosomes, endoplasmic *reticulum*, ribosomes…) in blue. In contrast, eosin stains the cytoplasmic proteins and a variety of extracellular structures from pink to red.

In addition to the widely, but not very specific used H-E staining procedure, other stain combinations and techniques are available. For example, trichrome procedures (including three dyes) such as Mallory's and Masson's, help to differentiate collagen from muscle cells.

Histochemical stainings

Specialized staining methods are used to illustrate particular features. Osmic acid reacts with fat to give a grey-black colour; the Periodic Acid –Schiff (PAS) reaction and Alcian Blue (AB) reveal different varieties of glycosaminoglycans of proteoglycans and glycoproteins that are present in some tissues and cells. Hematoxylin is the usual counterstain. The PAS positive si-

tes stain magenta/red; the AB positive components stain blue. Silver impregnation displays reticular fibers and some aspects of nervous tissue.

Immunohistochemistry & lectin cytochemistry

A highly specific interaction between molecules is the one between an antigen and its antibody. For this reason immunohistochemical staining methods have been developed for detection of a large array of biomolecules.

Histochemical methods involving the use of lectins have much in common with immunohistochemical techniques. Lectins are carbohydrate-binding proteins other than antibodies or enzymes. They are isolated from a wide variety of plant and animal sources. Their interest in histochemistry lies in their high affinity and specificity for individual glycan residues of proteoglycans and glycoproteins. Using a panel of biotinylated lectins and the avidin-biotin-peroxidase complex (ABC) technique, it is possible to obtain precise information about glycan moieties in vertebrate and invertebrate tissues.

Coverslipping

The stained section on the slide must be covered with a cover glass to protect the tissue from being scratched, to provide better optical properties for viewing under the microscope, and to preserve the tissue section for years to come. The stained specimen must go through the reverse process that it went through from paraffin section to water. The stained slide is taken through a series of alcohol solutions (70, 90 and 100%) to remove the water, then through clearing agents to a point at which a permanent resinous medium can be placed beneath the glass coverslip over the section. Ideally, to prevent any air intrusion, varnish will be applied all around the cover glass.

Pictures of gross anatomy

We have provided gross views of parasagittal and transverse sections of whole fish stained with the Masson's trichrome (Figs A to S). This allows a better understanding of the location of each organ. Note that the sections of *Danio rerio* are slightly oblique.

Key to the gross anatomy figures A to S

1 adipose tissue
2 epaxial muscles
3 hypaxial muscles
4 hypobranchial musculature
5 dorsal fin erector muscles
6 *gonopodium* erector muscles
7 *myosepta*
8 skull skeleton
9 vertebra(e)
10 rib(s)
11 neural arches
12 hemal arches
13 notochord
14 pectoral fin
15 pelvic fin
16 anterior cardinal vein
17 posterior cardinal vein
18 Cuvierian duct
19 heart (*sinus venosus*)
20 heart (*atrium*)
21 heart (ventricle)
22 heart (*bulbus arteriosus*)
23 pericardial cavity and *pericardium* (dotted line)
24 spleen
25 thymus
26 epidermis
27 scales
28 buccal cavity
29 pharynx
30 tongue
31 esophagus
32 keratinized (horny) esophagus epithelium
33 small intestine
34 posterior intestine (rectum)
35 liver
36 gall bladder
37 pancreatic tissue
38 peritoneal cavity
39 *peritoneum*
40 gas bladder
41 gas gland
42 gills
43 branchial cavity
44 gill rakers
45 *operculum*
46 ventral aorta
47 branchial arteries
48 dorsal aorta
49 pseudobranch
50 hematopoietic kidney
51 excretory kidney
52 ureter
53 urinary bladder
54 urogenital *sinus*
55 telencephalon
56 diencephalon
57 mesencephalon (optic lobes)
58 metencephalon (*cerebellum*)
59 myelencephalon
60 spinal cord
61 grey matter
62 Mauthner's neurons
63 Mauthner's axons
64 brain ventricle
65 pituitary gland
66 thyroid follicles
67 ultimobranchial gland
68 testis with cysts
69 *spermatozeugmata* in efferent ducts
70 *gonopodium*
71 ovary
72 yolk droplets
73 inner ear (semicircular canal)
74 inner ear (otolithic chamber)
75 lateral system
76 olfactory organ
77 nostril
78 *eye*
79 ocular muscle(s)
80 lens
81 retina
82 iris
83 choroid *rete*
84 optic nerve
85 connective tissue
86 striated muscle
87 spinal ganglion
88 caudal artery and caudal vein
89 anal fin
90 dorsal fin
91 stomach
92 branchiostegals

Alphabetical key to the figures A to S

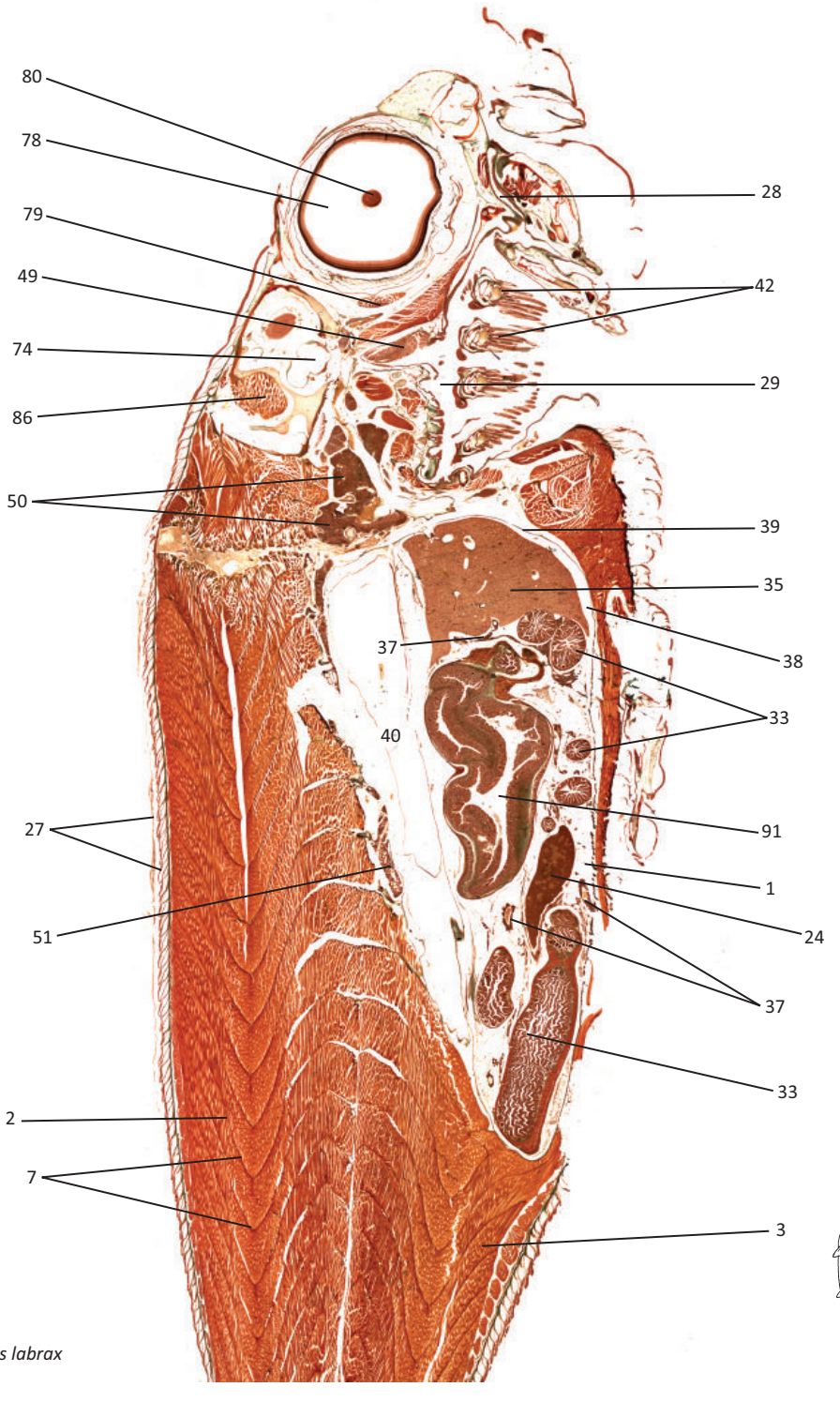


Fig. A: *Dicentrarchus labrax*

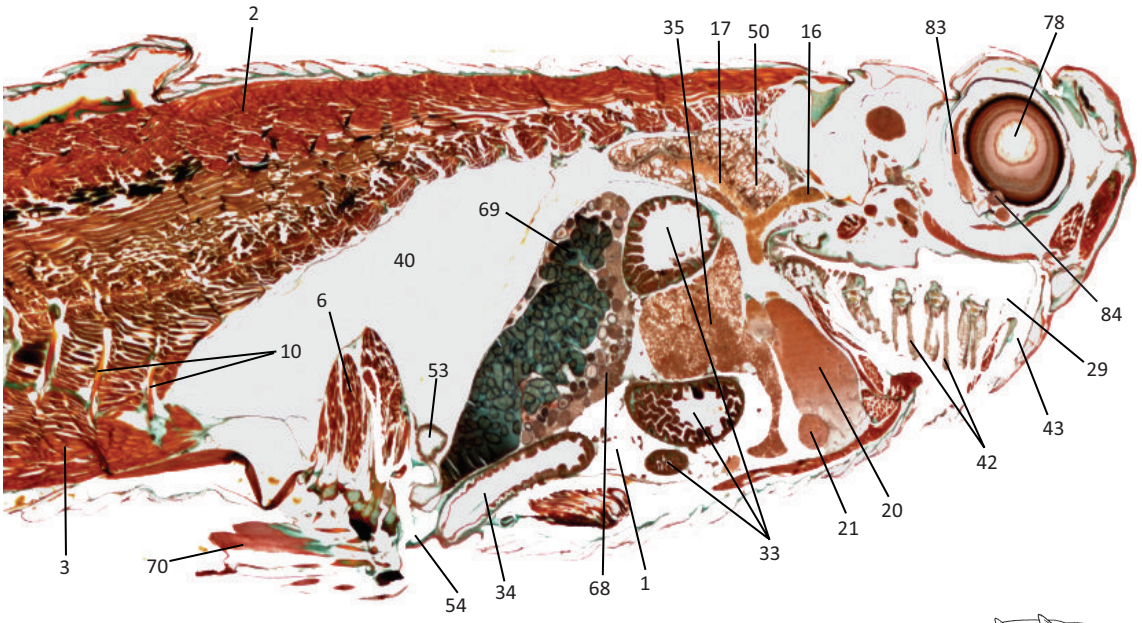


Fig. B: *Poecilia reticulata* var. Endler

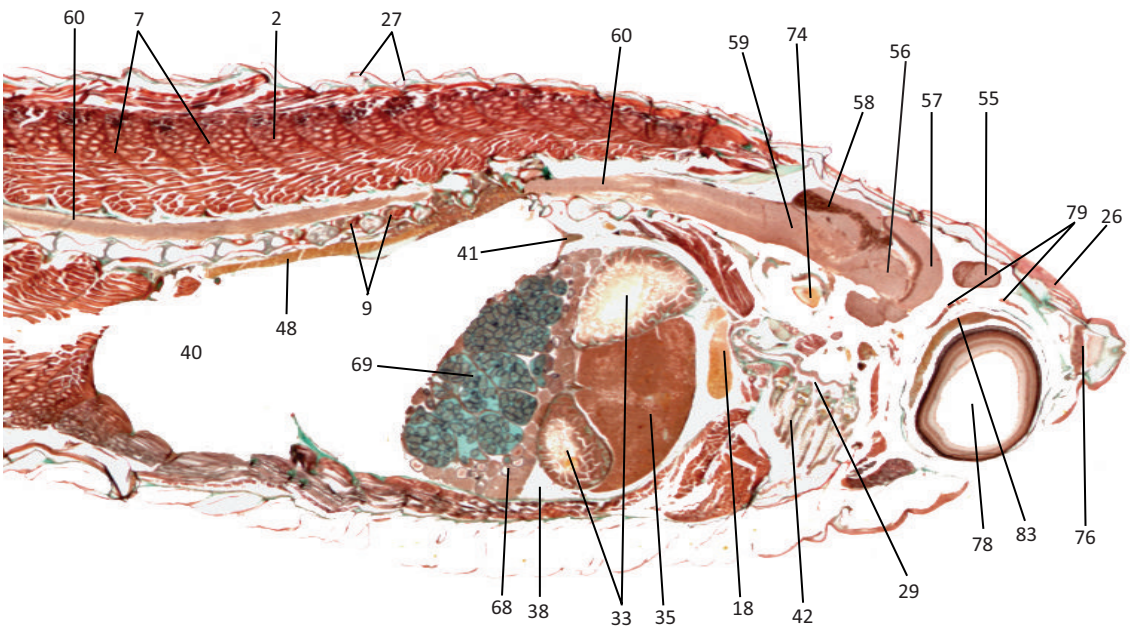


Fig. C: *Poecilia reticulata* var. Endler

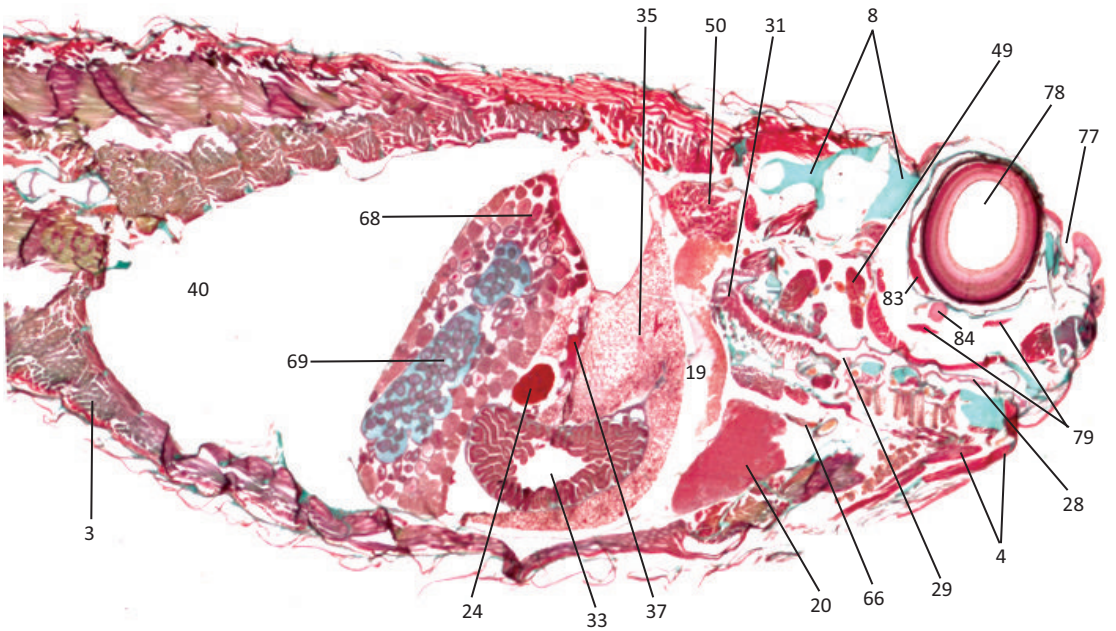
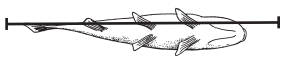


Fig. D: *Poecilia reticulata* var. Endler



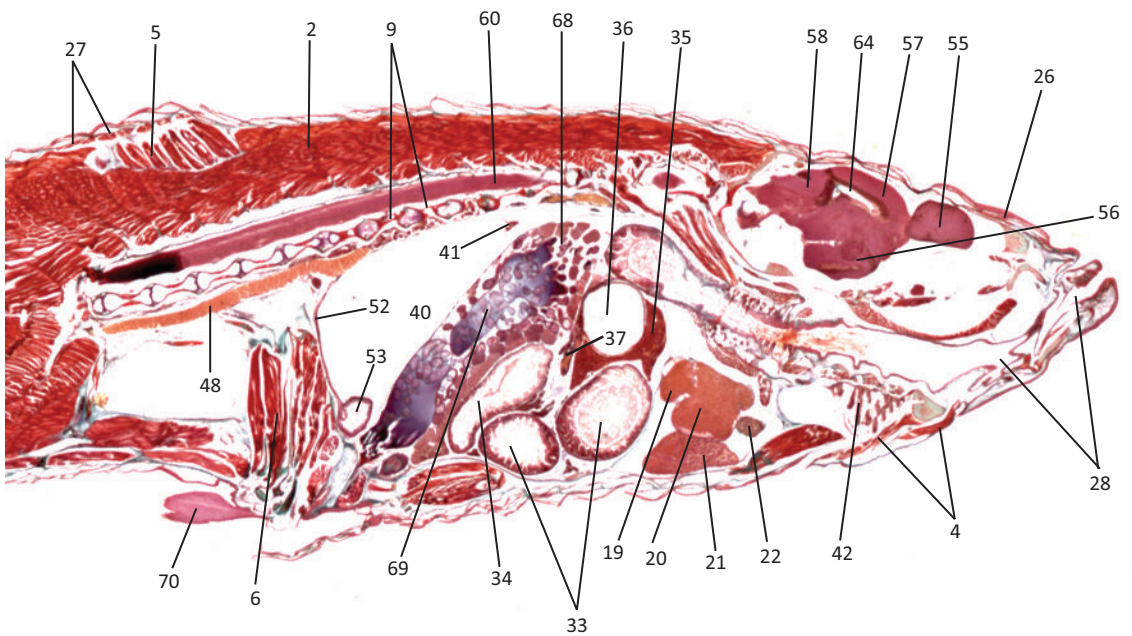
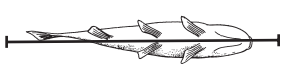


Fig. E: *Poecilia reticulata* var. Endler



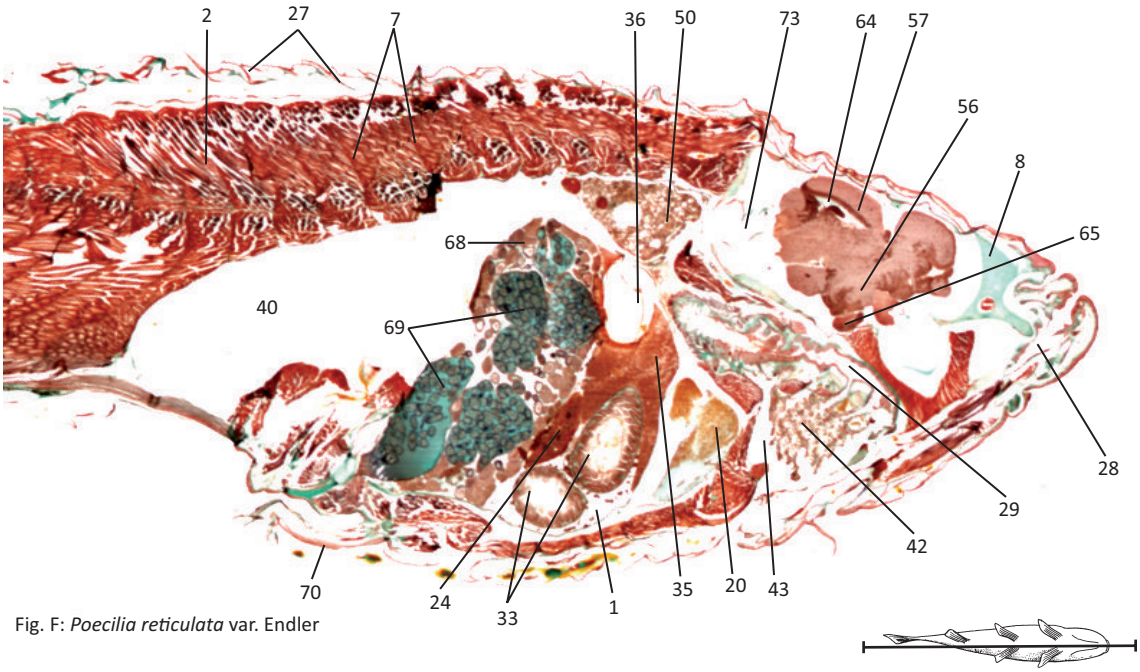


Fig. F: *Poecilia reticulata* var. Endler

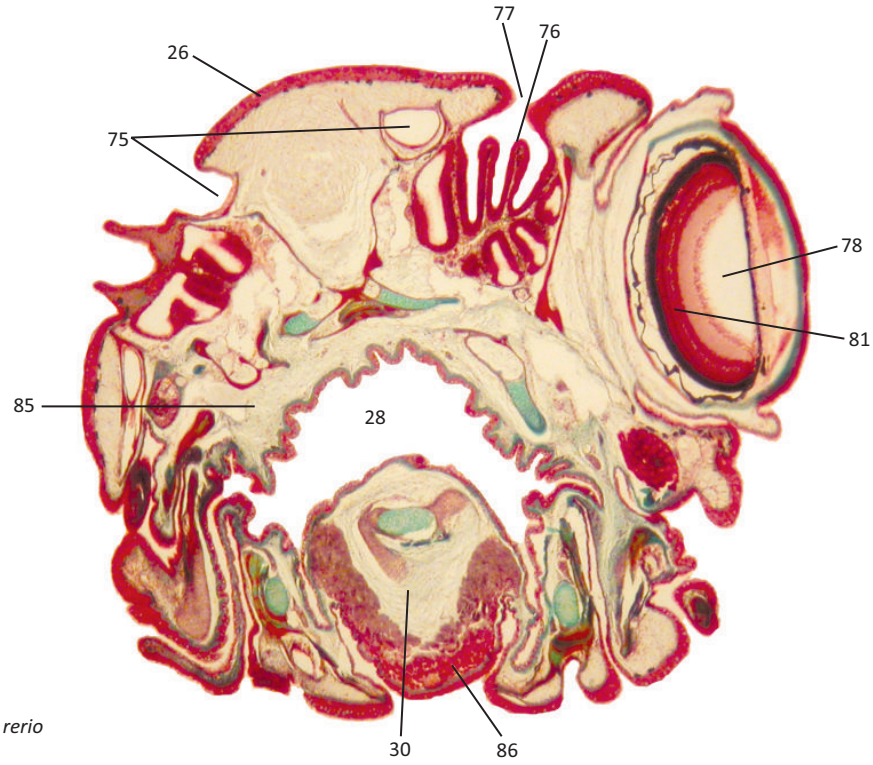
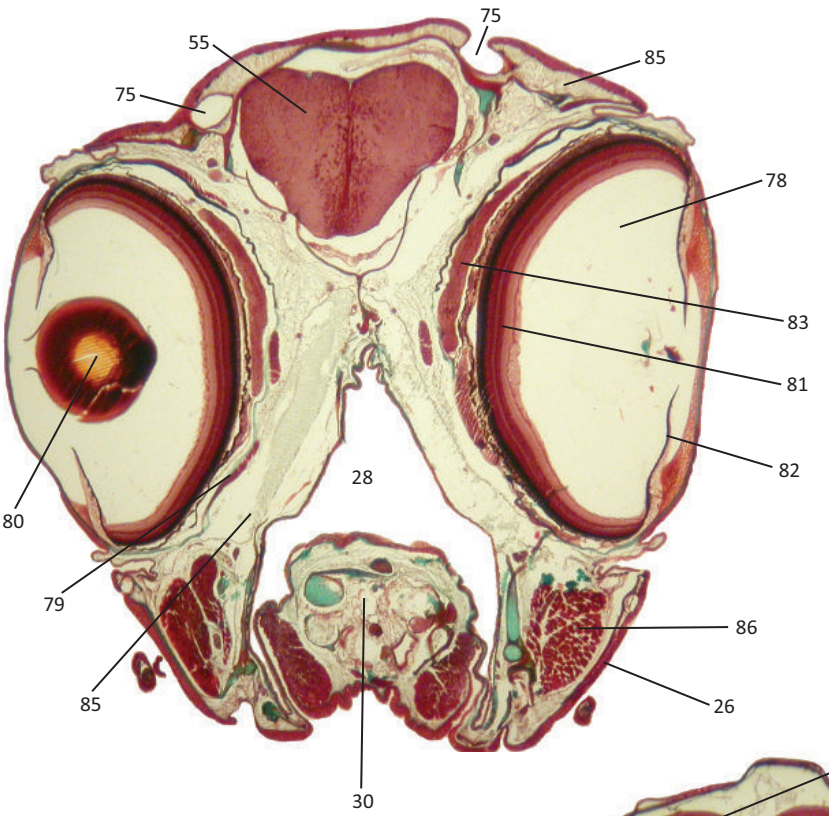
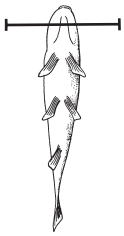
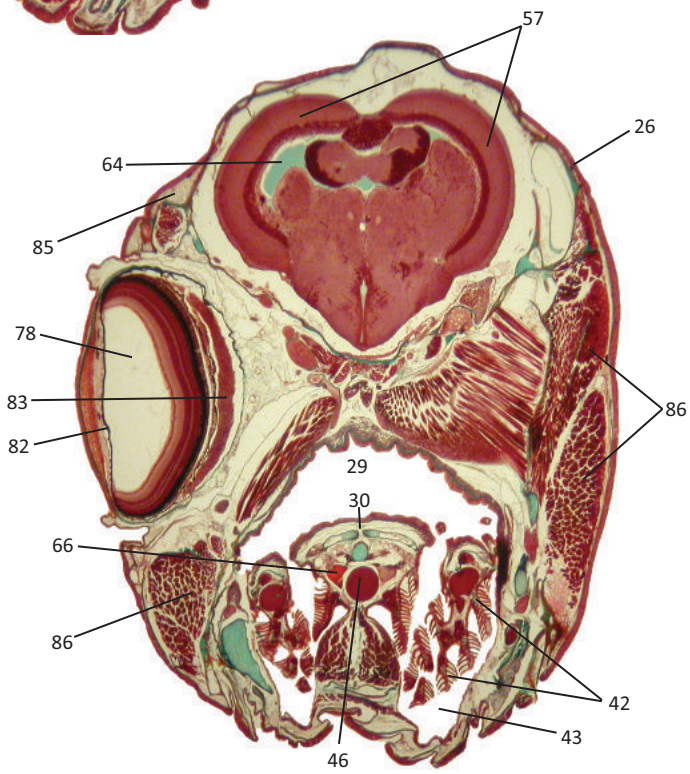


Fig. G: *Danio rerio*

Fig. H: *Danio rerio*







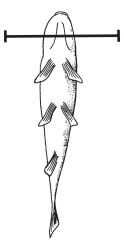
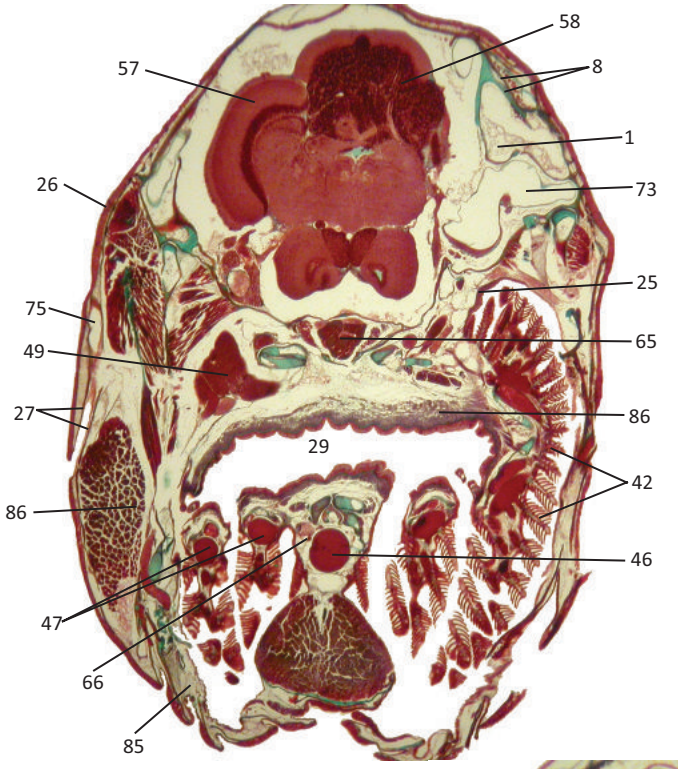


Fig. I: *Danio rerio*



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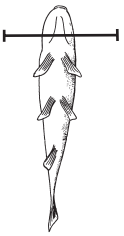
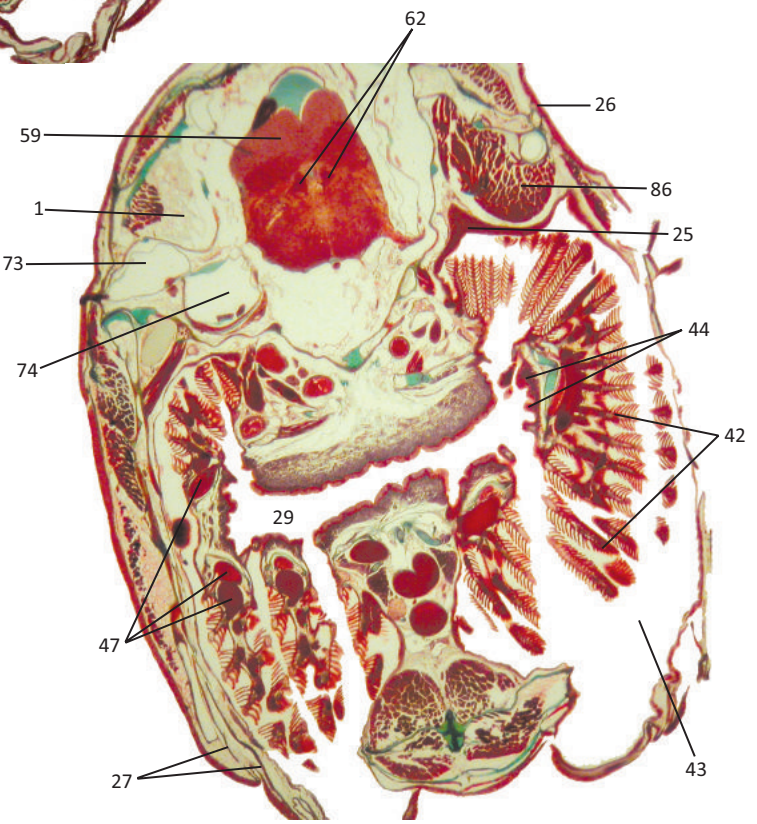




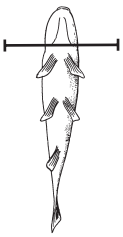
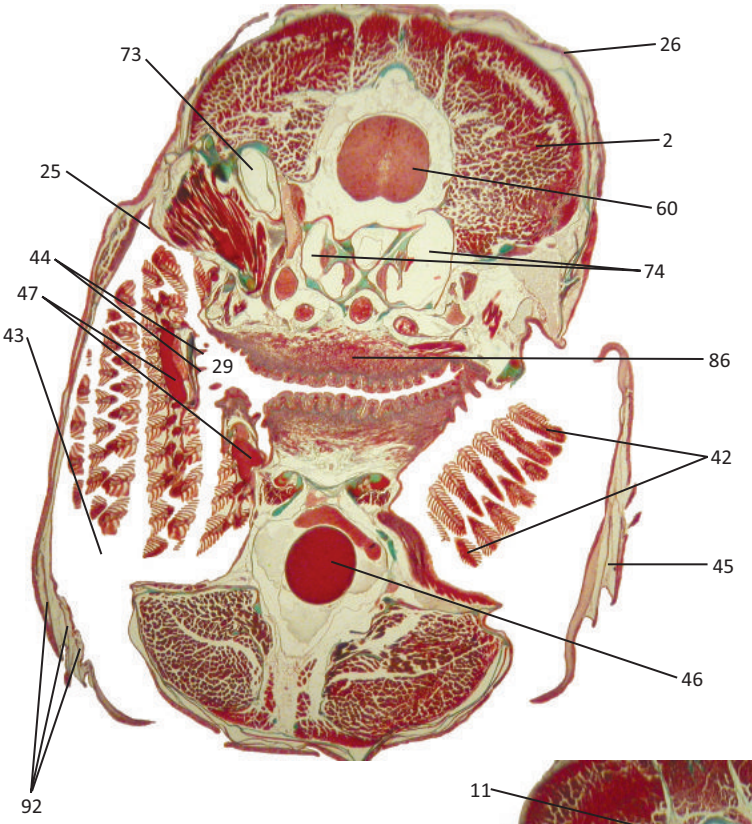
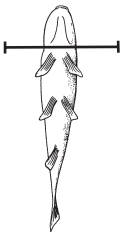


Fig. K: *Danio rerio*

Fig. L: *Danio rerio*





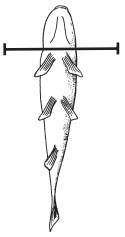


Fig. M: *Danio rerio*

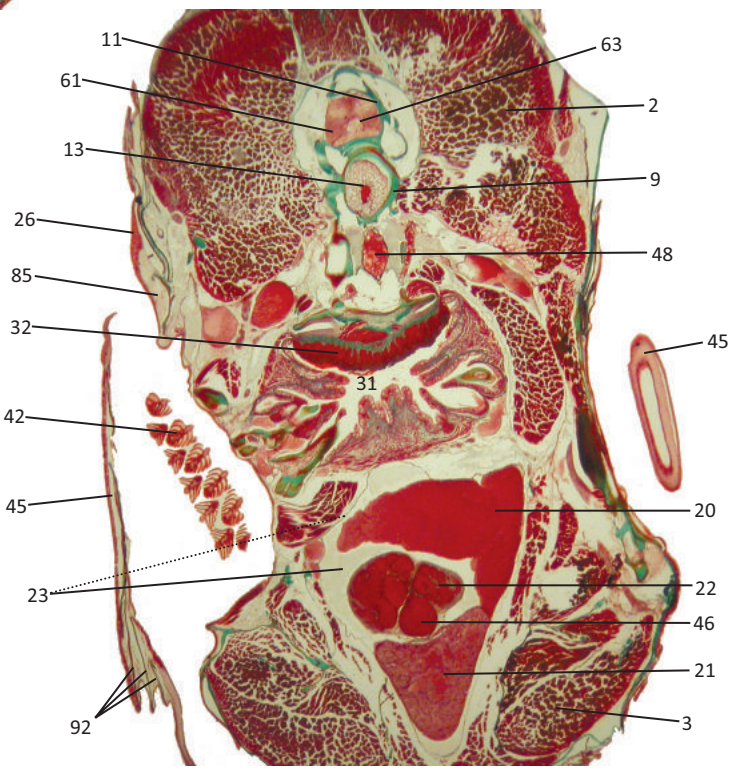
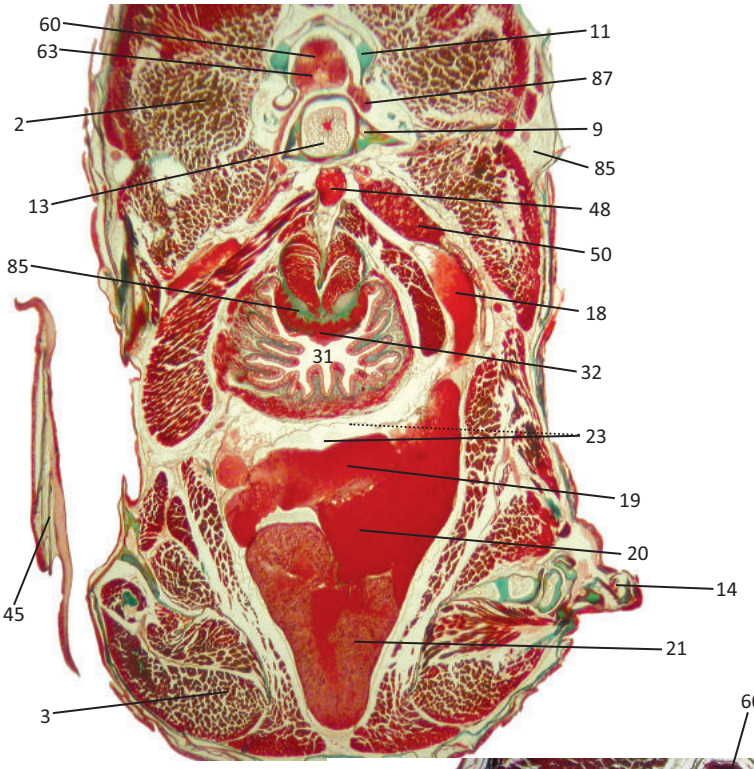
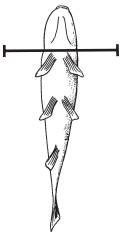
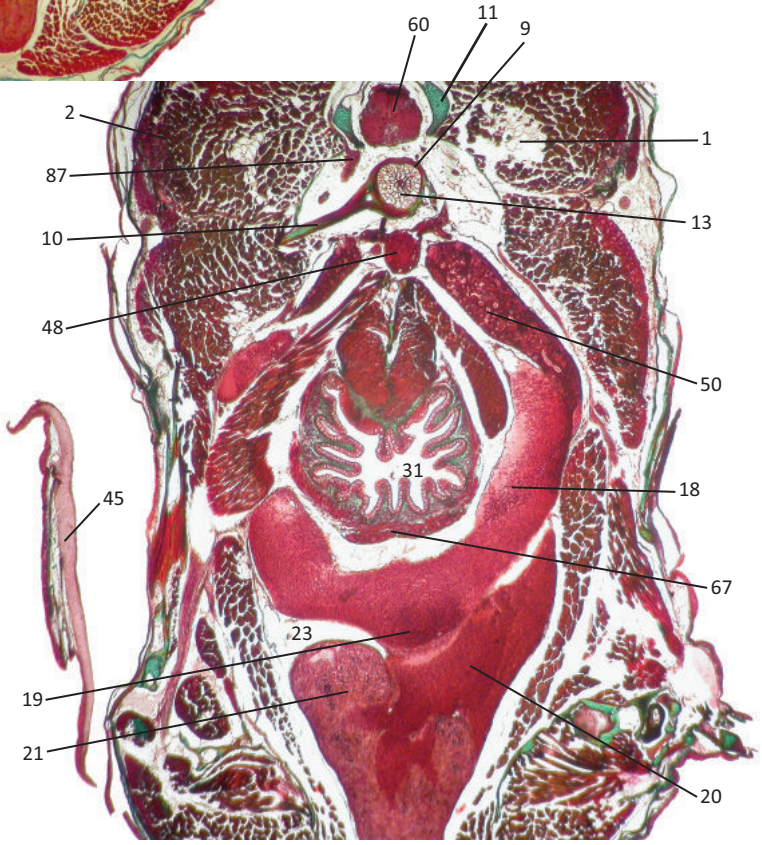


Fig. N: *Danio rerio*







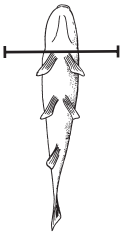
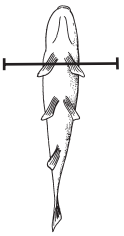
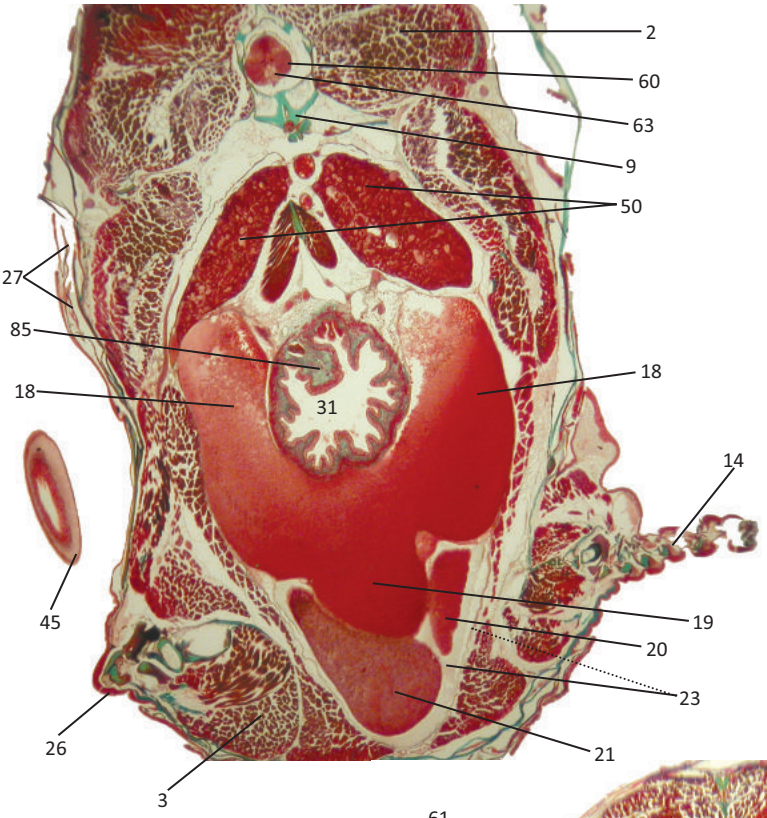
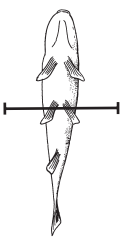


Fig. O: *Danio rerio*

Fig. P: *Danio rerio*







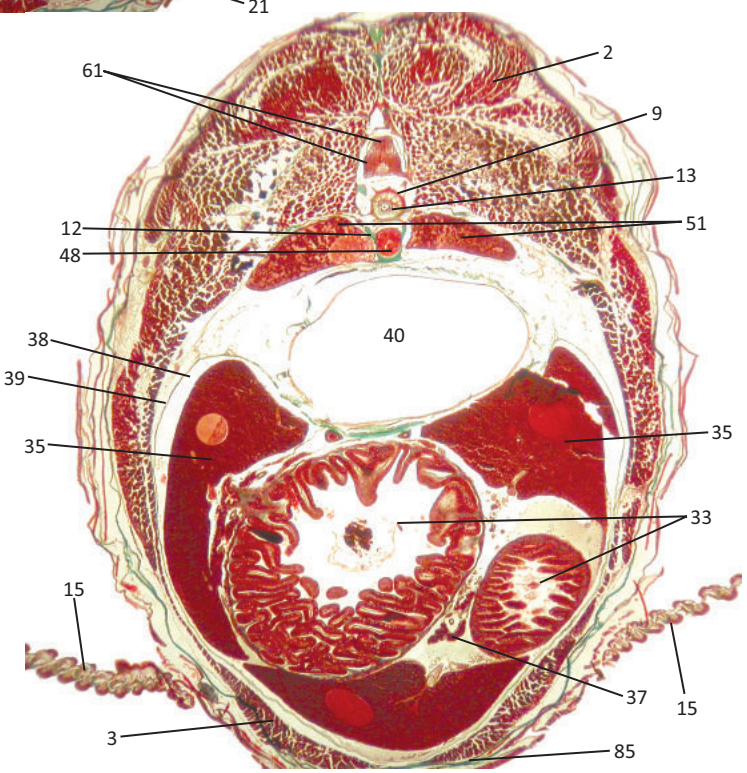
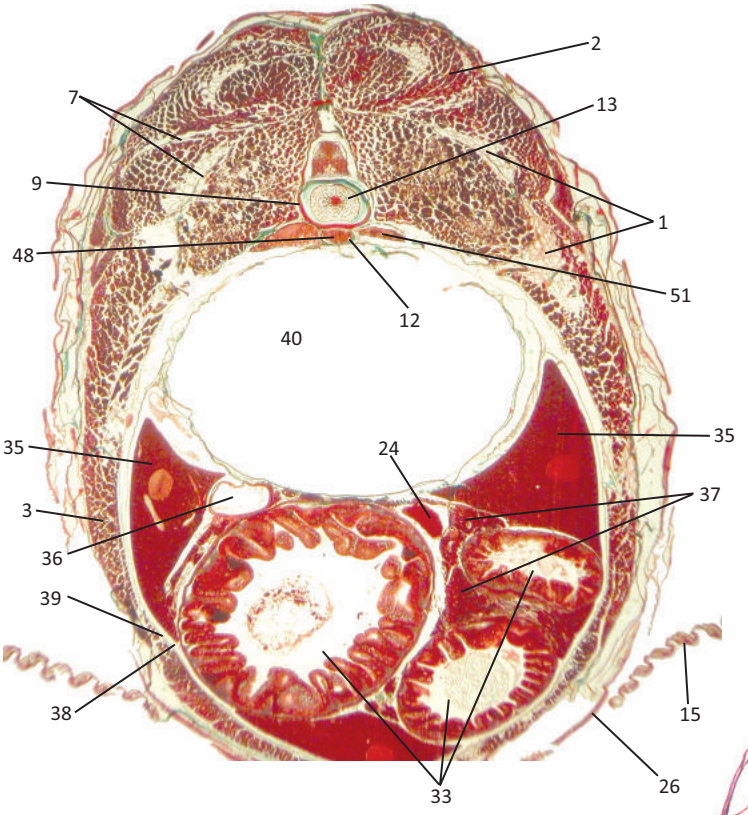
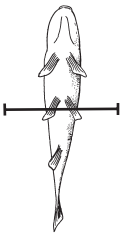
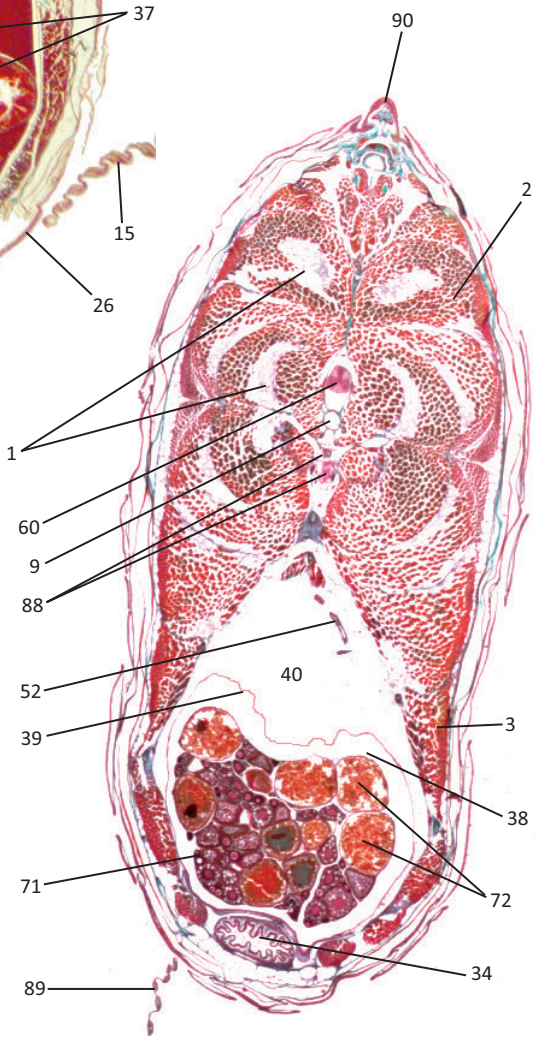


Fig. Q: *Danio rerio*

Fig. R: *Danio rerio*







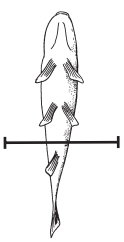


Fig. S: *Danio rerio*

1

Tissues of fishes

In fishes as in other vertebrates, cells are associated with one another and with extracellular components to form the five basic tissues specialized for different functions : epithelial tissue, connective tissue, skeletal tissue, muscular tissue and nervous tissue.

An epithelium (Figs 1.1 to 1.10) consists of contiguous cells, with a very small amount of intercellular matrix. Such tissue is found covering surfaces and lining cavities. It also forms the essential parts of the "glands", which are masses of cells specialized for secretion of a given product into the lumen of a duct or into a body cavity, or into the blood or lymph streams. When the secretion enters a duct or a body cavity the gland is known as an exocrine gland (Fig. 1.8), for example, the digestive glands. When it enters the blood or lymph to be carried to other parts of the body and affect distant structures, the organ which forms it is called an endocrine gland, for example the pituitary gland, corpuscles of STANNIUS, etc…Most endocrine glands consist of clumps or cords of secretory cells in close apposition to a dense network of small blood vessels. The thyroid gland (Fig. 1.9) is an unusual endocrine gland whose secretory product is stored within spheroidal cavities enclosed by secretory cells; these spheroidal units are called follicles.

Epithelial cells are supported by a basement membrane that separates them from the underlying connective tissue (Figs 1.1, 1.2 & 1.5).

Some glands are unicellular, they consist of specialized cells scattered throughout an epithelial lining. In fish, the most common unicellular gland is the mucus-secreting cell (Fig. 1.10) found within the skin epithelium or within the digestive tract epithelium. The mucoid substances are released onto the epithelial surface. As this secretory material is synthesized, it fills and expands the apical portion of the cell. The remainder of the cytoplasm and the nucleus are displaced to the narrow basal region of the cell.

In fish, chemosensory receptors (Fig. 1.7) populate the stratified epithelium of the integument and of the buccopharyngeal cavity. They function in the perception of smell or taste.

The connective tissues (Figs 1.11 to 1.13) are a diverse group of tissues that share a common origin from the mesenchyme of the embryo. They serve as connecting and filling tissues, lying beneath the epithelial tissues of the skin, of the digestive tract, and of tubular organs, between the muscles, between the masses of secreting cells in the glands, and in numerous other locations.

Each type of connective tissue consists of cells widely scattered in an abundant extracellular matrix consisting of fibers in an amorphous ground substance. The ground substance, composed largely of glycoproteins and glycosaminoglycans, forms a well-hydrated gel that fills the space between cells, fibers and blood vessels. Collagenous, reticular and elastic fibers occur in connective tissue. The various cell types of connective tissue are divided into two groups : one population of fixed cells, which includes fibroblasts and adipose cells, and another population of wandering or free cells (macrophages, plasma cells, mast cells…) whose presence primarily depends on the functional state of the tissue. The fibroblasts are responsible for the formation of both fibers and ground substance. Inactive fibroblasts, also called fibrocytes, are smaller and spindle-shaped. In loose connective tissue, the ground substance predominates; in dense connective tissue, the collagenous fibers form a compact meshwork. Ligaments (Fig. 1.13) are composed of dense bands of fibrous connective tissue whose collagen fibers are arranged in a very regular way.

Cartilage and bone (skeletal tissues) are connective tissues with a firm extracellular matrix specialized for support of the body as a whole.

The adipose tissue (Figs 1.14 & 1.15) has long been considered a type of connective tissue and can be described as a loose association of lipid-filled cells known as adipocytes with associated stromal-vascular cells, held in a matrix of collagen fibers. The adipocytes are characterized by one large lipid inclusion giving the cell a signet-ring shape that results from distension of the cytoplasm and apposition of the nucleus to the cell membrane. In sections, individual adipose cells appear as empty elements, because the fat was dissolved by the hydrophobic solvents used during the routine histologic preparation of the tissue.

The muscular tissue is composed of cells that specialize in mechanical work. Three chief types are classically described : smooth, cardiac and skeletal. The last two types are frequently referred to as striated because of the cross-striations on the cells, which are lacking in smooth muscle.

The cells of the nervous tissue have the ability to receive and transmit stimuli, that is, they possess the properties of irritability and conductivity.

In the nervous system of the vertebrates a long series of evolutionary stages can be seen as we pass from lower to higher forms. These concern chiefly, however, the pattern of nerve cell aggregations and *fiber* pathways. The essential structure of the units, the neurons, shows a great degree of similarity.

In fishes a clear separation between a central and a peripheral nervous system does exist. The central nervous system (CNS) consists of the brain and spinal cord and contains the neurons and a host of supporting cells called neuroglia. Nerve impulses pass to and from the CNS over long neuronal processes called axons and dendrites. The peripheral nervous system (PNS) comprises all of these processes which travel in cranial and spinal nerves and related clusters of outlying neurons called ganglia.

Skeletal tissues (cartilage and bone), muscular and nervous tissues of fishes will be the subject of separate chapters below.

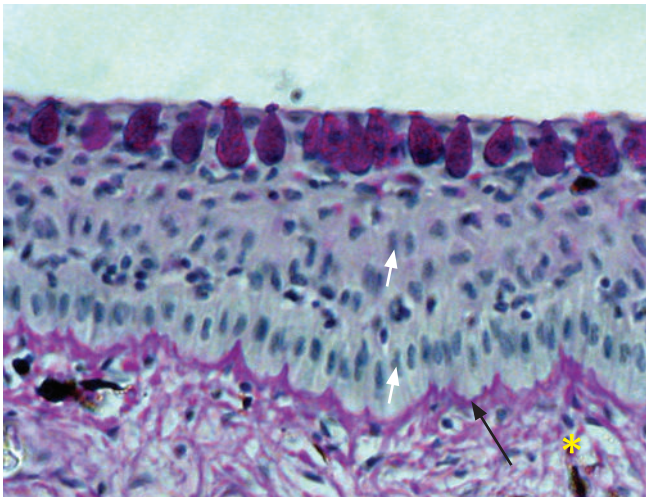


Fig. : 1.1 *Gnathonemus petersii* (PAS-H / MM)

Buccal cavity. The upper external surface is lined by a stratified epithelium and many PAS+ cells (unicellular glands - magenta) are located side by side at its surface. Cells of the bottom-most epithelial layer are usually cuboidal or columnar in shape and in contact with the basement membrane (black arrow). This one is mainly composed of glycosaminoglycans and (glyco)proteins and separates the epithelium from the underlying connective tissue (∗). The nuclei of the epidermal cells are stained blue (white arrows).

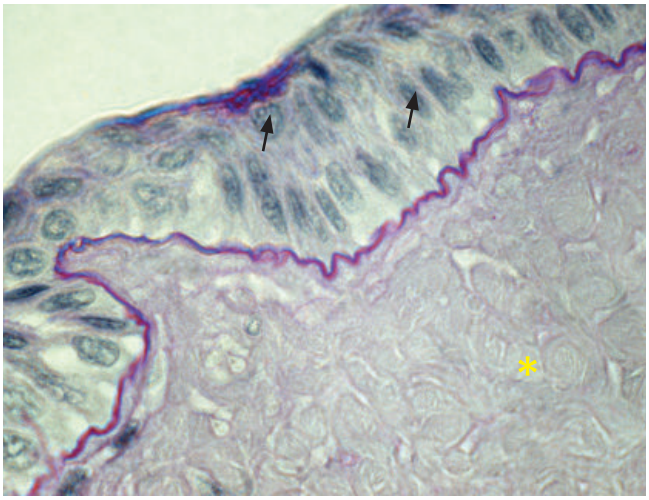


Fig. : 1.2 *Scyliorhinus canicula* (AB-PAS-H / HM)

Epithelium of the pharynx. The basement membrane is thick and colored in magenta by the AB-PAS reaction. Basement membranes are arrangements of extracellular matrix proteins which act as an interface between the supporting tissues (pale pink - ∗) and epithelial cells. The nuclei of the epithelial cells are stained blue (arrows) and more elongated at the basal level.

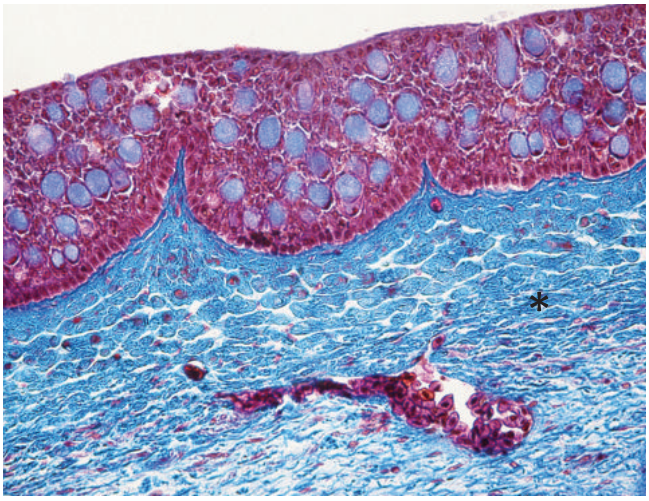


Fig. : 1.3 *Scyliorhinus canicula* (MT / MM)

Pharyngeal epithelium. Simple epithelia are formed of one layer of cells. This image shows a stratified epithelium (red) formed of multiple layers of cells separated by a very poor quantity of intercellular material. Mucus-secreting cells, in blue, are scattered amongst the epithelium. All supporting tissues are mainly composed of cells and extracellular matrix. The extracellular matrix is generally the dominent component (here in blue - ∗). A vein containing erythrocytes is seen at the bottom of the image.

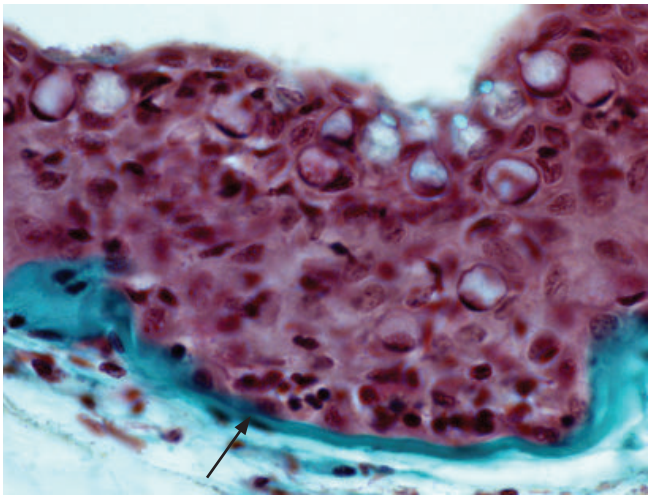


Fig. : 1.4 *Poecilia reticulata* (MT / HM)

Pharyngeal epithelium. In this high power view, note that the epithelial cells are closely bound to one another. Like it is often the case, the cell membranes are difficult to characterize. On this image, the basement membrane (in turquoise - arrow) is particularly thick. It is involved in epithelial differentiation.

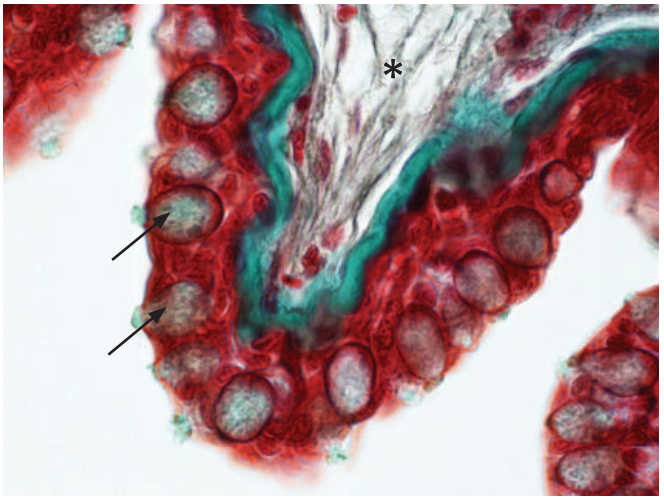


Fig. : 1.5 *Danio rerio* (MT / HM)

Buccal cavity. Stratified epithelium consists of a variable number of cell layers. In addition to the covering epithelial cells (red) many large mucus-secreting unicellular gland cells (arrows) are seen. All epithelia are supported by a basement membrane. Here the basement membrane is quite thick (green). Note the loose connective tissue (∗) at the centre of the fold.

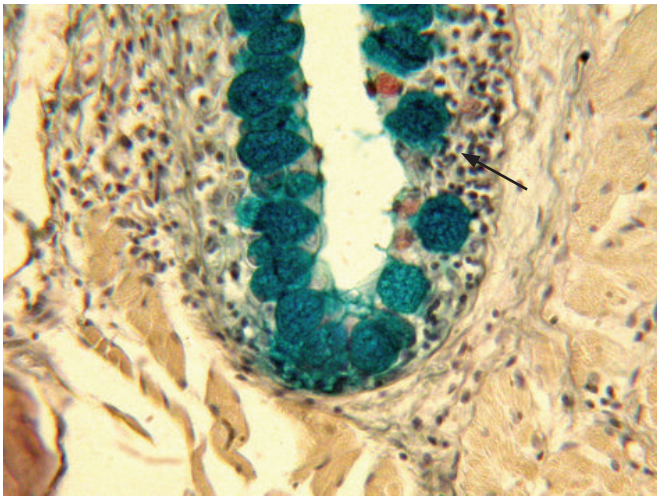


Fig. : 1.6 *Pelvicachromis pulcher* (AB-PAS-H / HM)

Pharynx. Portion of the pharyngeal epithelium showing at its centre an abundance of AB+ cells (in blue - mucous unicellular glands) amongst ordinary epithelial cells (arrow). This strong AB reaction emphasizes the presence of a lot of glycoconjugates which seem to play a part in food coating and epithelial lubrication.

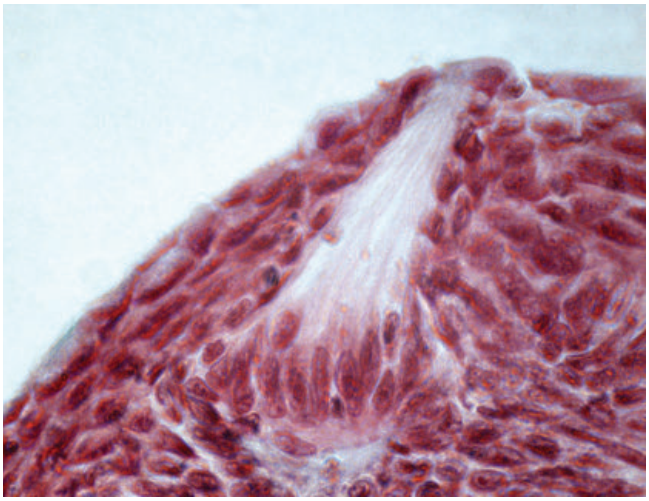


Fig. : 1.7 *Cyprinus carpio* (MT / HM)

In addition to ordinary cells, mucus-secreting cells and other cell types, intraepithelial structures composed of sensory cells and supporting cells may be located in various parts of the body (skin, barbels, fins, buccopharynx...). Such chemosensory receptor on a carp's gill raker (see chapter 10) is shown here at the centre.

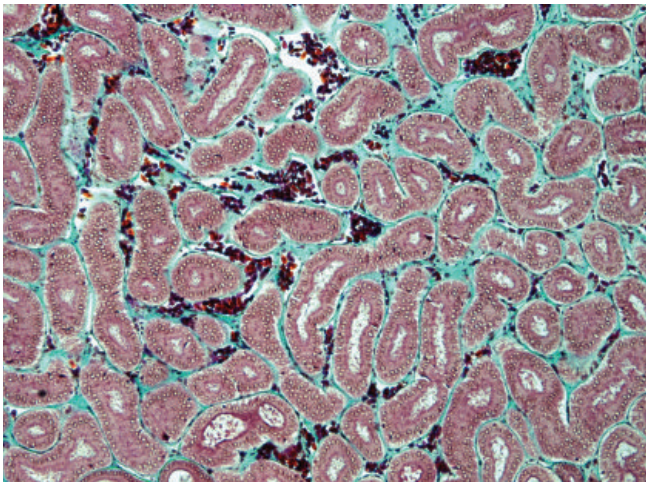


Fig. : 1.8 *Scyliorhinus canicula* (MT / MM)

Medium power view of tubular exocrine glands in cross section. Epithelia which are mainly involved in secretion often form structures called glands. This is shown here. The tubular sections, lined by a simple epithelium (pink) show a central unstained lumen. They are separated by connective tissue (in green) containing blood vessels (dark areas).

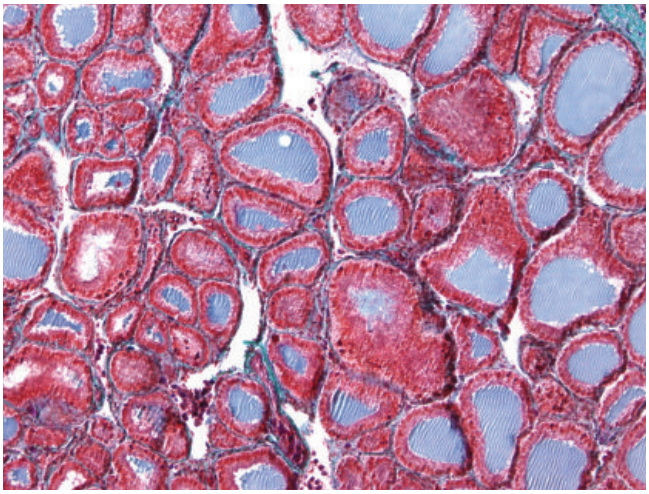


Fig. : 1.9 *Scyliorhinus canicula* (MT / MM)

The thyroid gland is a follicular endocrine gland composed of follicles which are spherical units storing hormones. The follicles are made up of a simple cuboidal epithelium (red) supported by a basement membrane. They are filled with a glycoprotein (sky blue) bound to the thyroid hormones. Ducts are lacking. The micrograph shows typical thyroid follicles of variable size.

In elasmobranchs the thyroid gland forms a discrete encapsulated organ whereas in most bony fishes the follicles are scattered (see chapter 13).

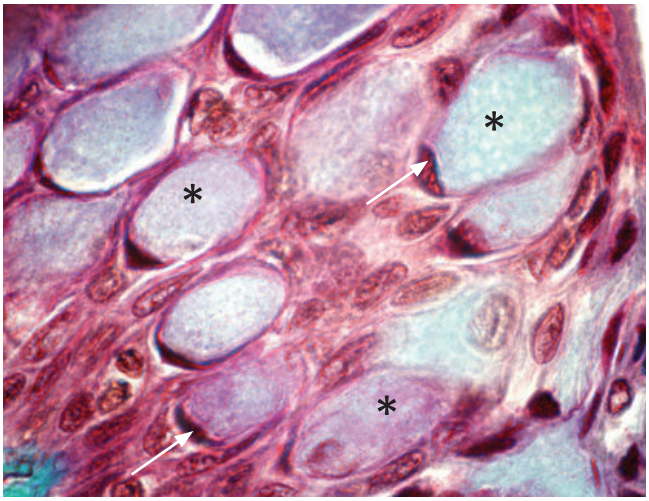


Fig. : 1.10 *Scyliorhinus canicula* (MT / IM)

Pharyngeal epithelium. In fishes the oral cavity and pharynx are lined by a stratified epithelium containing abundant mucus-secreting cells. The mucoid substance (∗) of the unicellular glands distends the apical portion of the cell and the highly condensed nucleus is moved to the narrow basal region of the cell (arrows). The other nuclei are those of the ordinary epithelial cells.

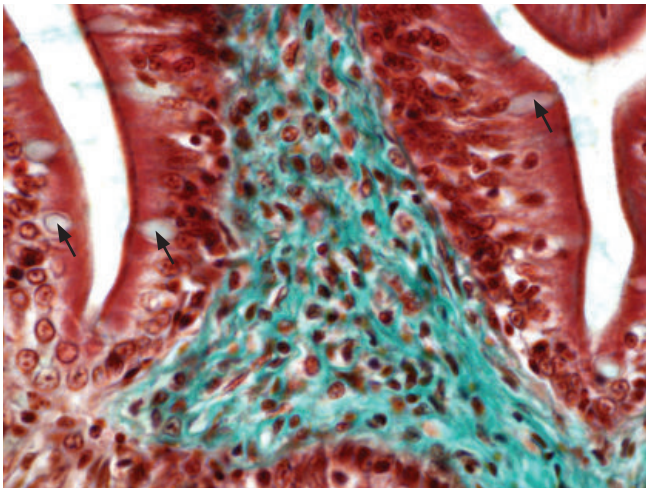


Fig. : 1.11 *Poecilia reticulata* (MT / MM)

Intestine. Collagenous tissue supports the epithelial linings of hollow organs. Such supporting tissue is illustrated in this micrograph. Fibrocytes and fibroblasts (at the center) lie in collagenous fibers (green). Fibrocytes are recognized by their condensed nuclei. Compare with the epithelial lining (red) composed of columnar cells and a few mucus-secreting cells (arrows).

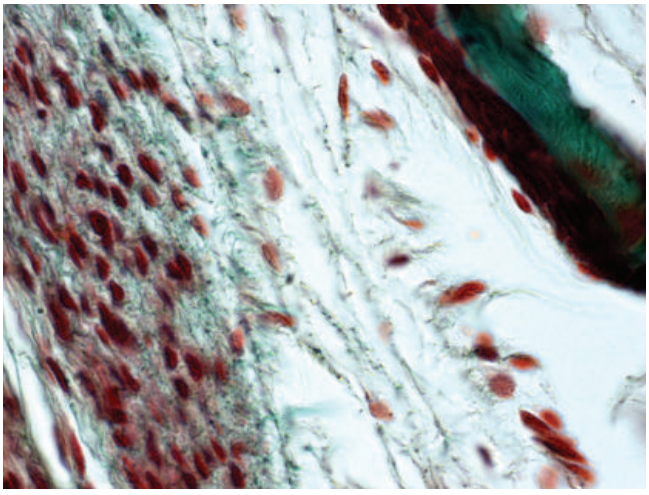


Fig. : 1.12 *Danio rerio* (MT / MM)

This micrograph illustrates loose (in the centre) and dense (left) connective tissue. In the former the open spaces between collagen fibers are filled with ground substance which is not stained in this type of preparation since it is dissolved during tissue processing. In the dense part the nuclei are clearly visible.

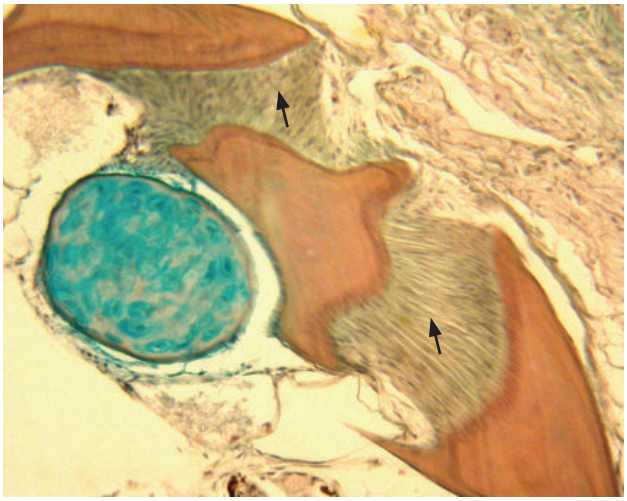


Fig. : 1.13 *Pelvicachromis pulcher* (AB-H-E / MM)

This micrograph shows portions of ligaments, composed of dense bands of parallel collagenous fibers (arrows). The three bone pieces (stained here in orange-brown) are maintained in the correct anatomical position by ligaments. Note, in turquoise, a piece of cartilage.

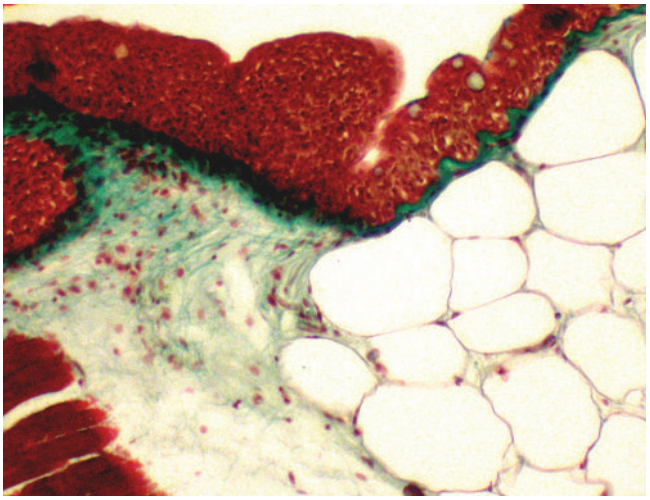


Fig. : 1.14 *Poecilia reticulata* (MT / MM)

Adipocytes are huge fat-storing cells which can be found in clumps or isolated in many supporting tissues. In adipose tissue they form the main cell type. In this micrograph, adipocytes are found in clumps (on the right - unstained) throughout loose connective tissue (green) supporting a stratified epithelium (red).

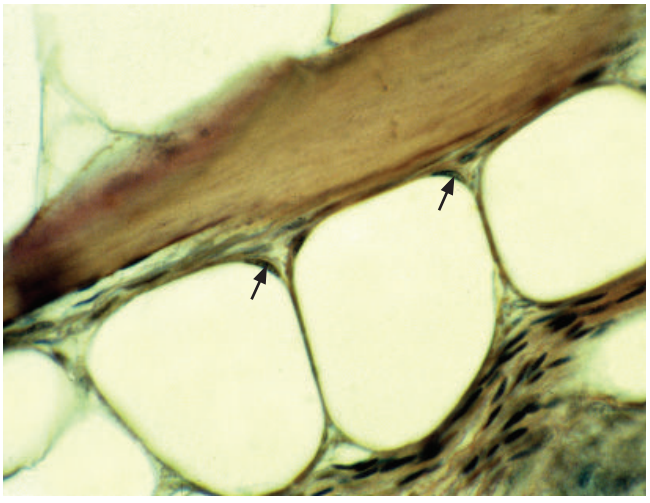


Fig. : 1.15 *Pelvicachromis pulcher* (AB-H / HM)

Three adipocytes seen at high magnification. Typically, little fat droplets stored in adipocytes conjugate to form one large drop. This latter fills up most of the cytoplasm, displacing the flattened nucleus at the periphery of the cell (arrows). As lipids are dissolved during the routine histological preparation, they appear unstained, as clearly seen on this micrograph.

2

Skeletal tissues

The skeleton is composed primarily of the skull, the vertebral column and the notochord, incomplete in the majority of fish. The vertebrae (Figs 2.1 to 2.4) are intermetameric and connected by elastic ligaments. They are composed of a vertebral body which encloses the notochord (except in chondrostei and dipnoi), neural arches (Figs 2.1 & 2.2) protecting the spinal cord and ventral arches (Fig. 2.1) around the caudal artery and vein. This axial endoskeleton comprises laterally spreading dorsal ribs and ventral ribs which surround and support the general cavity. Dermic fishbones whether or not attached to the vertebrae are found in the intermuscular connective. Fish have no sternum. The appendicular part of the skeleton includes pectoral and pelvic girdles with their related fin skeleton.

The fins are folds of skin supported by skeletal rays. *Lepidotrichia* (Figs 2.7 & 2.8) are bony segments of dermal (scale) origin located at the distal margin of osteichthyan fins. They originate from the base and extend distally, dichotomously branching towards the margin of the fin. Longitudinal sections show that *lepidotrichia* consist of a number of segments which are linked one to another by dense fibrous connective tissue. A cross section of the fin shows that each *lepidotrichium* comprises a series of opposing concave hemisegment pairs enclosing space for nerve bundles, loose connective tissue and blood vessels.

In the medial layer of hemisegments, collagen is arranged dorsoventrally, while the collagen in the lateral layer of hemisegments is oriented from the base of the fin distally, in a craniocaudal direction.

Each hemisegment is usually limited by an envelope of flattened cells that separates it from the surrounding tissues. It is either composed of an acellular matrix, or possesses very few cells.

Fin rays of cartilaginous fishes (Figs 2.5 & 2.6) are slender, unsegmented and horny, and are called *actinotrichia* (often erroneously called *ceratotrichia*).

Cartilage

Cartilage (Figs 2.9 to 2.17) is a firm, resistant tissue but is neither as hard nor as brittle as bone. It is found in all classes of vertebrates.

The resident cell of cartilage, the chondrocyte, is the main constituent cell of cartilage. It is isolated within a voluminous extracellular matrix which is neither vascularized nor innervated.

Chondrocytes, dispersed in cavities (*lacunae*), are metabolically active cells that have to synthesize and turnover a large matrix volume comprising collagens, glycoproteins, proteoglycans and hyaluronan.

Cartilage cells at the periphery of the piece are flattened in a plane parallel to the surface. Covering most elements of cartilage is a fibrous layer, the *perichondrium*, which comprises cells known as chondroblasts capable of forming a new cartilage matrix.

There are three main types of cartilage : hyaline, elastic and fibrous.

In hyaline cartilage the fibers are collagenous but the refractive indices of fibers and matrix are such that the fibers are invisible in ordinary histological preparations. In elastic cartilage the elastic fibers are visible in histological sections by means of a special stain (orcein). Fibrous cartilage contains a heavy meshwork of collagenous fibers which makes it very tough.

Fish have a variety of specialized cartilages that are somewhat different from those found in higher vertebrates; thus the classification of cartilages that modern texts of mammalian histology promote, is of relatively little value to

the comparative histologist who is confronted by a continuous *spectrum* of supporting tissues in fish. We have no intention of depicting in depth their full range. However, one type of supporting tissue is especially common and distinctive in teleosts and any classification must take it into account: we mean the hyaline-cell cartilage, also called pseudo-cartilage (Figs 2.13 to 2.15).

Hyaline-cell cartilage is typically an avascular tissue that consists of closely-packed cells with abundant, hyaline cytoplasm and little matrix. Its cellularity approaches that of an epithelium. When the cells are very large, the matrix may stain poorly. There is an enormous variation in cell size even within the same piece of tissue. There is no pericellular capsule and the matrix closely fills the gaps between cells. Some fish also have a chondroid covering the opercular bone, consisting of closely spaced round cells in a matrix with scattered fibers. This is considered a very primitive form of cartilage particularly present in the barbels of catfish and some Notothenioids. Another primitive cartilage can be found in the skeleton of Chondrichthyans and is transient in the gills of teleosts.

The entire endoskeleton of sharks, chimaeras and rays is cartilaginous, composed of chondrocytes in an extracellular matrix (ECM) surrounded by a fibrous *perichondrium*. The ECM may be mineralised (Figs 2.16 & 2.17), to varying degrees, with crystals of calcium phosphate hydroxyapatite. The majority of the skeleton is tessellated cartilage, comprised of a cortex of small blocks (*tesserae*) of calcified cartilage (both prismatic and globular) lying just beneath the fibrous *perichondrium* and overlying the uncalcified ECM.

Bone

Bone (Figs 2.18 to 2.28) is formed in all vertebrates except living agnathans (lampreys) and Chondrichthyes (sharks). It is a rigid calcified tissue of limited elasticity. Like cartilage, it is composed of a parenchymal cell, the osteocyte, embedded in a secreted acellular matrix. The osteocytes are present in "*lacunae*" in the matrix from which minute canaliculi (Fig. 2.20) radiate to form a complicated network connecting all the *lacunae* into a system of cavities.

Bone is not confined to the internal skeleton but is also found as hard plates or scales in the integument.

In addition to osteocytes, two other bone cells can be observed : osteoblasts and osteoclasts. It is accepted that osteocytes derive from osteoblasts (Figs 2.21 & 2.26) that have secreted the bone matrix around themselves. Osteoclasts (Figs 2.21 & 2.22) are large multinucleated cells (mononucleated in juveniles) with phagocytic properties. They dissolve the matrix by releasing lysosomial enzymes.

During bone remodelling, many osteocytes are released from their *lacunae* as osteoclasts actively reabsorb the bone. The relationships between bone, connective tissue and cartilage are more complex than in higher vertebrates. So, besides intramembranous ossification (when bone forms directly from mesenchyme) and endochondral (or intracartilaginous) ossification, we have to consider parachondral (Fig. 2.27) and perichondral (Fig. 2.28) ossification. In the former, bone forms in the connective tissue in proximity with a cartilaginous framework; in the latter, bone forms directly in the *perichondrium*.

In fish two types of bone tissues exist : cellular (Figs 2.18 to 2.20) and acellular (Figs 2.23 & 2.24) bones. The osseous endoskeletons of the higher orders of teleost fish (Perciformes) are noteworthy in that they are totally devoid of enclosed osteocytes (acellular bones). The osseous tissue of other fishes (*Clupeidae*, *Salmonidae*, *Cyprinidae*,…), like that of all other vertebrates, contains enclosed osteocytes, but their concentration is decidedly lower than that found in mammals.

In contrast to mammals, no hematopoietic tissue can be seen in the skeleton. Some of the bones of the cranium and gill arches contain spongy bone but, unlike mammals, this is not a site of hematopoiesis. Spongy bone (Figs 2.25 & 2.26) is laminated with spaces that give it a spongy-like appearance. Repair and remo-

delling of bone in teleosts involves osteocytes and osteoclasts. Bone reabsorption through the action of multinucleated osteoclasts plays an important role in skeletal development, in disease and healing in higher bony fish with cellular and acellular bone.

Notochord

When we survey the animal kingdom, we find that there are types of supporting tissue which can be described neither as cartilage nor as bone. The most primitive type of supporting tissue in chordates is that of the notochord (Figs 2.29 to 2.33). During development the notochord sheat or perichondral tube becomes completely invaded by bone and is built into a complex joined rod which allows for rigidity and flexibility.The notochord, of mesodermic origin, is normally surrounded by a thick inner fibrous sheath and a thin outer elastic one.

The notochord is persistent in some adult fish (chondrichtyans, dipnoi, chondrostei - Figs 2.31 to 2.33). However, in the majority of the species, it disappears partly, generally being strangled by the development of the vertebrae. Relics of notochord often persist, and are ensheathed by vertebral bone in the adult. (Figs 2.29 & 2.30).

The tissue of the notochord differs greatly from that of cartilage in histological appearance. It contains very tumescent cells lying closely pressed together. They resemble plant cells more than do most animal tissues. The notochord cells are thick-walled and their cytoplasm is filled with a homogeneous, semi-fluid content.

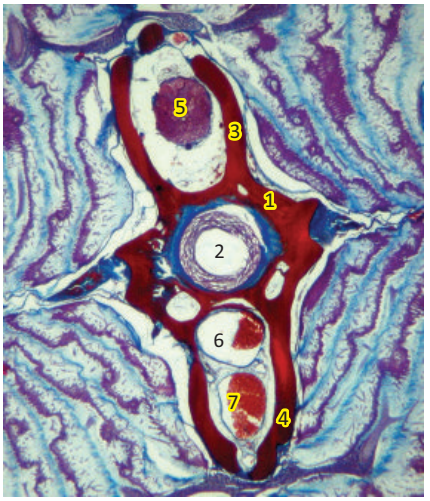


Fig. : 2.1 *Gnathonemus petersii* (MT / MM)

Cross section through the axial skeleton (caudal peduncle). The vertebra (red) bears processes above and below the *centrum* (1) which encloses remains of the notochord (2). These processes are the neural (3) and hemal (4) arches which protect respectively the spinal cord (5) and the dorsal artery (6) and vein (7), partially filled with blood. Note around the vertebra the electric organ (in blue and purple) characteristic of the mormyrids.

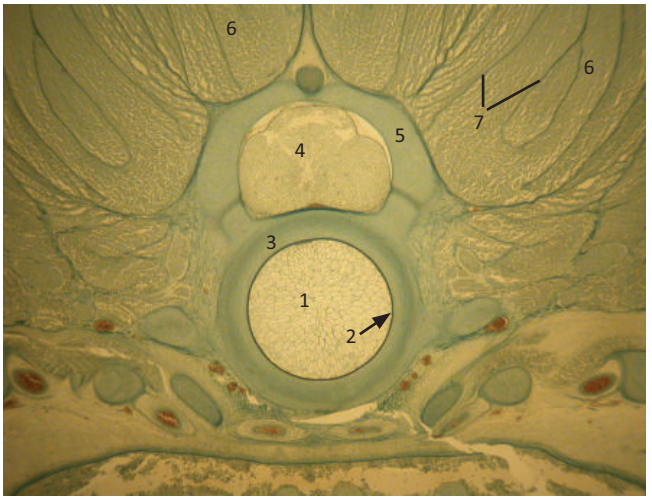


Fig. : 2.2 *Scyliorhinus canicula* (MT / MM)

Transverse section through the anterior trunk region (pharynx) of a young specimen. In Chondrichthyes endoskeletal tissues are mainly of two types : notochord and cartilage (no bone). Large vacuolated cells (1) fill the central core of the notochord, and together with the connective tissue sheath (2) give the notochord its characteristic flexibility. The vertebral *centrum* (3) encircles the notochord. Typically the spinal cord (4) is surrounded by the neural arches (5). The hemal processes are present only in the caudal region. Myomeres (6) separated by *myosepta* (7) are also demonstrated (see chapter 3). In reddish : blood vessels.

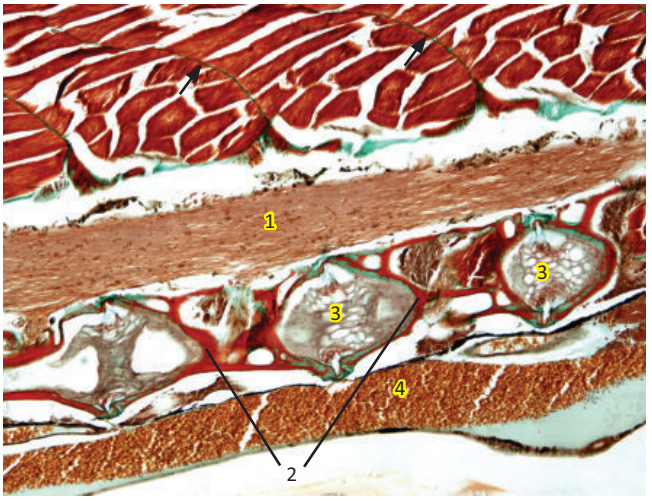


Fig. : 2.3 *Poecilia reticulata* (MT / MM)

Longitudinal section (close to the sagittal plane) through the median trunk region clearly showing the vertebral column lying between the spinal cord (1) and the dorsal aorta (4). Some vertebrae (2 - in red) surrounding remains of the notochord (3) are also visible. At the upper side of the picture note *fasciculi* of skeletal muscular fibers (myomeres - in red) separated by *myosepta* (arrows).

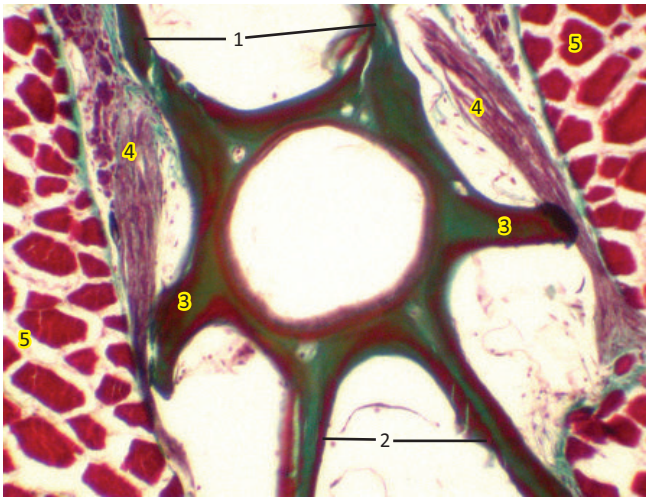


Fig. : 2.4 *Xiphophorus helleri* (MT / HM)

Transverse section of the caudal region. This micrograph shows different parts of a vertebra. (1) : neural arches; (2) : hemal arches; (3) : transverse processes. Nerves (4) of spinal ganglia as well as skeletal muscular tissue (5) are illustrated. In the vertebra differences of colors (green to red) are probably explained by variation in mineral deposits.

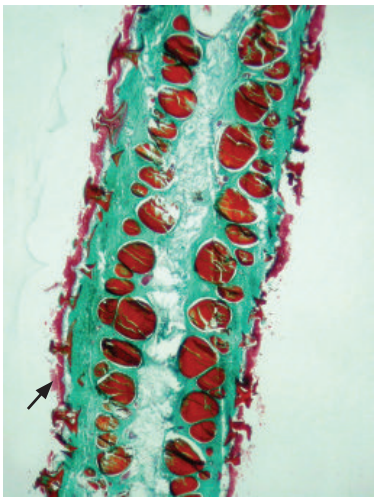


Fig. : 2.5 *Scyliorhinus canicula* (MT / LM)

Cross section of the caudal fin. Fin rays of elasmobranchs are slender, unsegmented, of a horny aspect and are called *actinotrichia* (sometimes called *ceratotrichia* - orange/red). These rays are disposed on both sides of the fins. Connective tissue, stained green, is covered by the epidermis (arrow).

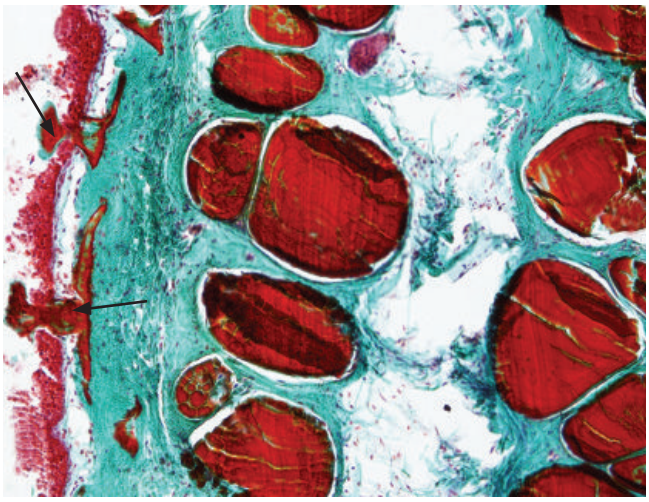


Fig. : 2.6 *Scyliorhinus canicula* (MT / MM)

Caudal fin. Higher magnification of the previous picture. *Actinotrichia* are long horny, acellular, cylindrical, flexible and unsegmented rays made up of elastoidin, supporting the fins of cartilaginous fish.

Two placoid scales (arrows) are seen at the left of the picture.

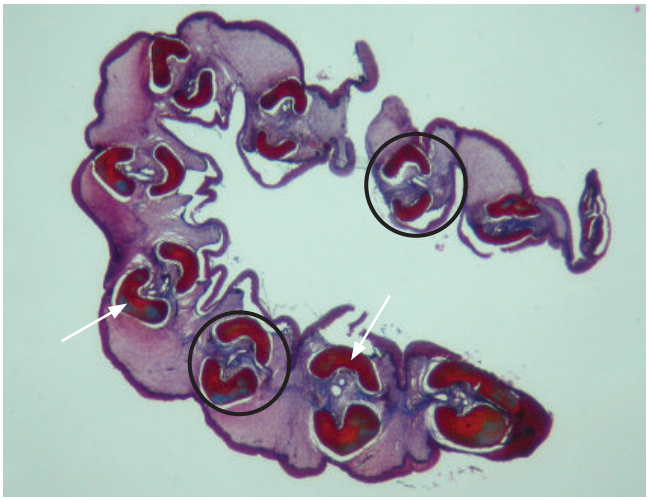


Fig. : 2.7 *Gnathonemus petersii* (MT / LM)

Cross section of the dorsal fin. In teleosts both the median and paired fins are provided with endoskeletal radials and with dermal fin-rays disposed on both sides of the fin: the bony *lepidotrichia* (circles). These have evolved from scales and each of them is formed by two *hemitrichia* (arrows) closely joined except at their base. *Lepidotrichia* can be either soft or hard and segmented.

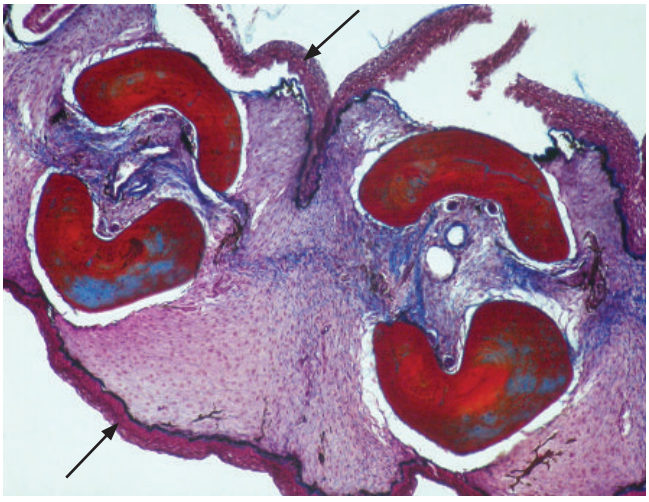


Fig. : 2.8 *Gnathonemus petersii* (MT / MM)

Dorsal fin. Each *lepidotrichium* is composed of two parallel rod-like structures, the *hemitrichia*, that appear as bowed strips. Segmented and often branched they are disposed bilaterally beneath the skin and resembling a pair of parenthesis. Each hemisegment presents a lateral convex surface and an inner concave surface that encircles a region containing loose connective tissue (collagen - in blue), nerve bundles and blood vessels (unstained spaces). Note the stratified epithelium (arrows) of the skin around the fin.

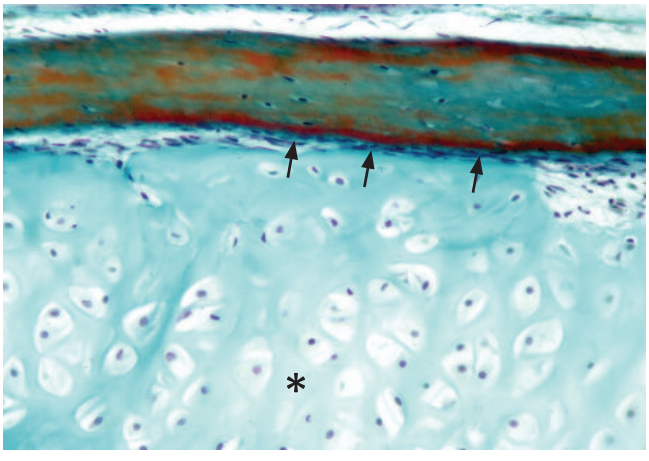


Fig. : 2.9 *Polypterus senegalus* (MT / MM)

Hyaline cartilage takes up the largest part of this micrograph. Cartilage is a semi-rigid form of skeletal tissue which contains neither blood vessels nor nerves. Chondrocytes (whitish with blue nuclei) are mature cartilage cells scattered in an abundant extracellular matrix. This one (∗) contains collagenous fibers (not visible) and a predominant ground substance. The *lamella* of cartilage shown here is lined by a thin *perichondrium* (arrows - see also Fig. 2.11) and covered by perichondral bone (orange/green).

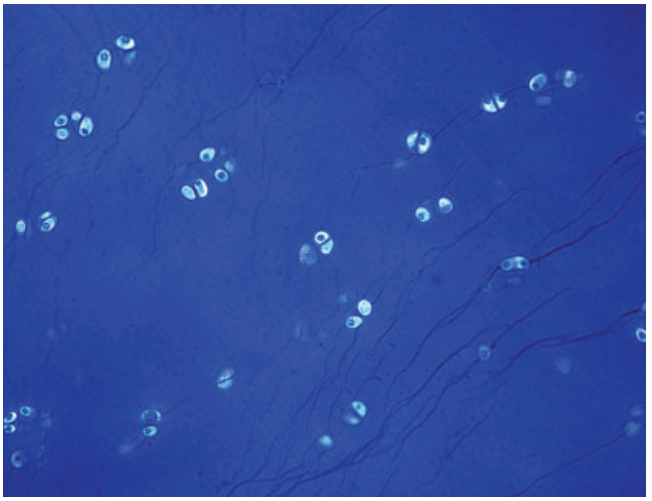


Fig. : 2.10 *Scyliorhinus canicula* (WR / MM)

Cartilage. Chondrocytes are housed in spaces, known as *lacunae*. Although the matrix contains many collagen fibrils, they are masked by the proteoglycans of the ground substance; hence the matrix (deep blue) appears homogeneous and smooth. The chondrocytes one can observe here are particularly sparse and mostly paired.

Striations in the extracellular matrix are artefacts.

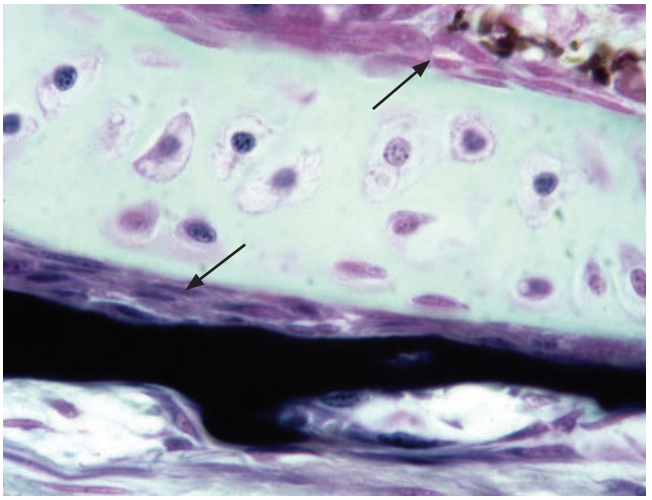


Fig. : 2.11 *Gnathonemus petersii* (MT / HM)

Hyaline cartilage. Covering most pieces of cartilage (pale green with well-marked chondrocyte nuclei) is a condensed fibrous layer, the *perichondrium* (arrows). This layer contains cells known as chondroblasts which are capable of forming new cartilage matrix. Bone (in black) is forming in the periphery of the cartilaginous piece.

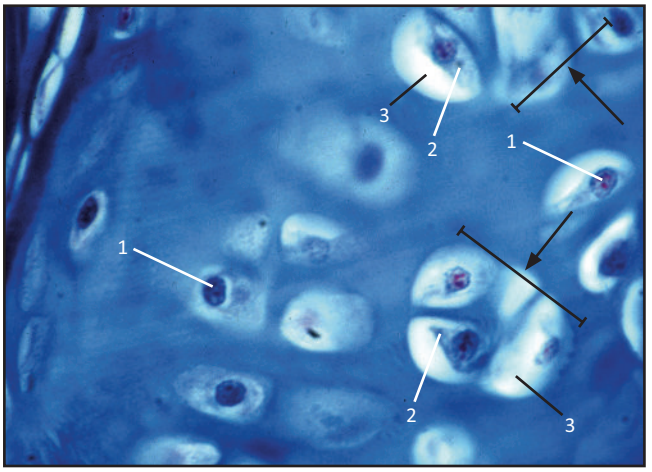


Fig. : 2.12 *Garra congoensis* (MT / HM)

Hyaline cartilage. This type of cartilage is similar as in mammals. Histological fixation may bring the chondrocytes to shrink in their *lacunae*, so they appear not to completely occupy their spaces in the matrix. This allows to distinguish clearly the nuclei (1), the cytoplasms (2) and the *lacunae* (3) of some chondrocytes in this image.

The chondrocytes frequently occur in groups, gathered together into what are called «cell nests» (arrows). Note the matrix (deep blue) and its apparent homogeneity.

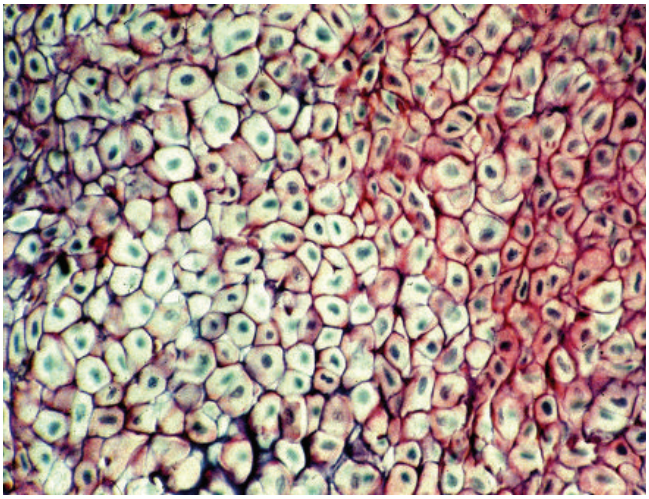


Fig. : 2.13 *Pelvicachromis pulcher* (MT / MM)

Hyaline-cell cartilage is typically an avascular tissue that contains tightly-packed cells with abundant cytoplasm. Its cellularity approaches that of an epithelium. The matrix is poorly developed and close to the plasma membranes.

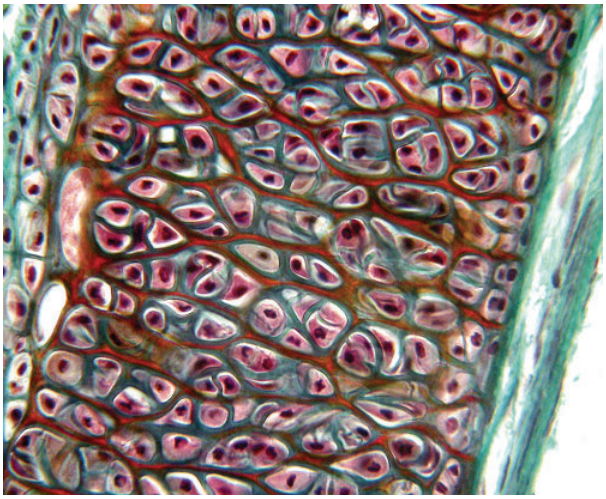


Fig. : 2.14 *Corydoras paleatus* (MT / MM)

This micrograph illustrates hyaline-cell cartilage containing close-packed cells (chondrocytes - pink with dark nuclei). Compared to the previous image the clusters of cells are better delimited and although scarce, the extracellular matrix (orange and blue) is more visible.

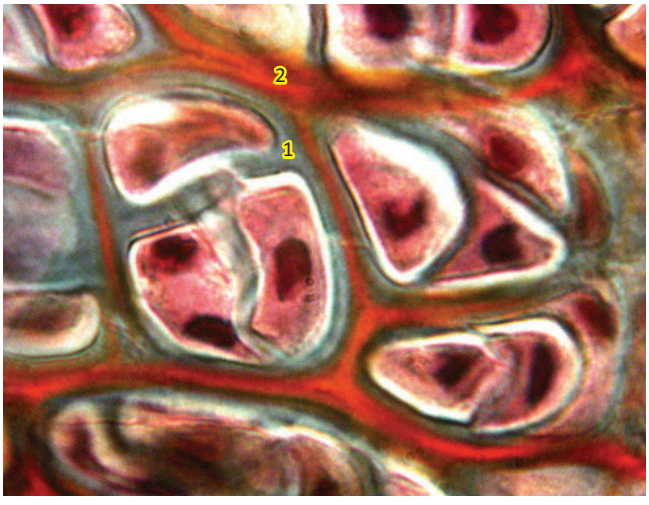


Fig. : 2.15 *Corydoras paleatus* (MT / HM)

This micrograph is a higher magnification of the previous picture. The chondrocytes are closely packed and their cytoplasm (pink) is well preserved. The matrix is stained grey within the nests (1) and orange around them (2). This typically non-vascularized type of cartilage is seen among others in the barbels of catfishes.

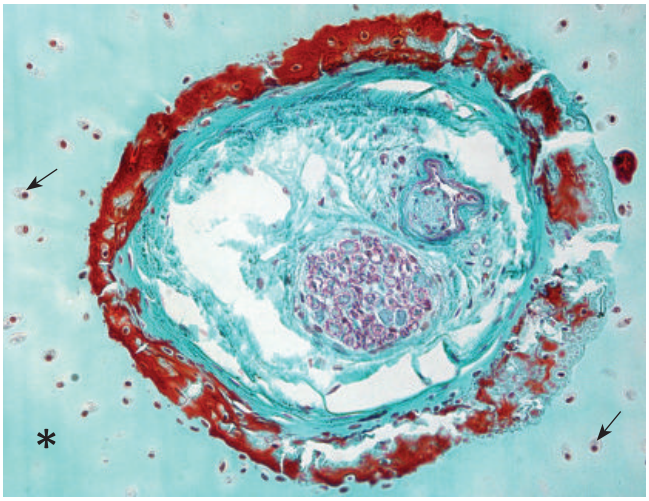


Fig. : 2.16 *Scyliorhinus canicula* (MT / MM)

Calcified cartilage. Cartilage is widespread as endoskeletal tissue in elasmobranchs. It shows the typical form of hyaline cartilage, with chondrocytes (arrows) in *lacunae* embedded in an extensive dense matrix (∗). In addition, deposits of small blocks (*tesserae*) of calcified cartilage (orange/red) in uncalcified matrix can also occur at the periphery of some skeletal pieces (here, skull). A nerve (pink) in cross section is seen in the centre of the picture. It is surrounded by connective tissue.

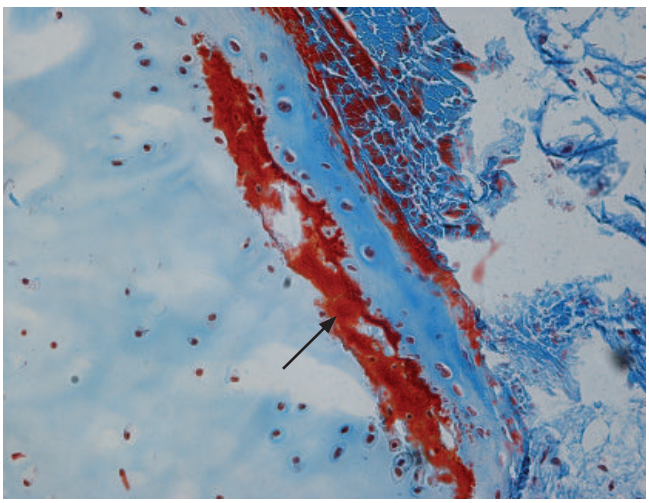


Fig. : 2.17 *Scyliorhinus canicula* (MT / MM)

Calcified cartilage of the «tongue». The tessellated cartilage comprises a cortex of small blocks *(tesserae*) of calcified cartilage (arrow) overlying the uncalcified matrix (pale blue on the left) containing chondrocytes. At the right, blue stained collagen.

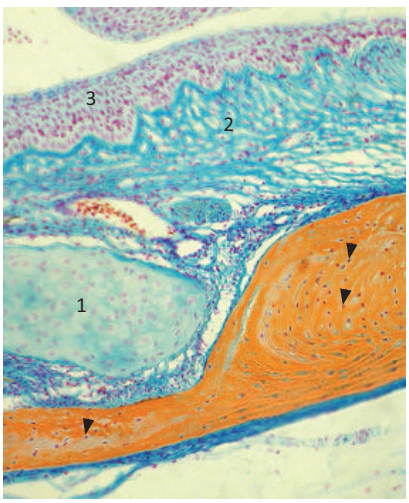


Fig. : 2.18 *Gnathonemus petersii* (FR-HB / LM)

Cellular bone (orange) and cartilage (1) are side by side within connective tissue (2) supporting a stratified epithelium (3). Numerous osteocytes (the cells responsible for maintenance of bone matrix) with densely stained nuclei are visible (arrowheads).

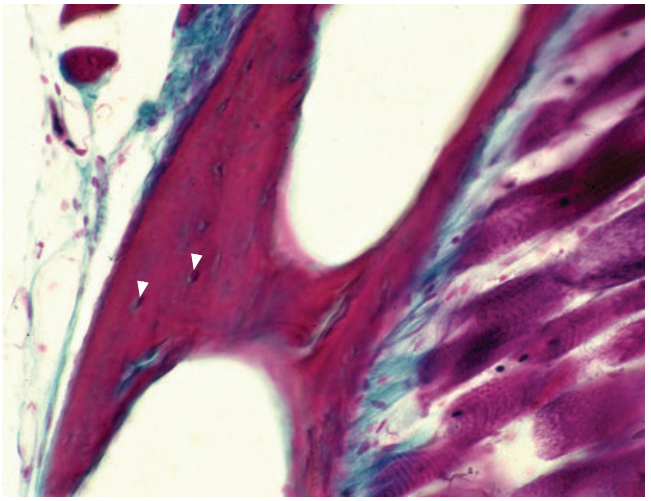


Fig. : 2.19 *Gnathonemus petersii* (MT / MM)

Cellular bone (dark-red, H-shaped) is seen in this elephant nose fish lower jaw element. Some small osteocytes are identified (arrowheads). Note *fasciculi* of skeletal muscle at the right of the picture and the blue stained collagen.

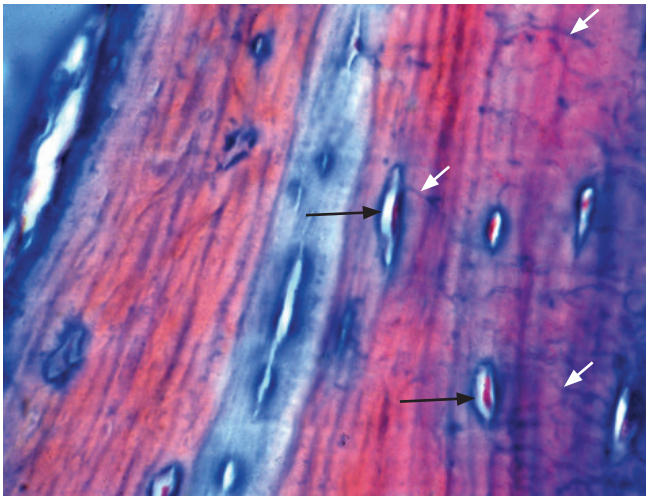


Fig. : 2.20 *Garra congoensis* (MT / HM)

Cellular bone. It is generally accepted that fish bones may be either cellular or acellular. This micrograph shows cellular bone containing a relatively great number of osteocytes (black arrows). Note numerous minute interconnecting *canaliculi* (white arrows) which contain fine cytoplasmic extensions of the osteocytes. The mineralized matrix is stained red.

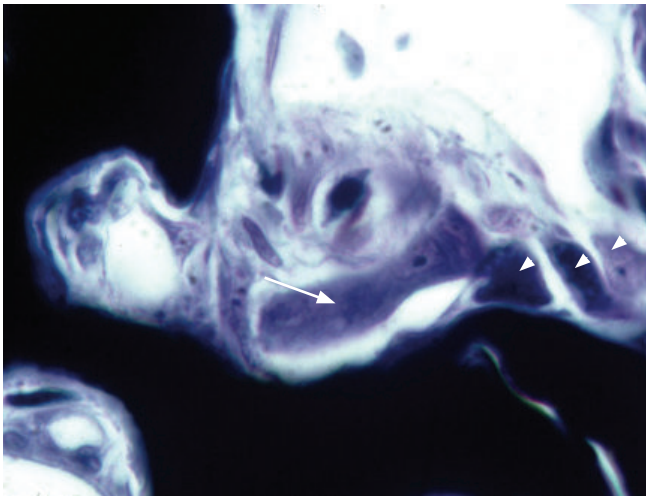


Fig. : 2.21 *Polypterus senegalus* (MT / IM)

In addition to the osteocytes, the two other bone cell types are osteoblasts and osteoclasts. Osteoblasts (arrowheads) are rather large and often plump cells, arranged in one or more layers along bony surfaces (in black). They are very actively synthetic, making collagen and proteoglycans. Their intense cytoplasmic basophilia indicates a high activity. Osteoclast (arrow) is a very large, multinucleate cell, closely applied to the surface of a bony spicule and often lying in a depression.

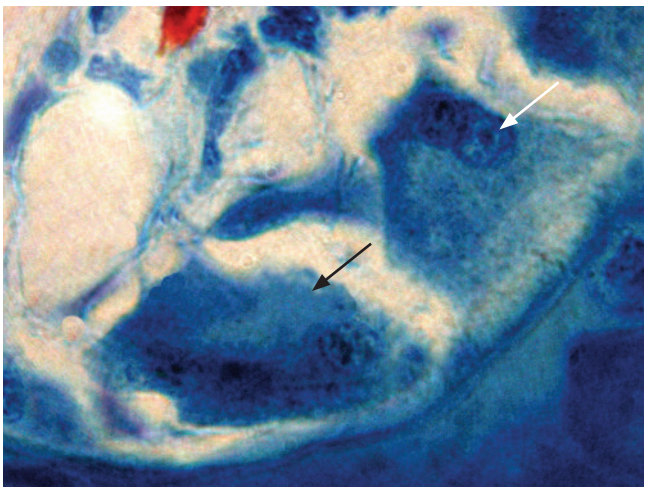


Fig. : 2.22 *Garra congoensis* (MT / IM)

Osteoclasts. This micrograph shows typical bone resorption by two multinucleate osteoclasts (arrows) which are actively involved in continuous bone remodelling. Their apical surfaces bear fine *microvilli* forming a brush border.

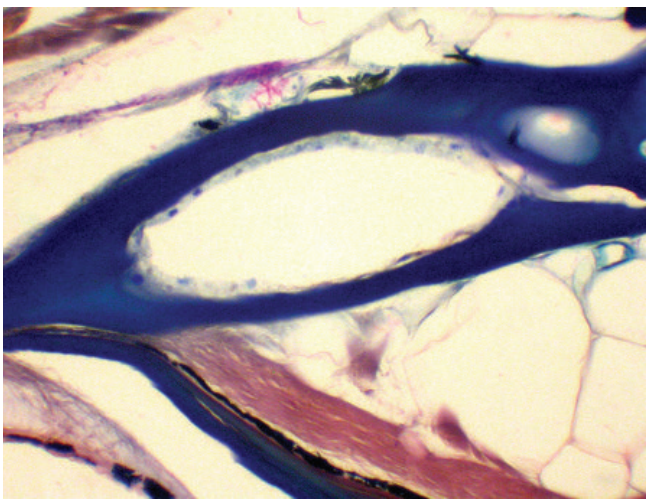


Fig. : 2.23 *Pelvicachromis pulcher* (MT / MM)

Acellular bone. The osseous endoskeleton of higher teleosts (Perciformes...) is devoid of osteocytes. This micrograph shows the typical appearance of such acellular bone (dark blue) surrounding a canal. The unstained cells at the right bottom corner are adipocytes.

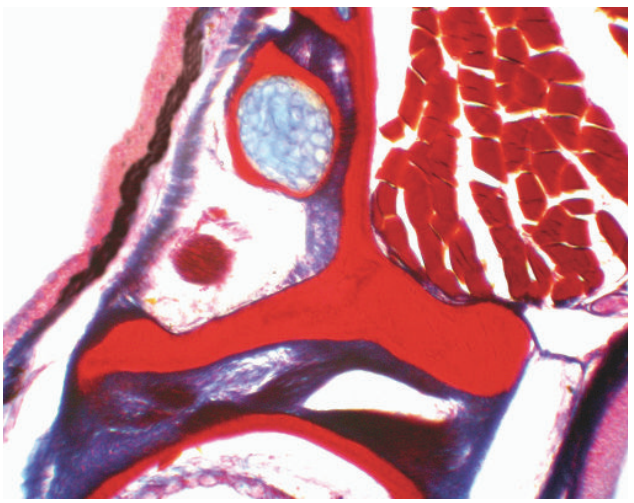


Fig. : 2.24 *Astatotilapia burtoni* (AZ / MM)

Fishes of the *Cichlidae* family, mainly native to Africa and South-America belong to the Perciformes. They are characterized by spiny fins and acellular bones. The azan trichrome stained this unique bone type in red at the centre of the picture.

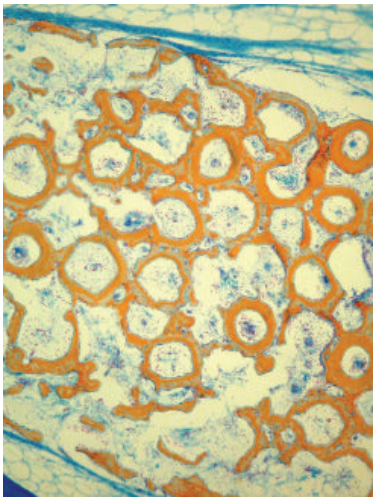


Fig. : 2.25 *Gnathonemus petersii* (FR-HB / LM)

Spongy bone. Low power view of anastomosing spicules of bone (orange). As more and more spicules form in the same vicinity, they will become interconnected. As they fuse with each other, they form cancellous (spongy) bone, but in fish unlike mammals this is not a site of hematopoiesis.

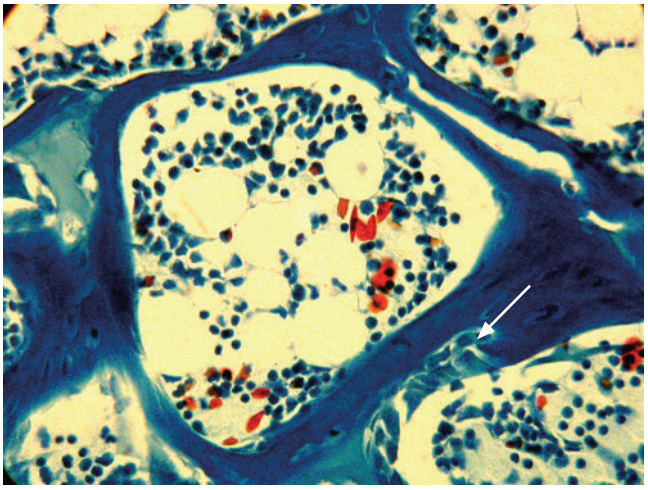


Fig. : 2.26 *Garra congoensis* (MT / MM)

Lower jaw cancellous bone. This micrograph illustrates centers of ossification in vascularized areas of connective tissue (blood cells are stained orange and blue) . The anastomosing spicules (blue) are obvious. As usual in routine sections, adipocytes are unstained.

The arrow points to a layer of osteoblasts.

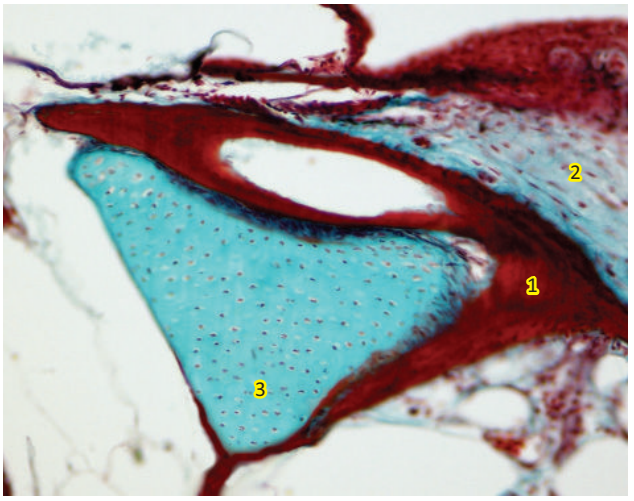


Fig. : 2.27 *Poecilia reticulata* (MT / MM)

Parachondral ossification. Fish osteogenesis is very complex. In this type of ossification bone (1) forms in the connective tissue (2) in proximity of a cartilaginous piece (3) harbouring numerous chondrocytes.

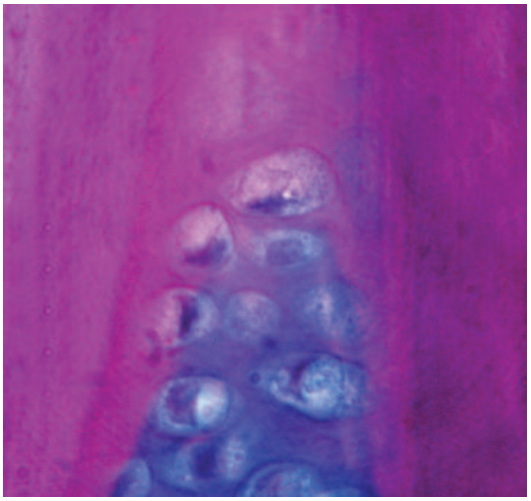


Fig. : 2.28 *Xiphophorus helleri* (AB-PAS / HM)

In the perichondral ossification bone (magenta) forms directly in the *perichondrium*. Cartilage (blue) containing chondrocytes will be gradually invaded by bone *trabeculae*.

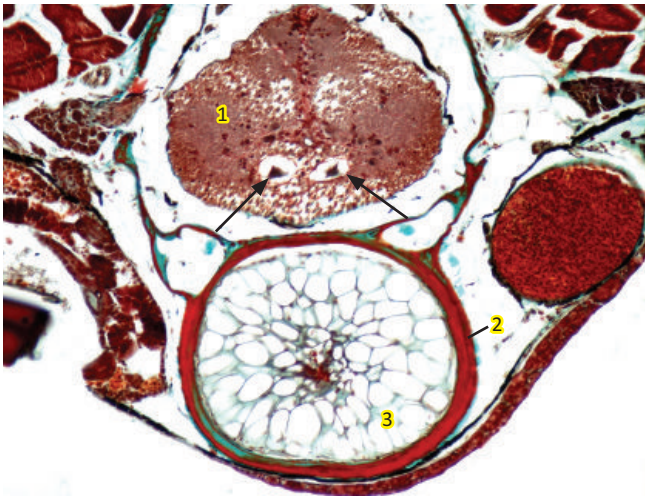


Fig. : 2.29 *Poecilia reticulata* (MT / MM)

Notochord. This transverse section through the anterior trunk region shows the position of the spinal cord (1) and of a vertebra whose centre (2) surrounds the notochord (large unstained vacuolized cells - 3). The notochord is a rather flexible rod partially persisting throughout life in lower vertebrates. Note in the spinal cord the giant axons (arrows) of the paired MAUTHNER's neurons (see chapter 12).

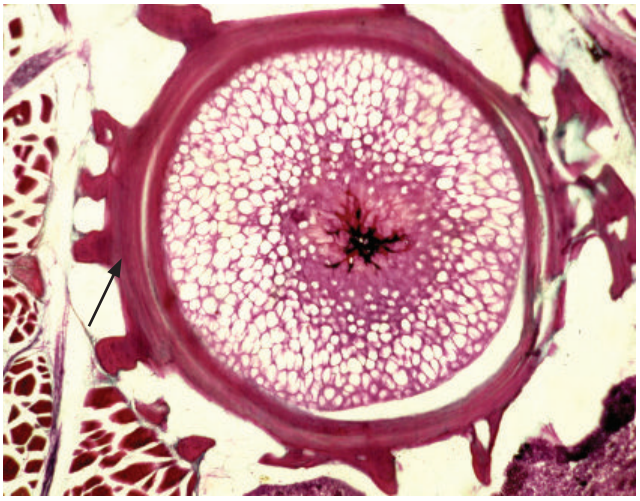


Fig. : 2.30 *Gnathonemus petersii* (H-E / MM)

Notochord. Transverse section through the notochord surrounded by vertebral body of the vertebra (*centrum* - arrow) with several bony processes. The notochord contains very tumescent cells lying closely pressed together.

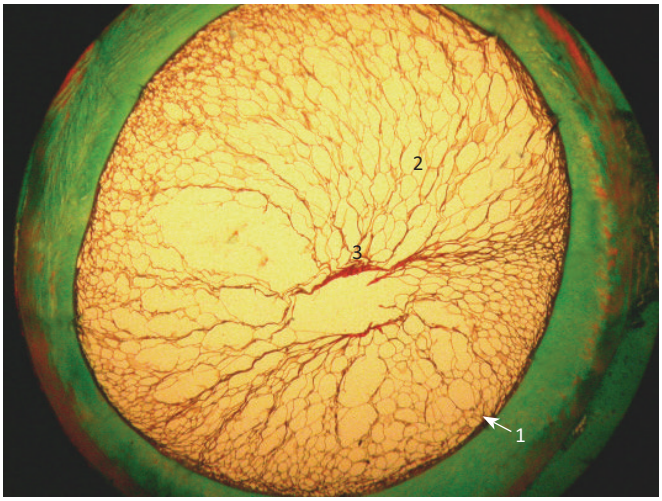


Fig. : 2.31 *Acipenser gueldenstaedtii* (MT / MM)

Transverse section through the notochord, persistent in this fish group (*Chondrostei*). The notochord sheath (in green) is composed of fibrous tissue. No centra are formed in living sturgeons. Turgidity of the individual cells of the notochord is obvious. Under the sheath three areas are found: a simple squamous or cuboidal layer (1), several layers of large vacuolized cells (2) and in the centre, cells (3) surrounding *lacunae*.

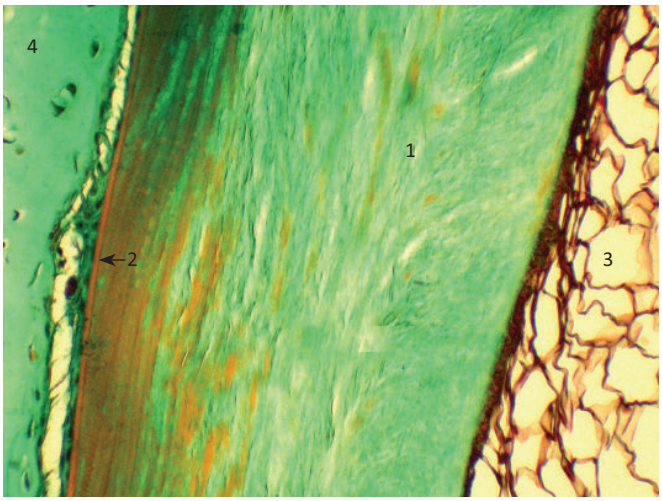


Fig. : 2.32 *Acipenser gueldenstaedtii* (MT / HM)

Transverse section of the notochord sheath. This perichordal tube is composed of a thick fibrous inner layer (1) and a thin outer elastic one (2). The notochord sheath surrounds vacuolized cells (3). Note the presence of hyaline cartilage at the left (4), very abundant in this fish skeleton.

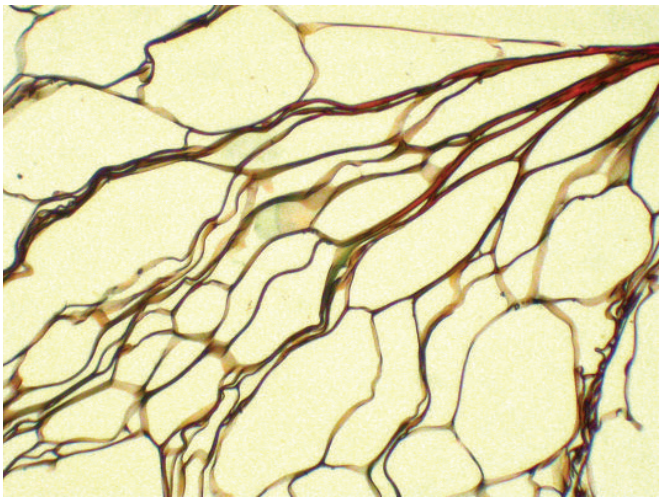


Fig. : 2.33 *Acipenser gueldenstaedtii* (MT / HM)

Notochord cells (higher magnification of area 3 of Fig. 2.32). The cells lie closely pressed together and the tissue resembles that of plants more than do most animal tissues. Thick-walled cells are well demonstrated.

3
Muscular tissue

In fish and other vertebrates muscular tissues are present in three principal areas of the body : the skeletal muscles, the heart, and the walls of hollow organs. Microscopically, longitudinal sections of skeletal and cardiac muscular tissue reveal that their cells (myofibers) have characteristic cross-striations, whereas the muscular tissue of hollow organs is composed of myofibers without cross-striations and hence has a smooth appearance. Therefore, three basic types of muscle cells are recognized : 1) striated skeletal myofibers (rhabdomyocytes), which comprise the skeletal muscles that originate at and insert on the bones of the skeleton, 2) striated cardiomyocytes, which comprise the walls of the heart, and 3) smooth myofibers (leiomyocytes), which form the contractile portion of the walls of most viscera.

Skeletal muscle

Skeletal muscle fibers (rhabdomyocytes) (Figs 3.1 to 3.10) are multinucleated *syncitia*. The oval nuclei are usually found at the periphery of the cell under the plasma membrane, called sarcolemma. Under the microscope, at higher magnification, we can see long thread-like myofibrils which run parallel to each other for the whole length of the fiber. The rhabdomyocytes that are sectioned transversally show a polyhedral appearance and peripheral nuclei. The spaces between individual myocytes are filled with loose connective tissue containing capillaries.

When viewed in longitudinal section (Figs 3.4 to 3.6), rhabdomyocytes show transverse striations of alternating light (I) and dark (A) bands, with an amazing degree of evenness and regularity. Each I band is bisected by a dark transverse line, the Z line. The two most distinctly different skeletal muscle types – red and white – have different degrees of vascularization and myoglobin content, which account for their color (Figs 3.9 & 3.10).

The white (fast) fibers constitute the bulk of the muscle mass, making up the deep skeletal muscles in the epaxial (dorsal) and hypaxial (ventral) myomeres. These light muscles are relatively poorly perfused but are considered useful in short, strong bursts of swimming during which they fatigue rapidly. The term myomere is commonly used in fish anatomy and refers to a separate muscle bundle, with parallel fibers running along the long axis of the body. Myomeres are connected by delicate connective tissue *septa* (*myosepta* - Fig. 3.1). There is an epaxial and a hypaxial myomere for each vertebra in most fishes.

Red muscle is generally found superficial to the epaxial and hypaxial white skeletal muscles of fish. The slow red fibers have a higher lipid content than white fibers and more mitochondria per cell. These narrower muscle cells are also supported by a better vascular supply than white muscle. Red muscle cells are usually most prominent in the areas underlying the fins of major propulsion. In salmonids, this is under the lateral line in a configuration to move the tail. In perch and other species that utilize their pectoral fins for locomotion, red muscle cells are abundant in the areas under these fins. In seahorses this is the case under the dorsal fin.

Let's also illustrate other muscles like those coordinating the movements of the fins, the mouth, the spiracle (Fig. 3.2), the eyes (Fig. 3.3), or the gills…

Sound production in many fish species, e.g., the *Carapidae*, results from the action of extrinsic muscles that insert into the air bladder. Sonic fibers (Fig. 3.11) are thicker than red and thinner than white epaxial fibers, and sonic fibers and myofibrils exhibit an unusual helicoidal organization : the myofibrils of the *center* are in straight line whereas they are more and more twisted towards the periphery.

Whereas the number of muscle fibers is fixed around the time of birth in mammals, in many species of fish, muscle *fiber* numbers increase throughout a large part of their life.

Cardiac muscle

Cardiac muscle (Figs 3.12 to 3.14) consists of a great *syncytium* of anastomosing fibers (cardiomyocytes) that lie parallel to each other. At sites of end-to-end contact are the intercalated discs (junctional complexes), structures found only in cardiac muscle.

Mature cardiomyocytes exhibit a cross-striated binding pattern identical to that of skeletal muscle. Unlike multinucleate skeletal muscle (see above), however, each cardiac muscle cell possesses only one, rarely two centrally located pale-staining nuclei. Surrounding the cells is a delicate sheath of endomysial connective tissue containing a rich capillary network.

Working cardiomyocytes from fish hearts are smaller than their mammalian counterparts. The sarcoplasmic *reticulum* is frequently reduced, sometimes to nothing more than peripheral *caveolae*, T tubules are also reduced. Cardiomyocytes are capable of regeneration, even in older individuals. They show both hyperplastic and hypertrophic growth in contrast to post-embryonic mammals where the growth is hypertrophic only.

Fish inhabiting environments with fluctuating temperatures have an amazing cardiac plasticity and capacity to maintain maximum cardiac output across a broad temperature range.

The specially modified conduction pathways (PURKINJE fibers) found in higher vertebrates seem to be lacking in fish hearts. However, rare cardiac pacemaker cells, sometimes seen by chance in histological sections, near the sinoatrial *ostium* (usually) or elsewhere in the *sinus* initiate the heart beat. This signal is then conducted into the *atrium* and produces the atrial contraction. Heart rate is mainly influenced by vagal inhibition and temperature.

Smooth muscle

Smooth muscle (Figs 3.15 to 3.18) forms a large part of the walls of the alimentary tract, of the arteries and veins, of ducts of various glands and is found in a variety of other locations.

The cells (or fibers or leiomyocytes) of smooth muscle are elongated, spindle-shaped structures, each with a centrally located nucleus (Fig. 3.18). They are enclosed by a basal *lamina* and a network of reticular fibers. The leiomyocytes are placed close together, forming layers or sheets, although single cells may be formed. The cell contains delicate contractile myofilaments, hard to see with the light microscope. In many tubular visceral structures, such as the gastrointestinal tract, smooth muscle is disposed in layers with the cells of one layer arranged at right angles to those of the adjacent layer. This arrangement permits a wave of contraction to pass down the tube, propelling the contents forward; this action is called peristalsis. When cut in transverse section (Figs 3.16 & 3.17), the central nuclei may appear absent in several sections due to sectional angle.

The contraction process of smooth muscle is slow and not subject to voluntary control.

Fig. : 3.1 *Scyliorhinus canicula* (H / MM)

Epaxial muscles. Transverse section through the axial trunk musculature. In fish this musculature is divided into a series of myomeres (here in grey and taking up 90 % of the picture) separated by collagenous sheets called *myosepta* (in blue - arrows).

In longitudinal section, these myomeres are V-shaped (see introduction Fig. A).

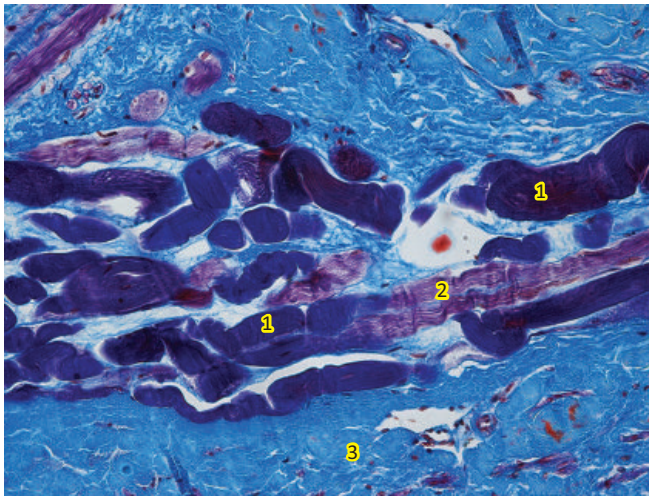


Fig. : 3.2 *Scyliorhinus canicula* (MT / MM)

Spiracular muscle. In addition to the well-developed trunk and caudal musculature, fish have other small voluntary muscles like extra-ocular, fin muscles or the spiracular muscle shown here. Opening/closing movements of the spiracle are controlled by the first dorsal constrictor muscle which regulates water entry into the pharynx. Portion of the apparent wavy course of this skeletal muscle is illustrated with more (dark blue - 1) or less (purple - 2) contracted areas. In blue, the connective tissue (collagenous fibers - 3).

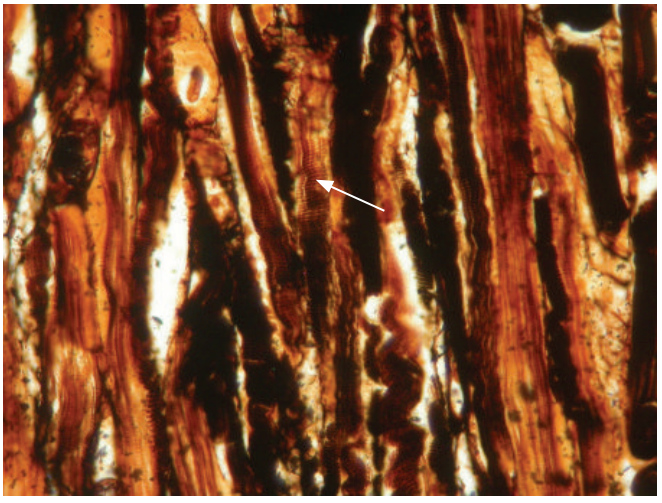


Fig. : 3.3 *Scyliorhinus canicula* (TI-Ag / MM)

Ocular muscle. Longitudinal section of some superior oblique rhabdomyocytes. This staining method allows to see the regular cross-striations especially at the centre of the image (arrow). These striations are composed of alternating dark A bands and light I bands.

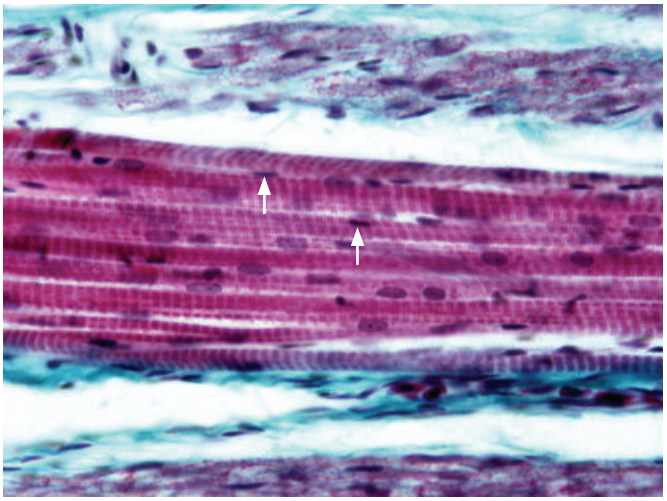


Fig. : 3.4 *Gnathonemus petersii* (MT / HM)

Skeletal muscle. This micrograph illustrates some characteristic histological features of skeletal muscle fibers cut longitudinally. About ten unbranched and elongated fibers are shown at the centre of the micrograph. Rhabdomyocytes are multinucleate cells whose flattened nuclei (arrows) are located at the periphery, just beneath the plasma membrane (*sarcolemma*). Regular cross-striations (A and I bands) are characteristic. The muscle fibers are grouped together into bundles (*fasciculi*) surrounded by loose collagenous tissue (*perimysium* - in turquoise).

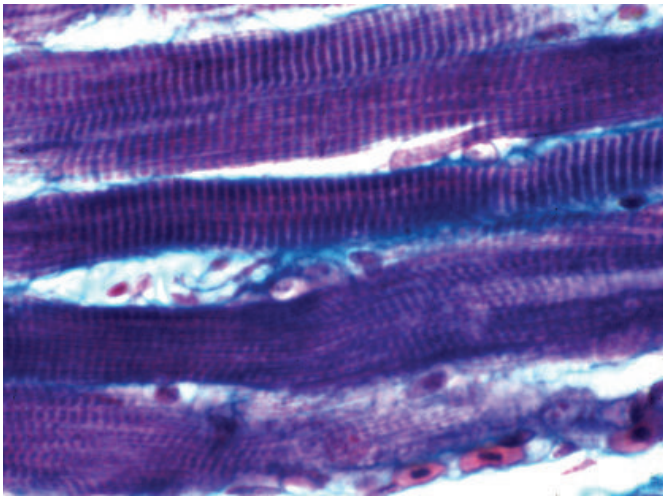


Fig. : 3.5 *Pangasius micronemus* (MT / HM)

Skeletal muscle in longitudinal section. Within the rhabdomyocytes, there are longitudinally running myofibrils. Each myofibril is made up of repeating structural units called sarcomeres (between two Z bands). Since the sarcomeres of all myofibrils in a single cell are in register, the result is a typical cross-striation clearly visible at high magnification with the light microscope. The striations of the rhabdomyocytes are composed of alternating light I bands and dark A bands, obvious on top of the image. Note the collagen in blue.

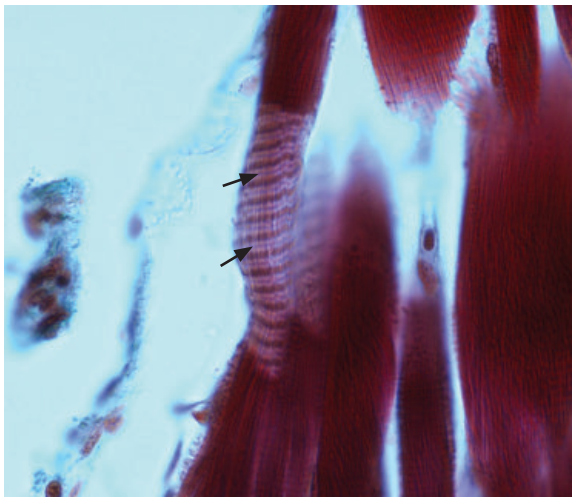


Fig. : 3.6 *Danio rerio* (MT / HM)

Portions of four skeletal myofibers (branchial rhabdomyocytes) in longitudinal section. Cross striations are evident in the centre of the picture. Thanks to the cutting angle and the low degree of contraction thin Z bands (arrows) are visible at the centre of the light I bands.

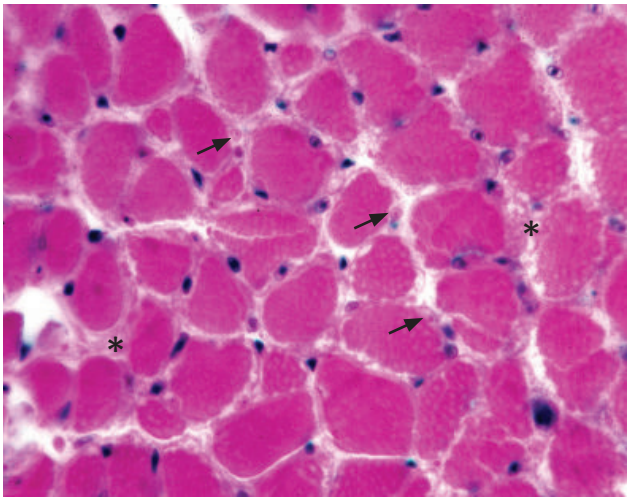


Fig. : 3.7 *Stomatorhinus puncticulatus* (MT / MM)

This micrograph demonstrates the typical appearance of skeletal muscle cut in transverse section and shows the peripheral location of the nuclei (pinkish - arrows). Don't confuse with the dark nuclei of the fibroblasts or red blood cells. Note the *endomysium* (loose connective tissue - ∗) containing numerous capillaries between the polyhedral cells (rhabdomyocytes).

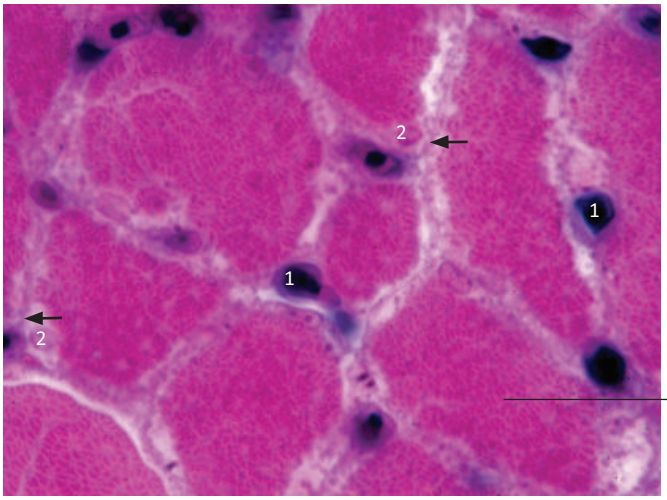
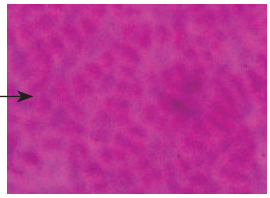


Fig. : 3.8 *Stomatorhinus puncticulatus* (MT / HM)

Higher magnification of the previous document. In fixed tissue preparations such as this, the rhabdomyocytes appear irregular and polyhedral. Each cell is separated by endomysial spaces of connective tissue containing capillaries with red blood cells (1). Some nuclei are seen (2) and are peripherally placed under the sarcolemma (arrows). Striations are only visible in longitudinal sections.

In this preparation, the sectioned rhabdomyocytes appear speckled with little dots. These are the myofibrils (actin and myosin myofilaments) cut transversally.



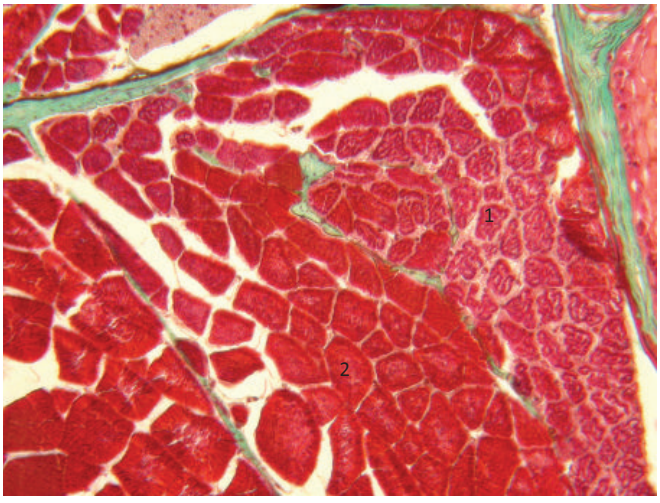


Fig. : 3.9 *Kryptopterus bicirrhis* (MT / MM)

Axial muscles, transverse section near the lateral line. This micrograph shows the presence of superficial red layers (slow red muscles - 1) and deep white layers (fast white muscle - 2). The former are small in cross section and correspond to aerobic muscle fiber type. The latter (anaerobic fibers) are large in cross section and are always predominant. They are used for intense contraction like required in escape or hunting situations. Connective tissue is stained green. The one on the upper left belongs to the transverse *septum*.

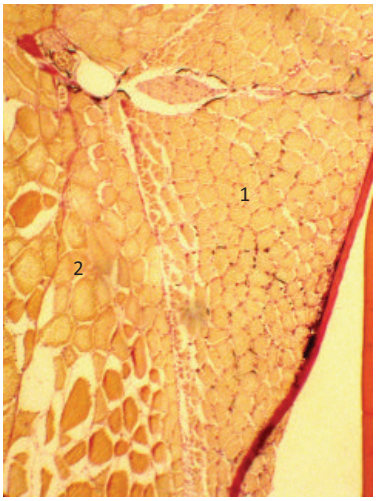


Fig. : 3.10 *Kryptopterus bicirrhis* (PAS-H-AUR / LM)

Cross section of axial myomeres in the lateral line region. The rhabdomyocytes that constitute the myotomal mass are composed of superficial small diameters red fibers (1) and inner large diameters white fibers (2). Most muscles are of intermediate type containing red and white mixed muscles.

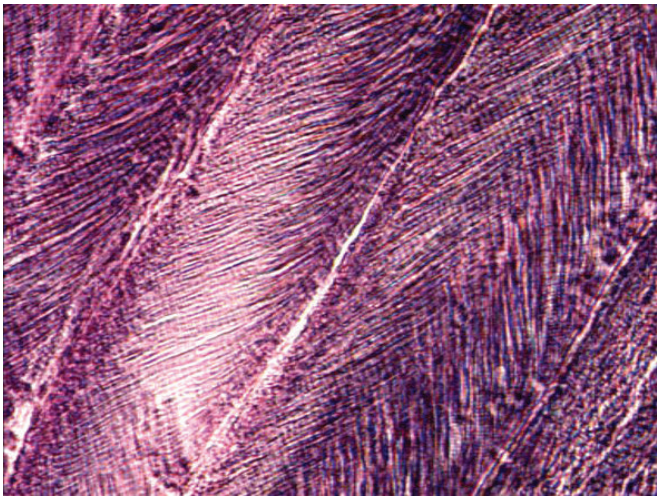


Fig. : 3.11 *Carapus acus* (H-E / HM)

Sonic fiber. Sound production in many fish species results from the action of extrinsic muscles that insert onto the gas bladder. These fibers are thinner than white and thicker than red fibers. This longitudinal section shows the extend twisted myofibrils in the periphery of the fiber.

Image: courtesy of Dr. E. Parmentier, Université de Liège, Belgium.

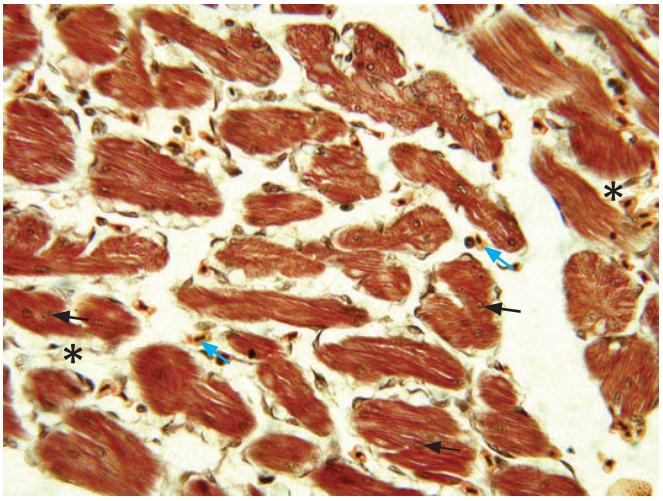


Fig. : 3.12 *Cyprinus carpio* (MT / MM)

Cardiac ventricle. Transverse and oblique sections in ventricular cardiomyocytes. Cardiac muscle fibers are cylindrical cells with one or rarely two central nuclei (black arrows). Note numerous erythrocytes (blue arrows) in the capillaries in the delicate collagenous *endomysium* (∗).

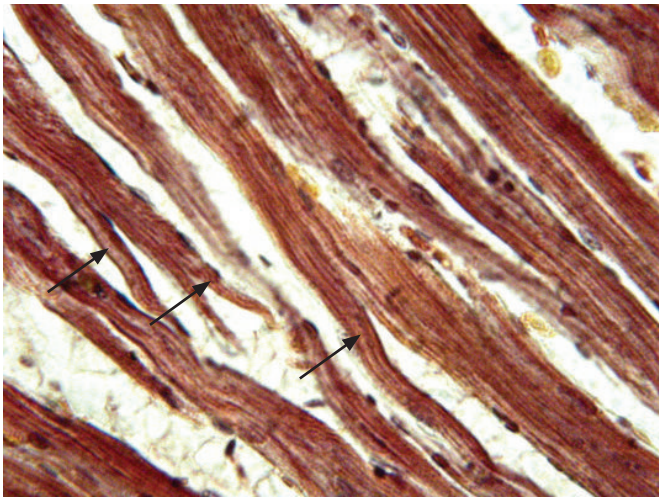


Fig. : 3.13 *Cyprinus carpio* (MT / MM)

Longitudinal section of ventricular cardiomyocytes. This micrograph shows branching of the myofibers (arrows) whose ends are in contact with adjacent cells. In routine preparation such as this, the cross-striations are not readily visible.

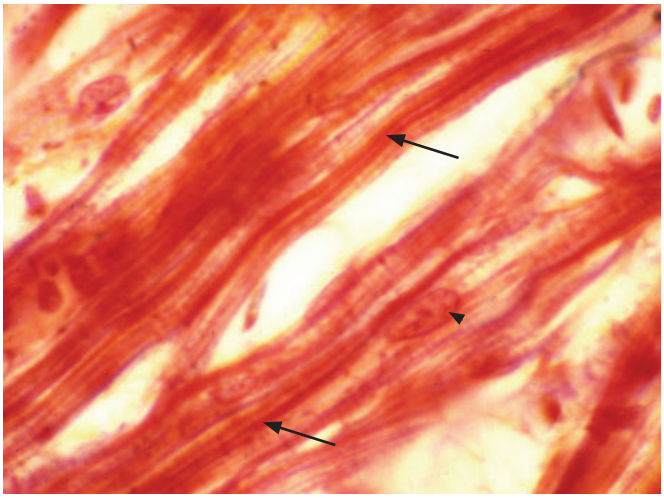


Fig. : 3.14 *Cyprinus carpio* (MT / IM)

Longitudinal section of ventricular cardiomyocytes. In this field cardiomyocytes containing parallel myofibrils (arrows) and central nuclei (arrowhead) are demonstrated.

Although myofibrils arrangement is similar to that of the skeletal muscle, striations are generally difficult to see in the cardiac muscle because of the irregular cell dichotomy.

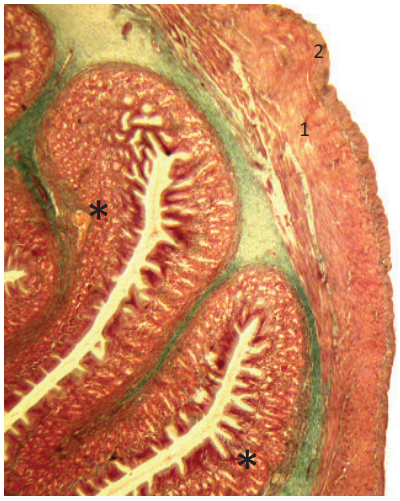


Fig. : 3.15 *Perca fluviatilis* (MT / LM)

Cross section through the wall of the stomach. This wall is composed of an outer *muscularis externa*, *submucosa* and inner *mucosa* (see chapter 7). The muscular coat is composed of a large inner circular (1) and an outer longitudinal (2) layer of smooth muscle (leiomyocytes). Note the two large mucosal folds (∗) surrounded by green connective (*submucosa*).

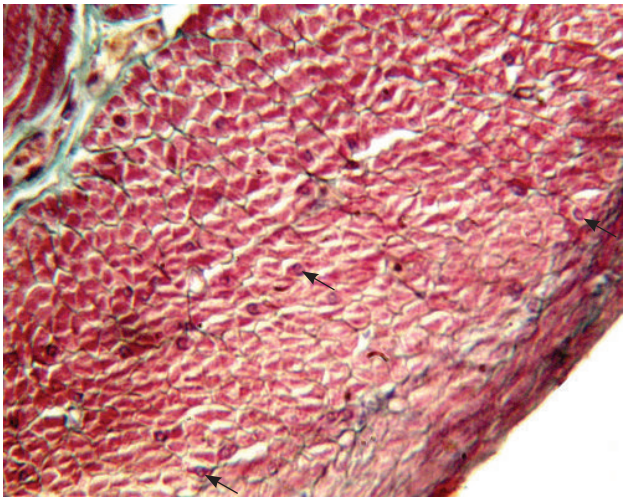


Fig. : 3.16 *Perca fluviatilis* (MT / MM)

This micrograph shows typical appearance of smooth muscle in cross section. This section cuts the spindle-shaped leiomyocytes at different levels. So they appear erroneously of various diameters. Leiomyocytes are rather small visceral muscle cells containing a single nucleus (arrows) centrally located in the cytoplasm.

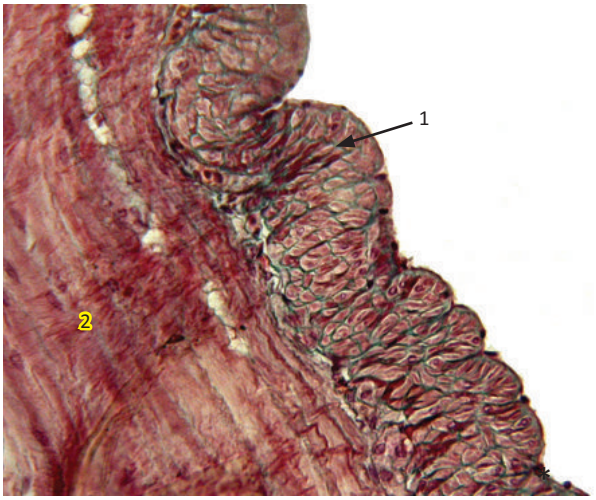


Fig. : 3.17 *Perca fluviatilis* (MT / MM)

In the stomach and in many digestive structures, smooth muscle fibers (leiomyocytes) are mostly arranged in two adjacent perpendicular layers: the outer longitudinal layer (1) and the inner cicular one (2). This arrangement permits peristalsis i.e. the forward propelling of the content of the tube. Collagen (*endomysium*) is delicately stained in green.

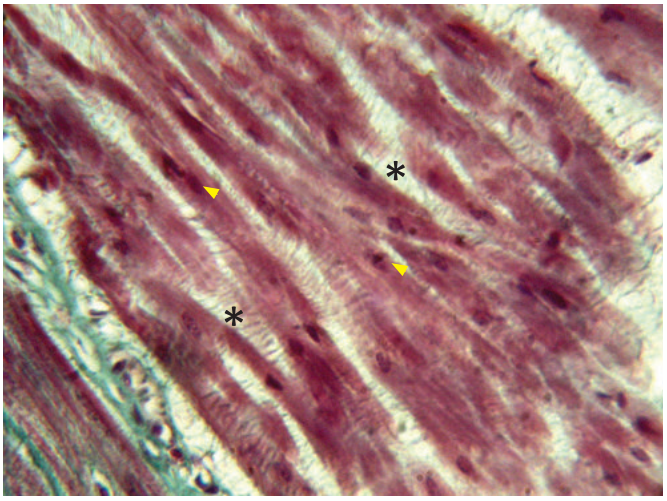


Fig. : 3.18 *Perca fluviatilis* (MT / HM)

This micrograph shows elongated, spindle-shaped leiomyocytes. The central nuclei (arrowheads) are located at the widest part of the cells. Smooth fibers are usually much smaller and shorter than skeletal ones. The fine intercellular lines (∗) are artefacts.

4

Cardiovascular system and blood

Heart

The fish heart (Figs 4.1 to 4.11) typically consists of four chambers (Fig. 4.1) coupled in series and located caudo-ventrally to the gill cavity. In teleosts the four chambers are (in sequence) : the *sinus venosus*, the *atrium*, the ventricle and the highly elastic *bulbus arteriosus* (or a contractile *conus arteriosus* in elasmobranchs, chondrostei and holostei).

The heart of fishes consist of three layers of tissue, the *epicardium*, *myocardium* and *endocardium*. The external *epicardium* consists of a single layer of flattened epithelial cells, the *mesothelium*, on a thin connective tissue layer, that merges with the pericardial cavity lining. The *myocardium* varies in thickness in different parts of the heart. It is thin in the *sinus venosus*, but it is contractile. The volume of the *sinus venosus* (Figs 4.2 & 4.3) is equivalent to that of the *atrium*, which is the next chamber. The muscular layer is somewhat thicker in the cardiac *atrium* (Fig. 4.4), where pectinate muscles radiate from the roof of the *atrium* forming a star-burst muscular net. The ventricle (Figs 4.5 to 4.8), as expected, has the thickest layer of cardiomyocytes. Its wall, which thickness is variable according to sex and age, can contain several layers of muscular fibers. It is also characterized by an abundant spongy *myocardium* that leaves in the lumen some *lacunae* in which blood circulates. In the ventricle, blood acquires a high pressure and passes, through valves (Fig. 4.10), into the last chamber of the heart.

The pear-shaped *bulbus arteriosus* has no valves and constitutes the thickened base of the ventral aorta, the main vessel leaving the heart and leading the deoxygenated blood into the gills. Its wall contains fibroelastic tissue (Figs 4.8 & 4.9) which acts as a “shock absorber” when the blood is pumped by the ventricle. In teleosts, a very short conus with two valves is present and is immediately anterior to the bulbus.

The *conus arteriosus* (Fig. 4.11) of the elasmobranchs has a thick and contractile wall containing numerous cardiomyocytes. It has a series of valvules, the number (from two to seven pairs) and the organization of which are very different according to the groups.

The *endocardium*, homologous to the *tunica intima* of blood vessels, consists of a one-cell-thick layer (endothelium) that may be highly phagocytic in some species (Atlantic cod, plaice). Unlike the mammalian heart, the teleost *myocardium* is capable of regeneration.

All the cardiac chambers of the fish heart are enclosed in a *pericardium* of fibrous tissue variably adhering to surrounding tissues, making a rigid space around the heart. The pericardial space is filled with fluid, an ultrafiltrate from plasma.

Blood vessels

Illustrated in Figs 4.12 to 4.23. The muscular arteries possess a basic structure (*tunica intima*, *media* and *adventitia*) similar to that found in higher vertebrates. The *tunica intima* comprises a one-cell thick endothelium lying on a subendothelial connective tissue layer. The *tunica media* is characterized by a more or less thick layer of smooth muscle fibers and the *tunica adventitia* consists of loose connective tissue. Elastic arteries (ventral aorta Fig. 4.12 ; gill arteries) are found near the heart, and their *media* is rich in elastin, easily stained with orcein. In general, the smooth muscle in fish arteries has less fibrillar material than in its mammalian counterparts, probably a reflection of the lower blood-pressure found in this group.

Capillaries (Figs 4.21 to 4.23) are histologically similar to those found in mammals, but they are much more permeable. They consist of a

single layer of squamous endothelial cells surrounded by a basement membrane.

Veins have a few valves (Fig. 4.18) and are structurally similar to those in mammals, but have thinner walls and less abundant smooth muscle.

Cellular components of blood

The blood of fishes is a specialized circulating tissue composed of cells suspended in a fluid intercellular substance (plasma). The main lines of blood cells in fishes are red blood cells (erythrocytes), white blood cells (leucocytes) and thrombocytes.

In fish, like in other non-mammalian vertebrates, erythrocytes are oval in shape and always contain a nucleus (Fig. 4.24). The cytoplasm is saturated with hemoglobin. The number of erythrocytes varies according to the species as well as the age of the individual, season and environmental conditions. Remarkably, in several Antarctic notothenioid fishes hemoglobin can be much reduced or completely absent, and there are no erythrocytes.

Like leucocytes in higher vertebrates, the fish leucocytes in peripheral blood are agranulocytes and granulocytes. Classification of fish white blood cells is replete with unsolved problems, partly because methods used in mammalian hematology often do not give good results when applied to fish blood smears. The agranulocytes have no granules (lysosomes) in the cytoplasm and their nucleus is unlobed. Agranulocytes come in two varieties : lymphocytes and monocytes. In teleosts, the average diameter of small lymphocytes varies between 5 and 8 μm (up to 12 μm in large lymphocytes). The nucleus occupies virtually the whole of the cell leaving only a narrow rim of basophilic cytoplasm. Fishes, like mammals, possess subclasses of lymphocytes which may be determined by use of mitogens (lectins).

The literature is extremely confused on the definition of "monocytes" in fishes. Some authors deny the existence of monocytes in teleost fish. The monocytes are not numerous (0,1-0,5% of the leucocytes population). They resemble mammalian monocytes histochemically, possessing a few fine and scattered granules which stain positively with PAS and acid phosphatase.

Among the granulocytes, neutrophils are the most plentiful. The numbers of circulating neutrophils reported in fishes vary over a considerable range (1-25% of leucocytes). The teleost neutrophil has a grayish granular cytoplasm (Romanowsky dye). In certain species, e.g. plaice, the eccentric nucleus is round or oval, though in some other species, e.g. salmonids, the neutrophils possess lobed nuclei.

Eosinophils in the blood of teleosts has been variably reported. They were observed in the blood of various species of salmon (*Salmo*, *Oncorhynchus*), but were reported rare or absent from the blood of rainbow trout. Several workers agree that eosinophils are present in the blood of *Carassius*. Typically, these cells have been identified by the presence of large cytoplasmic granules which stain bright red with Romanowsky's stain or eosin.

As with eosinophils, reports on the presence of basophils in the blood of fishes vary. When present (goldfish, salmonids, carp), the description of this granulocyte, stained by the Romanowsky dye, mentions a large eccentric nucleus with homogenous chromatin and large purplish-blue cytoplasmic inclusions.

The thrombocytes are predominantly fusiform with a nucleus conforming to the cell shape. The cytoplasm is hyaline when stained with a Romanowsky-type dye. Transmission electron microscopy shows that thrombocytes clearly differ from all other leucocyte populations in possessing vesicular and microtubular structures in their cytoplasm. Glycogen granules are also found. In addition to taking part in blood clotting, it has been reported that the piscine thrombocytes are blood macrophages that form one of the protective barriers against foreign agents and might be considered true digestive cells, because they may remove circulating cell fragments directly by phagocytosis.

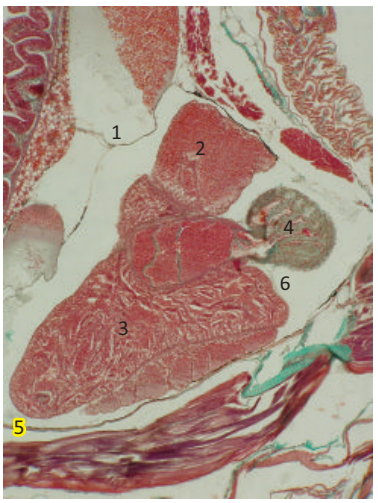


Fig. : 4.1 *Poecilia reticulata* (MT / LM)

This micrograph clearly shows that the teleost heart is a four-chambered pump. The four chambers are (in sequence) : the *sinus venosus* (1), the *atrium* (2), the ventricle (3) and the highly elastic *bulbus arteriosus* (4). The *pericardium* (5) and the pericardial cavity (6) are also demonstrated.

Note that the elasmobranchs have a *conus arteriosus* (Fig. 4.11).

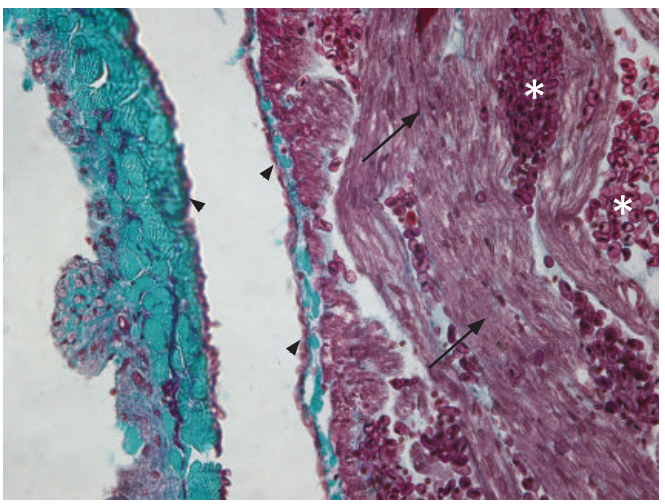


Fig. : 4.2 *Scyliorhinus canicula* (MT / MM)

This picture shows the wall of the *sinus venosus* on the left (blue) and the *atrium*, on the right (pink). The *sinus venosus* is a thin-walled sac mainly composed of connective tissue (collagen in blue). The *atrium* contains a loose, spongy *myocardium* (elongated cardiomyocytes - arrows). Numerous erythrocytes are seen (∗). The arrowheads point to the *epicardium*.

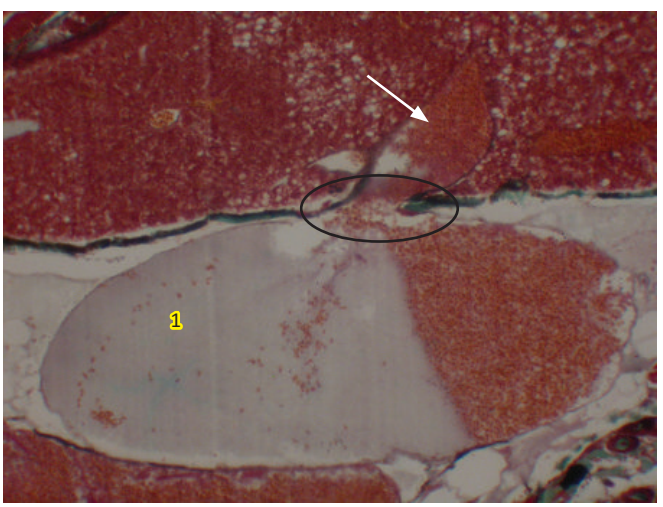


Fig. : 4.3 *Poecilia reticulata* (MT / MM)

The oval structure depicted in this picture is part of the *sinus venosus*, a thin-walled chamber mainly composed of connective tissue into which systemic venous blood is collected. A large hepatic vein (arrow) filled with red blood cells drains (ellipse) into the *sinus* (1) where erythrocytes are gathered at the right. Note in the half upper part of the micrograph liver tissue.

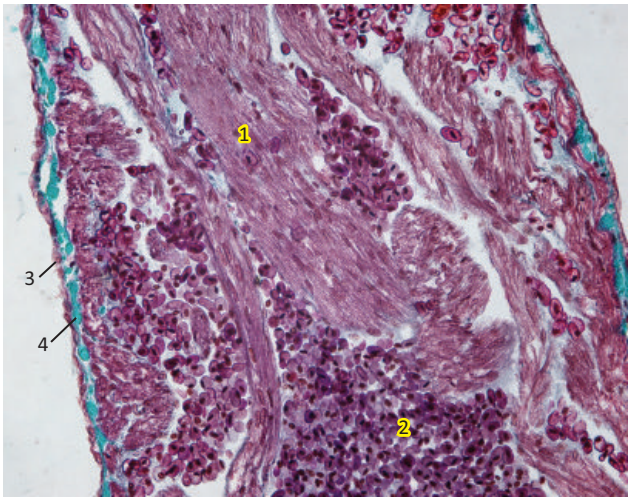


Fig. : 4.4 *Scyliorhinus canicula* (MT / MM)

The *atrium* is also thin-walled, although thicker and with more cardiomyocytes (1) than the *sinus venosus*. The spongy wall of the *atrium* shows numerous spaces filled with erythrocytes (2) and delimited on all sides by muscular strands running in various directions. The free surface of the *epicardium* consists of a single layer of flattened epithelial cells (*mesothelium* - 3) lying on a thin layer of fibrous tissue (turquoise - 4).

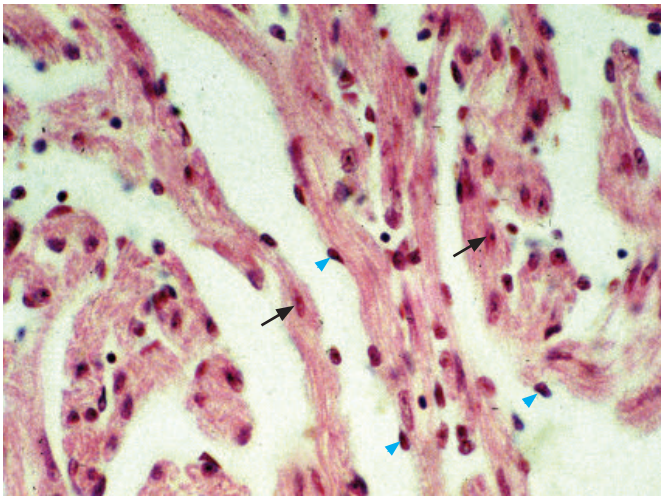


Fig. : 4.5 *Oncorhynchus mykiss* (H-E / HM)

Ventricular cardiomyocytes. Blood is drawn from the *atrium* via the atrioventricular valves into the ventricle as it is dilated. The ventricle generates a high blood pressure. Some cardiomyocytes are illustrated with thin elongated nuclei centrally located (arrows). Dense nuclei (arrowheads) of the *endocardium*, the innermost layer of the heart, are also demonstrated.

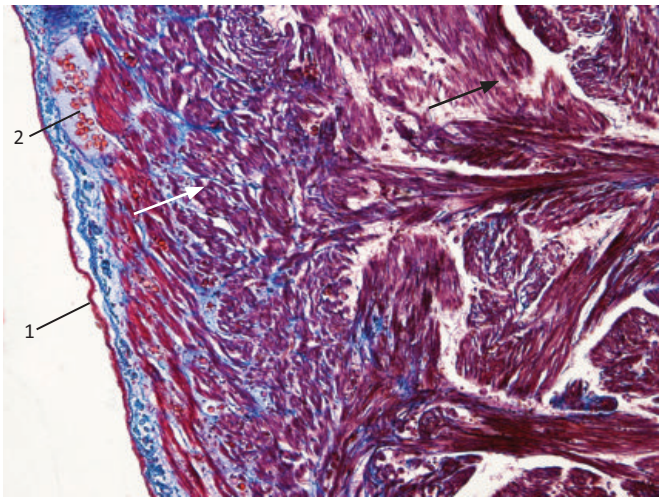


Fig. : 4.6 *Scyliorhinus canicula* (MT / MM)

The ventricle is a highly muscular chamber with generally an thick outer muscular layer (white arrow) and an abundant spongy trabecular *myocardium* (black arrow) with a narrow lumen in between. In this picture bundles of cardiomyocytes are mainly longitudinally arranged. Collagenous fibers are stained by anilin blue. Note also the *pericardium* (1) and a small coronary vessel (2) containing plasma (pale blue) and erythrocytes (orange).

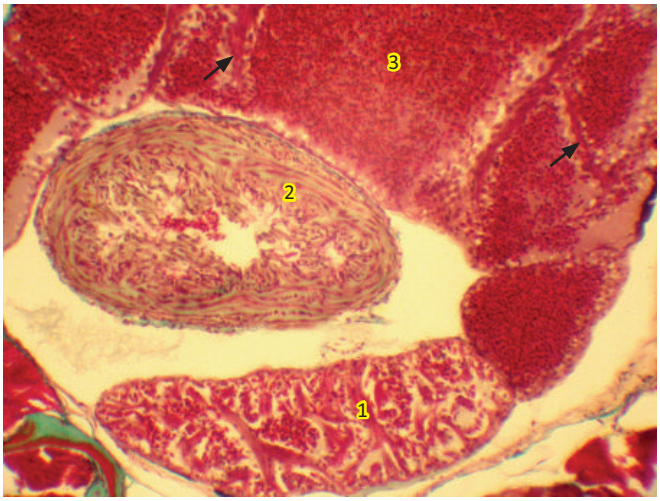


Fig. : 4.7 *Poecilia reticulata* (MT / MM)

In this picture three chambers of the heart are displayed: the muscular ventricle (1), the elastic *bulbus arteriosus* (2) and the *atrium* (3). The latter is filled with erythrocytes and some cardiomyocytes are observed (arrows).

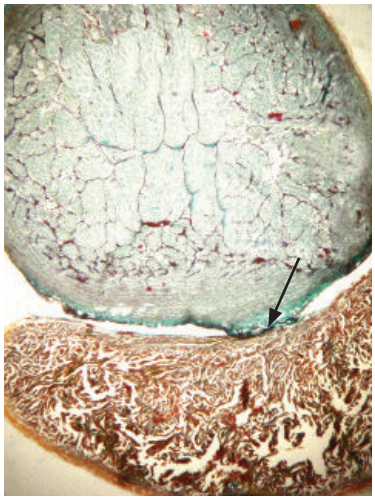


Fig. : 4.8 *Rutilus rutilus* (MT / LM)

This micrograph shows the wall of the *bulbus arteriosus* and the ventricle (below). As compared to the latter, the wall of the *bulbus arteriosus* consists of fibroelastic tissue (without cardiomyocytes). One can perfectly guess the place (arrow) where the ventricle leads into the *bulbus arteriosus* through the ventriculo-bulbar valves which cannot actually be seen here.

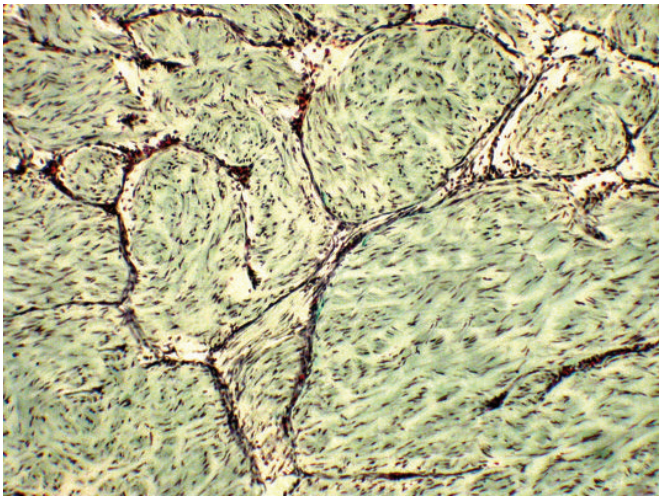


Fig. : 4.9 *Rutilus rutilus* (MT / MM)

In teleosts there is no contractile *conus*, but instead a fibroelastic *bulbus arteriosus* at the base of the ventral aorta. The *bulbus* is separated from the ventricle by a pair of valves and serves to smooth the pressure created by the ventricle beat. Numerous elongated fibrocyte nuclei are visible everywhere in this image. Elastic fibers cannot be easily distinguished from collagen (green) in routinely stained preparations.

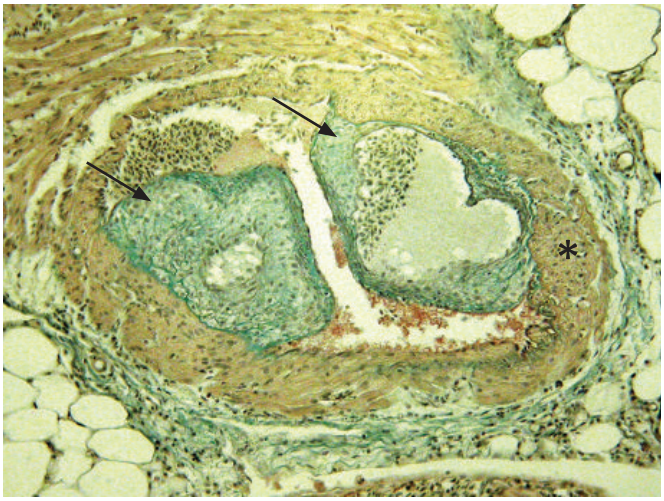


Fig. : 4.10 *Haplochromis multicolor* (MT / MM)

In teleosts a very short *conus* is present and immediatly precedes the *bulbus*. The former is lined by a thin muscular ring (∗) with two valves (arrows). Cardiomyocytes are stained in light-brown, collagen in green and the large adipocytes are unstained.

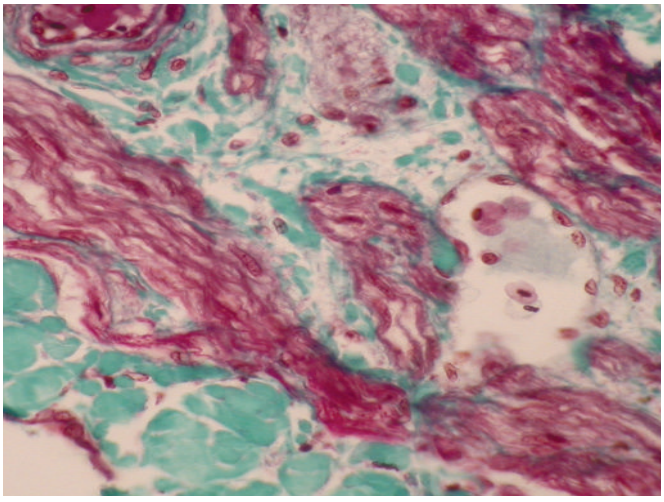


Fig. : 4.11 *Scyliorhinus canicula* (MT / HM)

Wall of the *conus arteriosus*. In elasmobranchs the *conus arteriosus* is contractile as shown by the presence of cardiomyocytes (red) in connective tissue (green). The *conus* is equipped with many valves (two to seven pairs). The presence of a muscular coat is considered as a primitive feature.

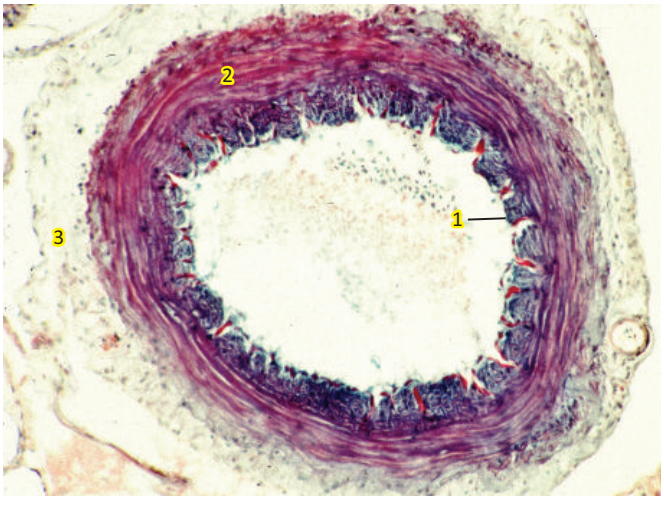


Fig. : 4.12 *Chromidotilapia guentheri* (MT / MM)

Transverse section of the ventral aorta. As in other vertebrates, arteries show three main layers : an inner layer or *intima* (1) which consists of an endothelium and varying amount of connective tissue; a *media* (2) consisting of smooth muscle and some connective tissue and an *adventitia* (3) forming the outermost coat and consisting of collagenous and elastic fibers. The internal elastic membrane separating the *intima* from the *media* is present but hard to observe (undulating aspect).

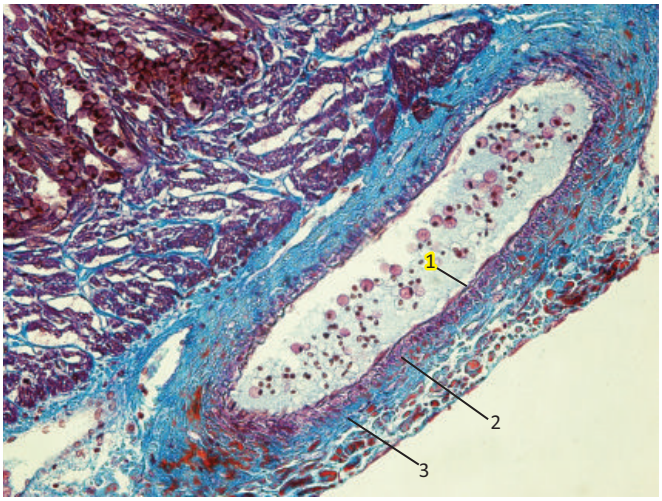


Fig. : 4.13 *Scyliorhinus canicula* (MT / MM)

This micrograph shows a coronary artery within the compact outer layer of the ventricle. The *intima* (1) is very thin and comprises the endothelial lining; the *tunica media* (2) is composed of smooth muscle cells; the *tunica adventitia* (3) merges with the surrounding collagenous tissue. In the lumen of the artery various blood cells bath in plasma (pale blue).

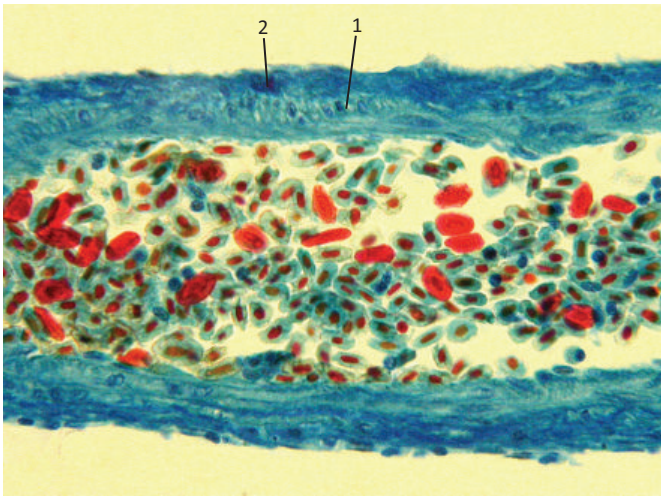


Fig. : 4.14 *Garra congoensis* (MT / HM)

Longitudinal section of a peripheral artery filled with erythrocytes. The *media* (with some leiomyocytes in cross section - 1) and the *adventitia* (2) are recognized but the *intima* layer is indistinct here.

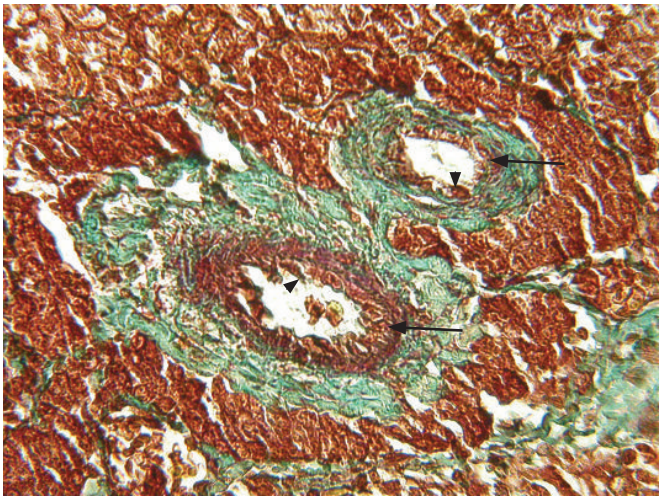


Fig. : 4.15 *Cyprinus carpio* (MT / MM)

This micrograph illustrates two small arteries in the collagenous tissue of a muscle fascicle. All that can be seen of the *tunica intima* are the nuclei of flattened endothelial cells (arrowheads). The *tunica media* (arrows) of each artery consists of one or two layers of leiomyocytes. The *adventitia* merges imperceptibly with the surrounding supporting tissue (green).

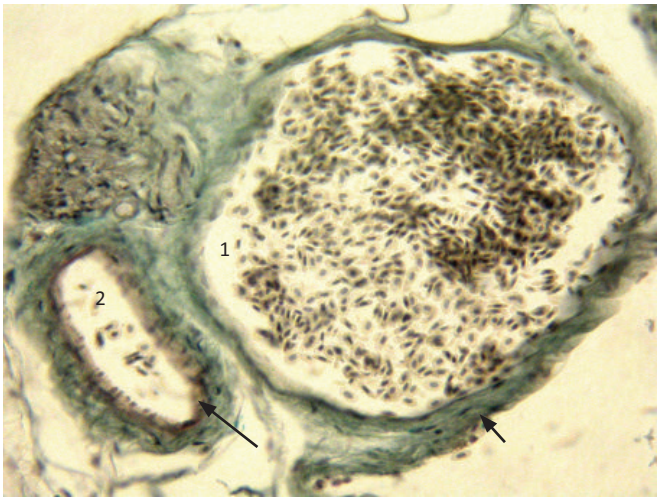


Fig. : 4.16 *Pelvicachromis pulcher* (AB-H / MM)

This micrograph allows to compare a medium-sized vein (1) and a small artery (2) both filled with erythrocytes. The *tunica adventitia* (short arrow) is the broadest layer of the vein wall and is composed of longitudinally collagen fibers which merge with the surrounding supporting tissue. Though of a lower diameter, the artery has a thicker wall (specially the *media* - long arrow) as compared to the vein. Note a portion of a peripheral nerve above the artery.

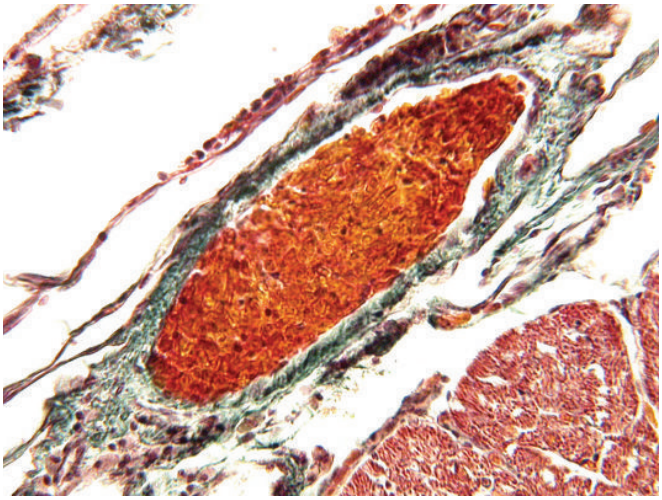


Fig. : 4.17 *Cyprinus carpio* (MT / MM)

Characteristic view of a vein. The wall is very thin and the layers are indistinct. The lumen is filled with erythrocytes. Collagen is stained green.

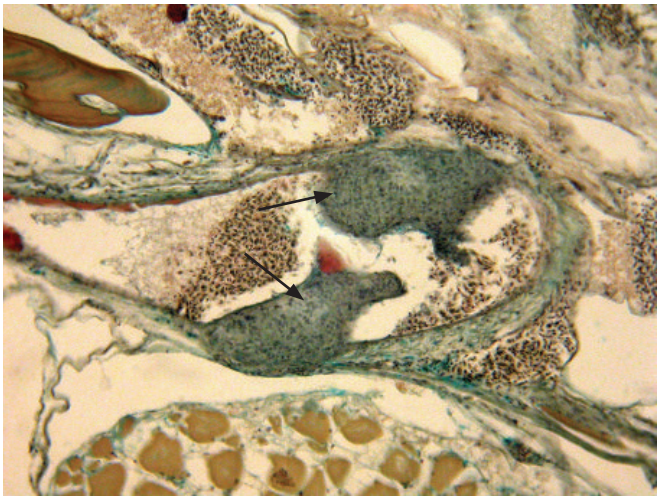


Fig. : 4.18 *Astatotilapia burtoni* (AB-PAS-H / MM)

The centre of this image shows a valve in a vein. The valve consists of two projections (arrows) of the *tunica intima* of the vein wall. The projections are composed of fibroelastic tissue covered by endothelium.

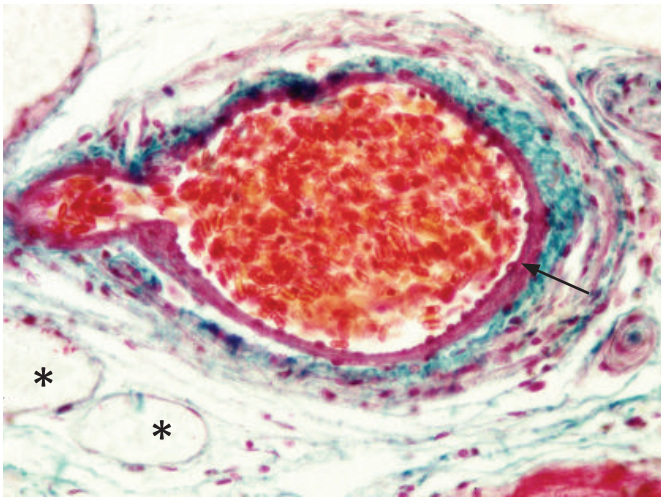


Fig. : 4.19 *Cyprinus carpio* (MT / HM)

This image illustrates the confluence of a small venule (left) with a larger vein cut in transverse section. The *tunica media* (arrow) is composed of one to two layers of leiomyocytes. Two lymphatic vessels (∗) are also found: they have a structure similar to that of a same diameter veins but their wall is even thinner. Note that the *tunica adventitia* of the vein is continuous with the surrounding collagenous supporting tissue (turquoise).

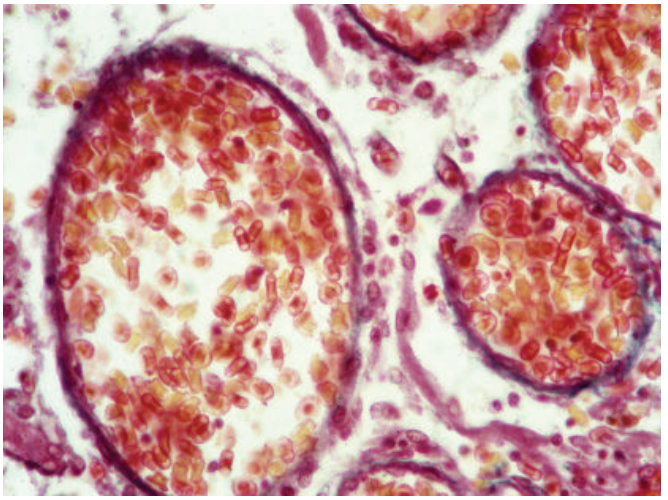


Fig. : 4.20 *Cyprinus carpio* (MT / HM)

This micrograph shows small veins and venules in transverse section. A classification of veins is difficult especially because layers in their walls are difficult to distinguish. Erythrocytes (orange) are obvious.

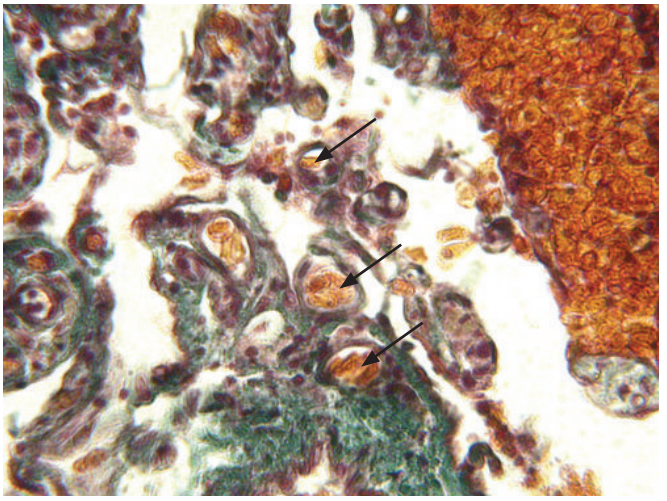


Fig. : 4.21 *Cyprinus carpio* (MT / HM)

This micrograph illustrates small vessels (capillaries or arterioles - arrows) travelling through connective tissue (green). Note a vein filled with blood cells on the right side of the picture.

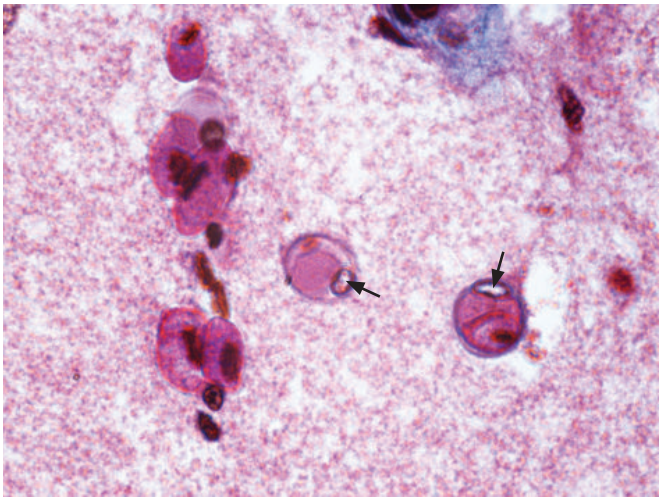


Fig. : 4.22 *Scyliorhinus canicula* (MT / IM)

White matter of the mesencephalon. Capillaries are histologically similar to those found in mammals. They consist of a single layer of squamous endothelial cells (arrows) surrounded by a basement membrane (blue).

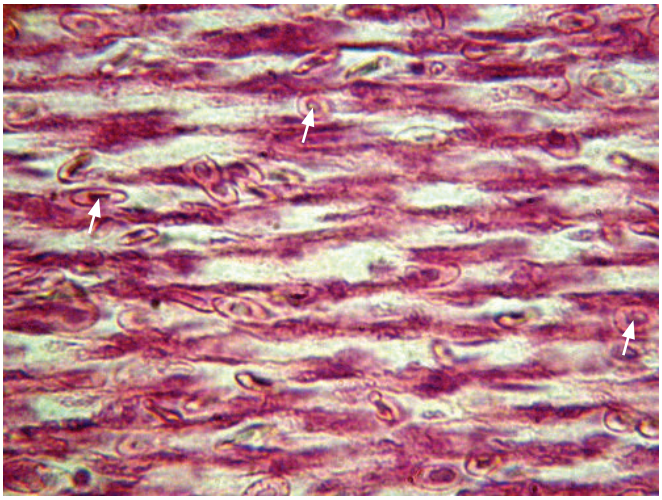


Fig. : 4.23 *Anguilla anguilla* (H-E / HM)

In many teleost species the circulatory system of the swim bladder is characterized by the presence of a countercurrent arrangement of arterial and venous capillaries, termed the *rete mirabile* (see chapter 9) which supplies the bladder with gases. The red blood cells are easily recognizable by their central nuclei (arrows) .

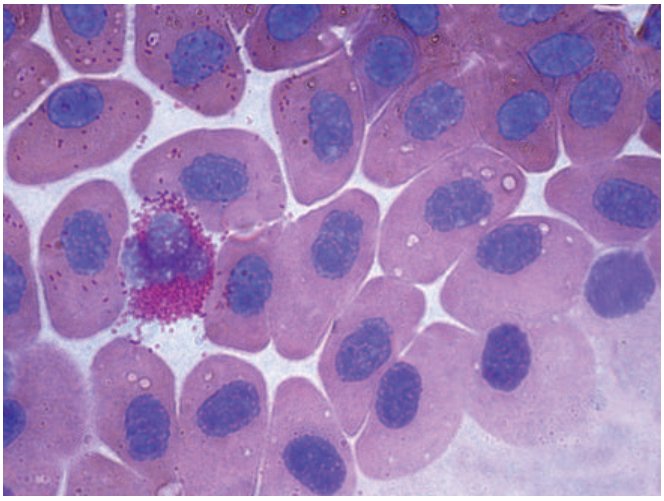


Fig. : 4.24 *Scyliorhinus canicula* (WR / HM)

Blood smear. In fish, like in other non-mammalian vertebrates, the erythrocytes are oval in shape and always contain a central nucleus. In dogfish the erythrocytes are large (20 µm). On the left a granulocyte is seen. It possesses a lobed nucleus and a cytoplasm filled with red granules.

5

Immune system and hematopoiesis

The spleen, thymus and kidney are regarded as being the major immune organs in fishes, albeit with slightly various roles between species. The spleen is the main erythropoietic tissue in elasmobranchs (sharks, rays), holocephalans (rabbit fish : *Chimaera*) and a few teleosts (*Perca, Scorpaena*). In most teleosts, in Chondrosteans (sturgeon, paddlefish) and in Holosteans (gars, bowfin) erythrocytes are produced within the kidney (see this chapter below).

Spleen

Teleosts lack lymph nodes and the spleen, with the kidney, form the two major filtering organs removing foreign agents and effete blood cells from the vascular system.

The spleen (Figs 5.1 to 5.5) is usually a solitary, dark red organ in the peritoneal cavity adjacent to the gut wall. The same basic elements as in higher vertebrates are typically present : blood vessels, red and white pulps, and ellipsoids. The spleen is covered by a thin, fibrous capsule with little evidence of contractile ability. Red pulp is an extensive, interconnecting system of splenic cords and sinusoid capillaries (open capillaries), consisting mainly of erythroid cells and thrombocytes, and usually comprises the majority of the splenic parenchyma. Splenic cords are a mesh of fibroblast-like cells with foci of various blood cells. White pulp, consisting mainly of lymphoid cells, typically surrounds arterial vessels, melanomacrophage *centers* (MMC - Figs 5.1, 5.4 & 5.5) and ellipsoids, or forms small clusters in the parenchyma. The melanomacrophage (MM) is a characteristic immune cell type of teleosts, and is prevalent in spleen. The MM is a phagocyte containing varying amount of pigment, including melanin (black-brown), hemosiderin, ceroid or lipofuscin (yellow-pink to golden brown) localized in vacuoles. MM and MMC are also found in kidney and liver. The MMC are thought to be a scavenger structure but their role in the immune system is ambiguous. Chronically stressed fish, including those that are unhealthy, tend to have more and larger MMC. The size and number of MMC also increase with fish age. Ellipsoids are periarterial sheaths of macrophages and fibrocytes, supported by reticulin fibers, that are formed at the end of splenic arterioles. A few species do not have ellipsoids. Foreign bodies, such as bacterial cells, are trapped by the ellipsoids and may be seen within the reticular meshwork or intracellularly within macrophages of the sheaths.

Thymus

The fish thymus (Figs 5.6 & 5.7) is a paired lymphoid organ situated in the dorsal region of each branchial cavity. It is usually covered by a thin capsule. Epithelial-type cells form, in the thymic parenchyma, a three-dimensional network that supports the thymocytes. A cortex with a large amount of thymocytes and a *medulla*, with more epithelial cells as in higher vertebrates are infrequently seen. Definite HASSALL' s corpuscles are absent but macrophages can be recognized. The thymus appears to function, as it does in mammals, as a site of differentiation of lymphocytes (thymocytes) involved in cell-mediated immunity, prior to their migration to peripheral lymphoid tissues.

Renal hemopoietic system

The cranial (head) kidney contains a variety of tissues that have no function in the urinary system. This head kidney (Figs 5.8 to 5.11) is almost exclusively hemopoietic. The blast cells are situated within a stroma of tissue (fibers and endothelial cells) similar to that of the bone marrow of the mammal. The endothelial cells line numerous discontinuous capillaries, through which blood from the renal portal vein is passed for filtration of spent cells and addition of new ones. Another cellular structure, found throughout teleost hemopoietic tissue is the MMC (see above).

Phagocytic system

The system of phagocytic cells (Fig. 5.12) is widely dispersed throughout the body. It is responsible for the removal of spent cells, particles or macromolecular aggregates from its surroundings. The phagocytic cells of the teleost fish are the promonocytes of the hemopoietic organs, the monocytes of the blood and lymph, the macrophages of loose connective tissue, the free and fixed macrophages of the spleen and kidney and, in many species, the fixed macrophages of the atrial lining of the heart.

An interesting feature of the teleost macrophages is their capacity to form aggregates once they are replete. Usually these aggregates are in the areas of the MMC of the hemopoietic tissues but such aggregates are also found, frequently pigmented, within or around chronic inflammatory lesions. These pigments, melanin and related pigments, are considered to play a defensive role in many organisms, in their capacity for generating hydrogen peroxide.

Epigonal organ

In the elasmobranch fish (sharks, rays) the gonads are directly associated with a unique lymphomyeloid tissue, the epigonal organ (Figs 5.13 & 5.14). This is an elongated, paired, pinkish-white structure located beneath the dark red kidneys. The anterior part of the epigonal organ is wrapped around the gonads, hence its name. A *peritoneum* covers the epigonal organ. The parenchyma consists of large amounts of leucocytes in various stages of development, in the meshes of a stroma formed by connective tissue, walls of blood vessels and blood *lacunae*. Recent data indicate that the epigonal organ is the site of T-cell differentiation in elasmobranchs, and thus plays an important role in the immune system of these fishes. In addition some studies provide evidence that the epigonal organ may play direct roles in the regulation of reproduction.

Fig. 5.15 shows the spiral valve (see chapter 7, the digestive system), a longitudinal fold of the intestine which can produce various types of white blood cells.

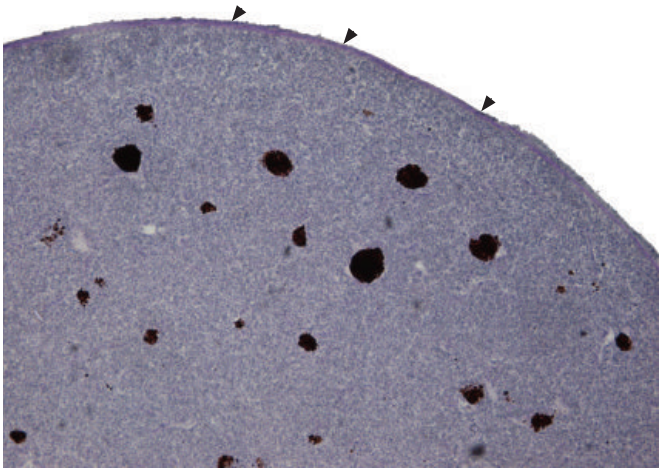


Fig. : 5.1 *Sparus aurata* (AB-PAS / LM)

Spleen. The fish spleen is covered by a thin fibrous capsule (arrowheads) and contains the same elements (red pulp, white pulp, ellipsoids, blood vessels) as the spleen of other vertebrates. However centers (dark spots) of pigment-containing cells called melanomacrophages occur in the spleen of most teleostean fishes.

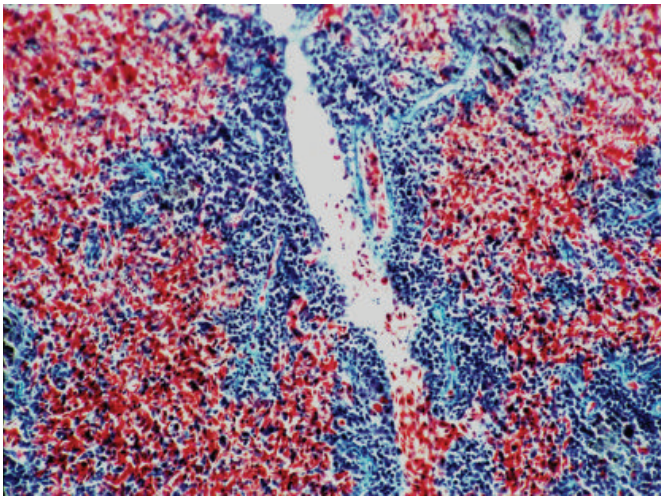


Fig. : 5.2 *Ctenopoma ansorgii* (MT / MM)

Spleen. The spleen of teleosts is a lymphoid organ filtering and removing foreign agents and defective blood cells from the vascular system. It is composed mainly of red pulp (red), whereas the lymphoid areas (white pulp) are less developed (here in blue). The red pulp consists of fibroblasts-like cells intermingled with various blood cells (erythrocytes, thrombocytes...).

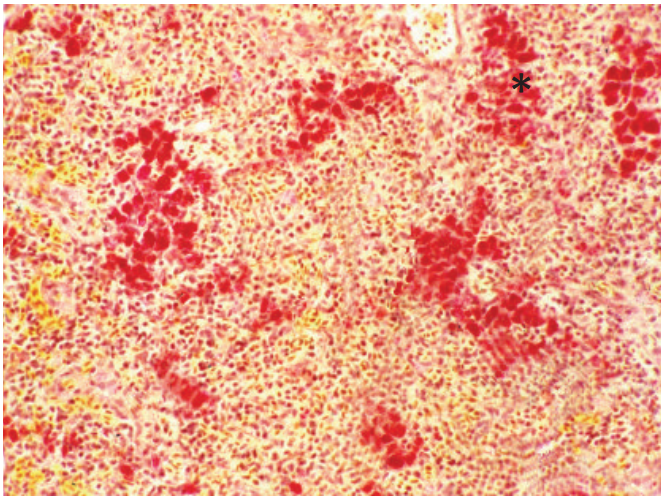


Fig. : 5.3 *Carassius auratus* (PAS-H-AUR / MM)

The nucleate erythrocytes and thrombocytes of teleosts are produced in the spleen and the anterior kidney. In this micrograph the erythrocytes are colored in orange among a lot of small lymphocytes. Melanomacrophage centers stained magenta (∗), due to their pigments which consist of melanins, lipofuchsin and hemosiderin.

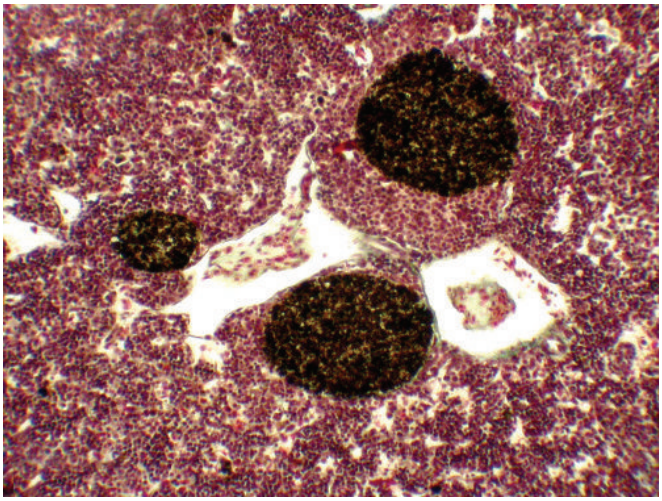


Fig. : 5.4 *Symphysodon aequifasciatus* (MT / MM)

In the spleen, the lymphoid tissue is diffuse and not well defined, in contrast with higher vertebrates. Melanomacrophage centers (dark spots) are often seen in the spleen sections : they are characteristic units of the teleosts' immune system (see below Fig. 5.5).

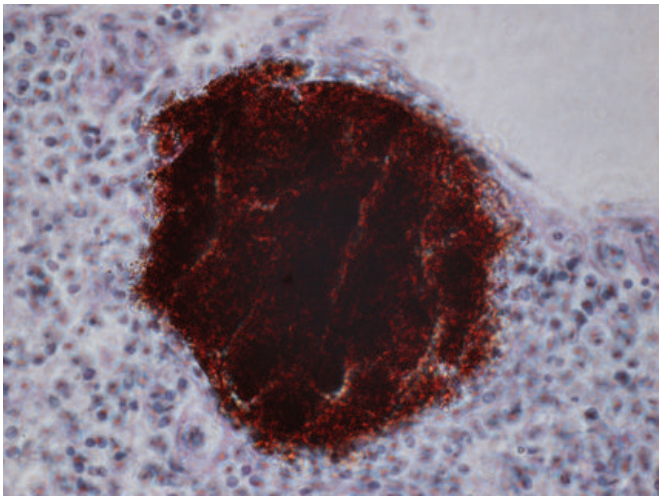


Fig. : 5.5 *Sparus aurata* (MT / HM)

Spleen. The melanomacrophage centers are thought to be scavenger structures in lower vertebrates, but their role in the immune responses is equivocal. Histological examination revealed the melanomacrophage centers to be composed mainly of macrophages and pigments. Melanin (brown) is obvious.

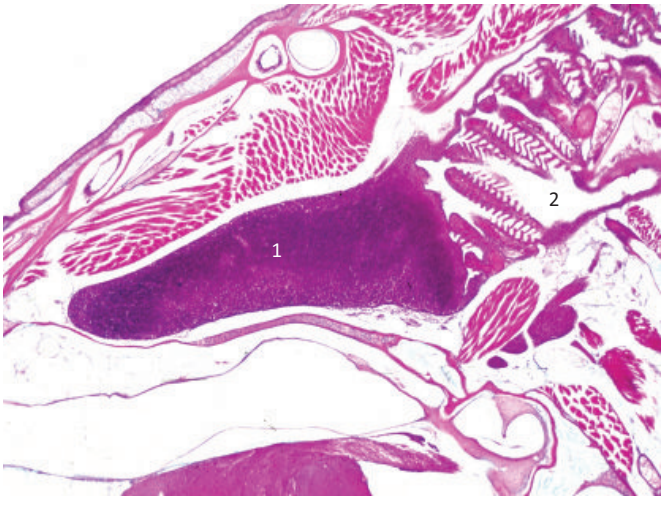


Fig. : 5.6 *Barbus caudovittatus* (H-E / LM)

Thymus (1) is a large paired distinct organ in the dorsolateral wall of the branchial cavity (2). In contrast to higher vertebrates, the teleost thymus appears to be only diffusely divided into cortical and *medulla* regions. It is mainly composed of small lymphocytes (thymocytes) and some epithelial-like cells.

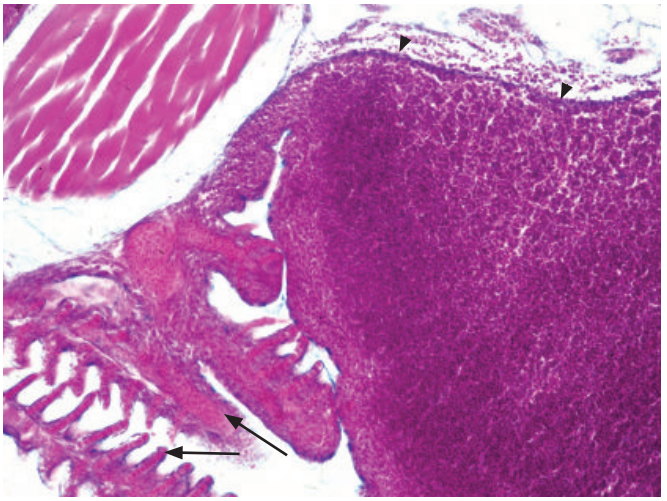


Fig. : 5.7 *Barbus caudovittatus* (H-E / MM)

Thymic tissue. The dark red color is due to aggregates of thousand of small lymphocytes called thymocytes. The arrows point to the gills and the arrowheads show the thin thymic capsule.

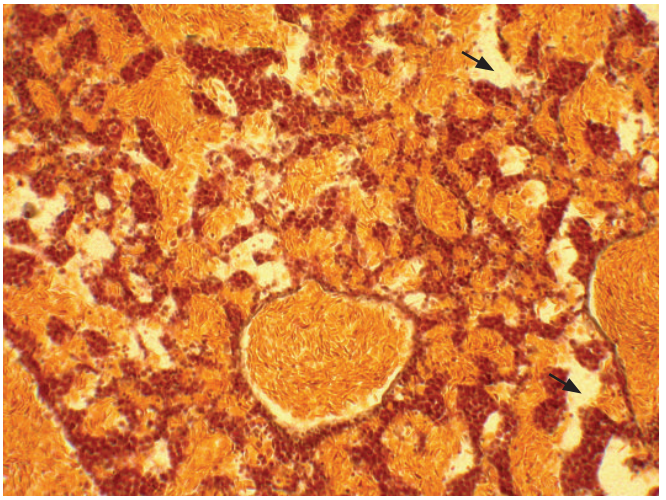


Fig. : 5.8 *Cyprinus carpio* (MT / MM)

Head kidney. In teleosts the anterior part of the kidneys generally predominates as blood-forming organ, with no or few excretory parts. Histological observation of the head kidney reveals a functional specialization for hemopoiesis (orange areas) and lymphopoiesis (red-brown areas). Note the scarce presence of nephron tubules (poorly colored - arrows).

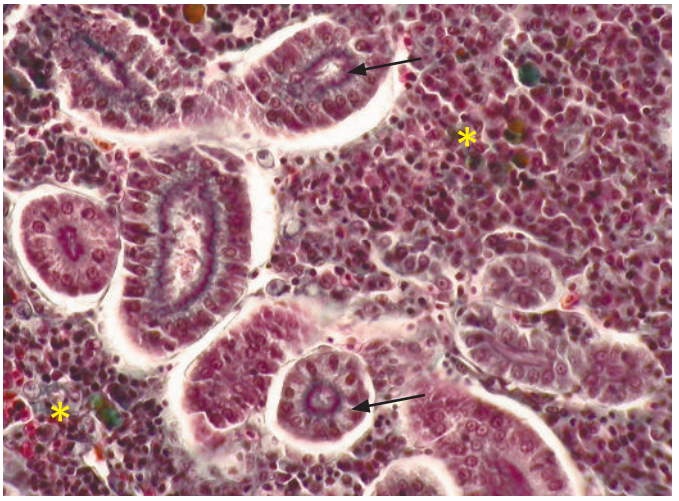


Fig. : 5.9 *Cyprinus carpio* (MT / HM)

This micrograph shows the cranial (head) kidney displaying extensive areas of hemopoietic tissue (∗) among a few nephron tubules (arrows).

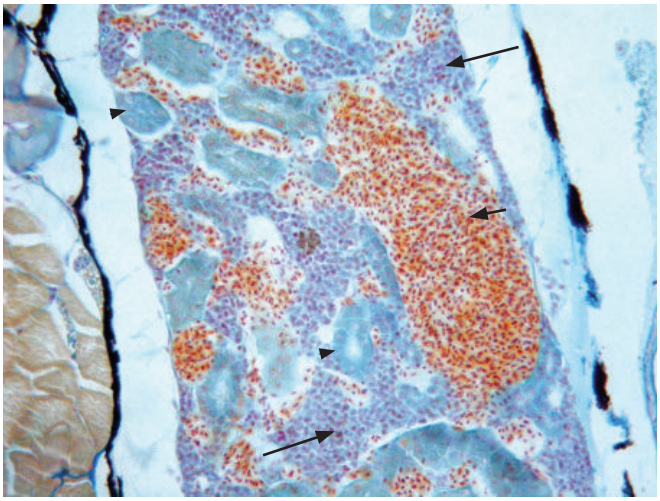


Fig. : 5.10 *Poecilia reticulata* (FR-HB / MM)

Cranial kidney. Areas of hemopoietic tissue (long arrows) and numerous blood vessels (short arrow) with erythrocytes are illustrated in this micrograph. Between these structures some nephron tubules are seen (arrowheads).

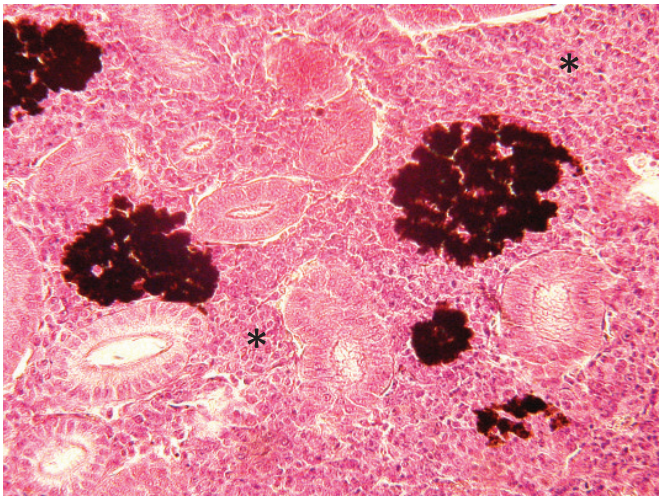


Fig. : 5.11 *Anguilla anguilla* (H-E / MM)

In addition to the spleen (and the liver), the hemopoietic tissue (numerous packed-cells - ∗) of the kidney harbours melanomacrophage centers (dark). Usually they increase in frequency and in size in environmental stress conditions. Note some nephron tubules with a central unstained lumen.

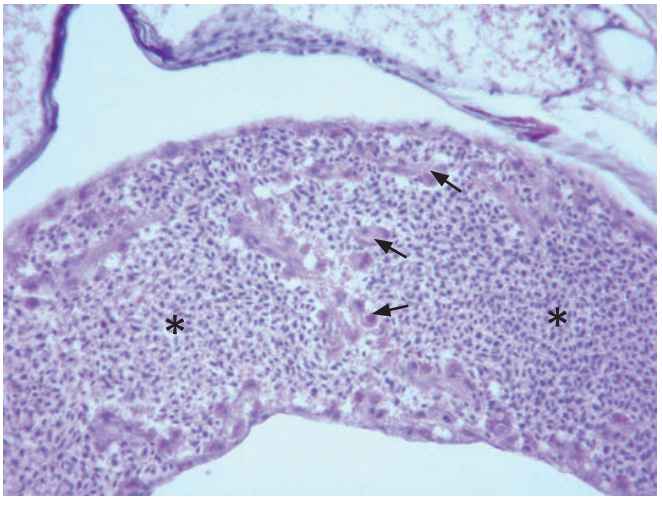


Fig. : 5.12 *Chromidotilapia guentheri* (MT / HM)

Phagocytic system. In many species of fish fixed macrophages can be observed along the atrial lining of the *myocardium* (arrows). These cells, globular in shape form an important part of the fish phagocytic system. Numerous erythrocytes (∗) circulating in the *atrium* are visible.

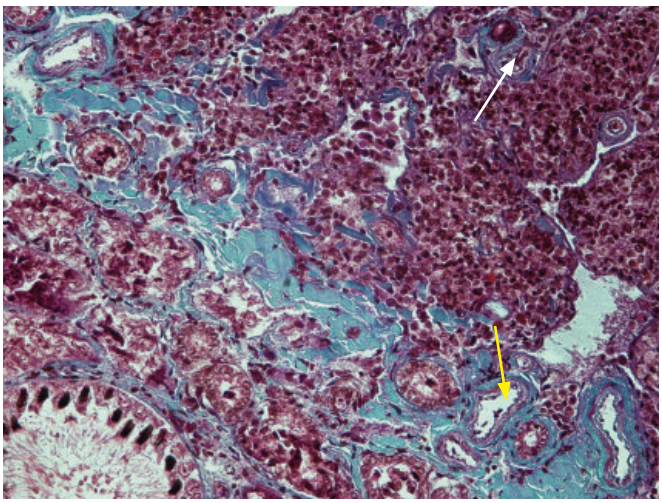


Fig. : 5.13 *Scyliorhinus canicula* (MT / MM)

Epigonal organ. In elasmobranchs gonads are associated with an elongated lymphoid tissue, the epigonal organ. The parenchyma is filled with leucocytes which are demonstrated in the meshes of stroma formed by connective tissue (blue) and the wall of blood vessels (arrows). At the lower left corner, part of a testis can be seen (see Fig. 14.17).

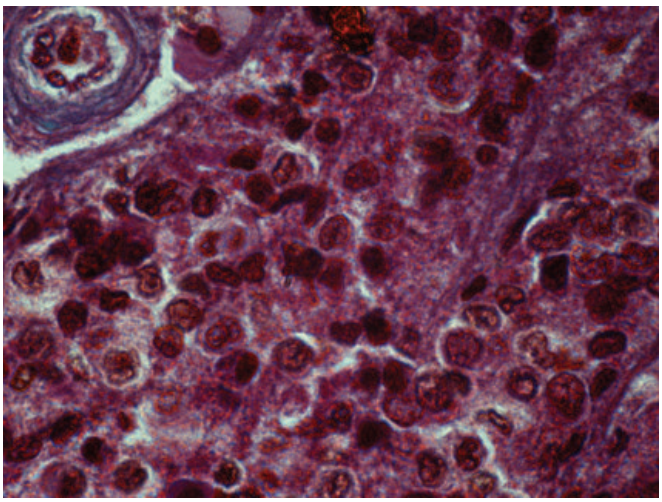


Fig. : 5.14 *Scyliorhinus canicula* (MT / HM)

Epigonal organ. The parenchyma consists of large amounts of leucocytes, in various stages of development. A small artery is seen at the top left. The epigonal organ seems to be the site of T-cell differentiation and may thus play a key role in the fish immune system.

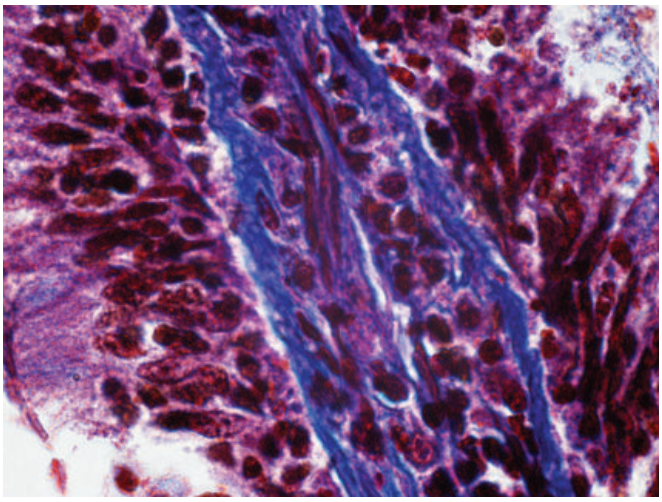


Fig. : 5.15 *Scyliorhinus canicula* (MT / HM)

The spiral valve of elasmobranchs intestine which serves to increase surface area for digestion really constitutes a special leucocytopoietic organ which produces various types of white blood cells. These leucocytes, mainly lymphocytes, are present at the centre of the image among the connective tissue (collagen) stained in blue. On both sides the epithelium covering the valve is visible.

6
Integument

The integument or skin (Figs 6.1 to 6.27) forms the external covering of the body. In fishes it protects against mechanical injury and noxious agents on the one hand and helps in respiratory, excretory and osmoregulatory functions on the other.

The skin in teleosts shows some inter-species differences; some species have no scales while others have special large epidermal alarm substance cells (club cells). The skin is composed of two layers, an outer epidermis and an underlying dermis (Fig. 6.1).

Epidermis

The epidermis (Figs 6.1 to 6.5 & 6.8 to 6.15) is a non-keratinizing stratified squamous epithelium that varies in thickness from 3-5 cells (caudoventral region) up to 20-25 (cephalodorsal region). A major difference from mammals is that in teleosts, the outermost epidermal fusiform cells remain viable (Fig. 6.2), and retain the capacity to divide; this has obvious implications for healing processes.

Cells found within the epidermis include : the filament-containing or malpighian cells that represent the major component; mucous cells (Figs 6.3 to 6.5) responsible for secreting primarily glycoproteins (mucus), forming a slimy protective coat. Functions attributed to this coat include drag reduction, predator evasion, and isolation of superficial epithelial cells from bacteria. Immunoglobulins, also present in mucus, provide additional protection against infection. In addition, the skin contains wandering leucocytes and macrophages.

The *Ostariophysi* (Cypriniformes, Siluriformes, Characiformes, Gonorhynchiformes and Gymnotiformes) have large eosinophilic (acidophilic) epidermal cells releasing fright substances when ruptured (the club cells - Figs 6.8 to 6.11). Other cellular types (Figs 6.12 to 6.14) may also be present : sensory cells, glandular cells and also granular complex cells, with various roles, not always fully understood.

In mormyrids the electroreceptive epidermis consists of three layers : the superficial polyhedral cells, the flat cells of the intermediate layer and the basal polyhedral layer.(Fig. 6.15).

Alevins of some cichlids have on their head a pair of glands made up of mucous prismatic cells. Mucus, PAS-positive (Figs 6.6 & 6.7), is secreted in the glandular lumen, hardens in contact with water and thus allows the adhesion of the young larvae to the substrate during a few days.

Dermis and hypodermis

The dermis (Fig. 6.1) contains two *strata* : *spongiosum* (*laxum*) and *compactum*. The thickness of *stratum spongiosum* situated at the base of the epidermis is variable in different parts of the body and contains collagen and reticulin fibers, nerves, capillaries, fibroblasts and pigment cells.

The *stratum compactum* is more developed than the *stratum laxum* and is formed by densely compressed bundles of collagen fibers that run parallel to the skin surface. Sometimes only the *stratum compactum* is present (Fig. 6.16).

Beneath the dermal layer is a looser, adipose tissue, which is generally more vascular than the overlying dermis. This is the hypodermis. It is a frequent site of development of inflammatory processes. However, the hypodermis is not distinguishable in many regions, and some authors say that this layer does not exist in fish and is part of the deep dermis.

Skin coloration

In fishes, the skin coloration is well marked. A large number of teleosts are brightly and brilliantly colored, while others are of a more

uniform and sober shade. The pattern of coloration relates to the life style of the animal and has functional significance. Coloration has been attributed to the presence of large numbers of a variety of pigment-cells types (chromatophores - Figs 6.17 to 6.20) that are present at different levels within the dermis : these include melanophores, xanthophores, erythrophores and iridophores. Melanophores are the most common class of fish pigment cells. The pigmented material of melanophores, called melanin, is deep brown in color. Melanin synthesis involves conversion of the amino acid tyrosine, by means of copper containing enzymes. Within melanophores, melanin accumulates in vesicles known as melanosomes. The melanophores are often star-shaped, of neuroectodermal origin. Light microscopy is often unable to identify xantophores by histologic observation, perhaps because of xanthophore pigments, which consist mainly of carotenoids and pterines. These are, respectively, fat- and water-soluble and may be lost during sample preparation. Iridophores are also difficult to detect in routine histology. Color patterns of a majority of fishes are due to the combined effects of chromatophores containing different kinds of pigments. The coloration in fishes performs adaptative functions and is useful to the animal in a variety of ways such as camouflage, aggressive purpose, courting patterns, etc.

Scales are a major feature of most species of teleosts but they can be very reduced (eels). Some groups of fish (loaches, catfish, gobies...) lack scales completely (Figs 6.8 & 6.9).

Scales, of dermal origin, are major features of most species (Figs 6.21 to 6.23, 6.26 & 6.27). They are of several types.

The placoid scale (Figs 6.24 & 6.25) is found in the skin of elasmobranchs and consists of a spine and a basal plate ; it contains a pulp cavity and is composed of a layer of dentine covered by enamel.

Polypteridae and *Lepisosteidae* are the only fish which possess ganoid scales, often rhomboidal in shape, with thick outer ganoine layer (enamel-like substance).

Cosmoid scales are scales with four layers, and are characteristic of *Sarcopterygii*.

The teleost scale comprises an outer osseous part, and an inner layer that consists of parallel collagen fibers embedded in an organic matrix. They originate in scale-pockets (Figs 6.21 & 6.22) in the dermis and extend toward the exterior of the animal in an overlapping way and are covered by the epidermis. Teleosts have ctenoid scales (with small spines on the posterior edge – Figs 6.26 & 6.27) or cycloid scale (round or oval scale composed of acellular dermal bone without spines).

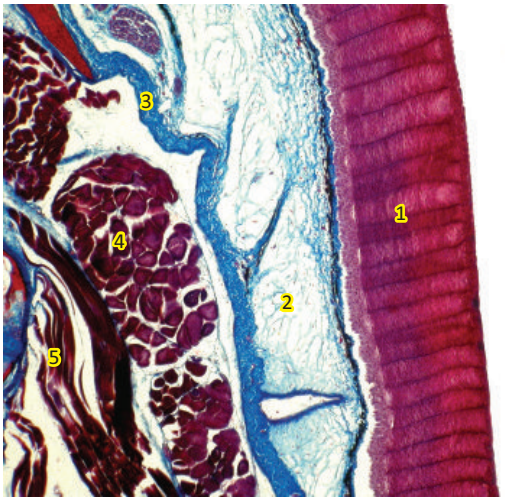


Fig. : 6.1 *Gnathonemus petersii* (MT / LM)

The skin has to maintain a physiologic balance between the external (outside medium) and the internal (body) environments. It is the first protective barrier against pathogen agents and mechanical injuries. The typical two layers of teleost skin comprise epidermis (1) and dermis. This latter usually is made up of two *strata*: an upper *spongiosum* (2) right beneath the epidermis and a deeper *compactum* (3). Collagen is stained blue. On the left striated muscles in cross (4) and longitudinal (5) sections.

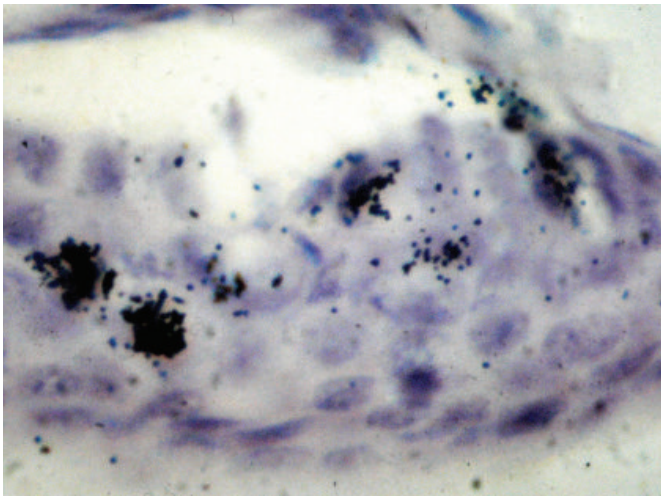


Fig. : 6.2 *Danio rerio* (^{3}H-TdR / HM)

An autoradiograph of section of zebrafish skin from a specimen intraperitoneally injected with tritiated thymidine two hours previously. The silver grains (dark dots) covering the nuclei of dividing cells are found at all levels of the epidermis. This is a major difference compared to mammals where only the basal layer cells (*stratum germinativum*) undergo mitosis!

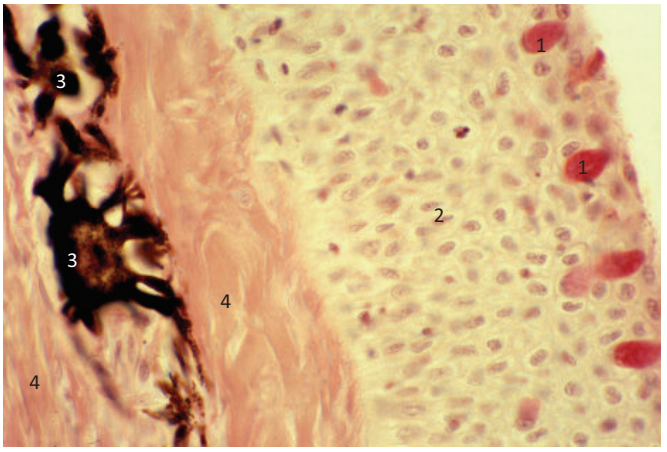


Fig. : 6.3 *Pelvicachromis pulcher* (PAS-H / HM)

Since the teleost epidermis is non-keratinized the protective role of this epithelium seems to be limited primarily to the deposition of the outer mucous coat by the mucus-secreting cells (magenta - 1). The other epidermis cells are filament-containing or Malpighian cells (2) always in majority. One can see chromatophores (3) in the dermis (4).

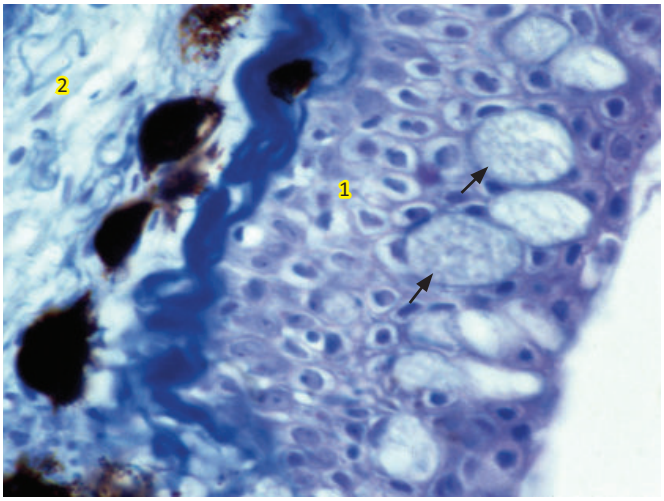


Fig. : 6.4 *Astronotus ocellatus* (AB-H / HM)

This section shows the stratified epidermis containing Malpighian cells (1) and mucous cells (arrows). The basement membrane (dark blue) is particularly thick in this image. Melanophores (dark-brown) are located in the dermis (2).

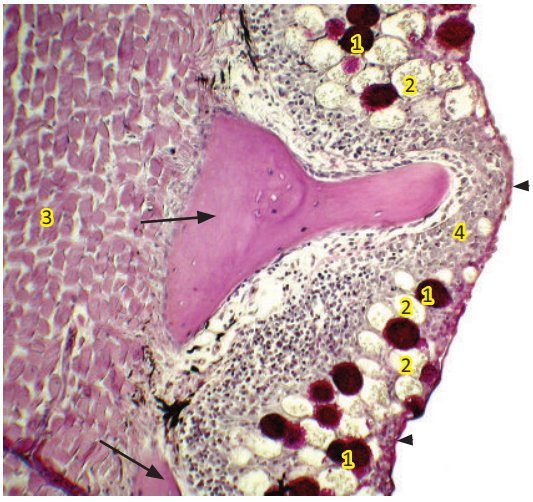


Fig. : 6.5 *Acipenser gueldenstaedtii* (PAS-H / MM)

This micrograph illustrates the thick epidermis of a young specimen. This stratified layer contains numerous mucous cells still filled with secretion (magenta - 1) as well as other ones (unstained - 2) having secreted their mucus to form the mucous coat (arrowheads). The endoskeleton of the sturgeons is cartilaginous but their skin contains many bony scutes (arrow) with some osteocytes. 3 : dense connective tissue of the dermis. 4 : malpighian cells.

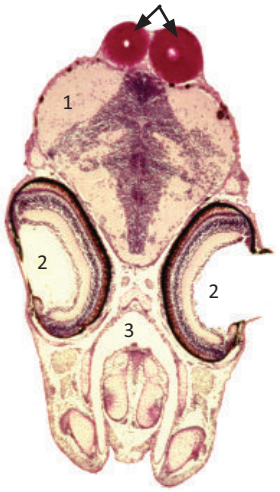


Fig. : 6.6 *Symphysodon aequifasciatus* (PAS-H / LM)

Transverse section in the head of a two-day-old specimen. The larvae of some cichlid *substratum* spawners have a pair of specialized organs which enable them to anchor to the breeding substrate. These organs are called “head glands”(arrows). The glands persist only during the early stages of development and disappear as the larvae become free-swimming (after 60 to 70 hours in this species). 1 : brain; 2 : eyes; 3 : oral cavity.

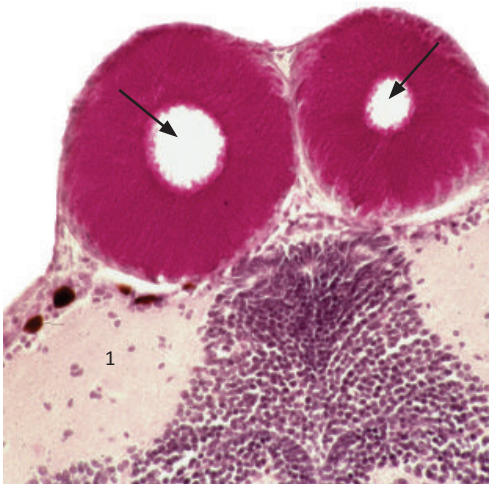


Fig. : 6.7 *Symphysodon aequifasciatus* (PAS-H / HM)

Higher magnification of the previous document. Each gland consists of several cells which secrete into a central cup (arrows) from which the secretion passes into water and hardens to form a distinct thread. The adhesive material (PAS +, magenta) is secreted by tall columnar cells, difficult to see here because of the abundant mucous secretion. 1 : brain.

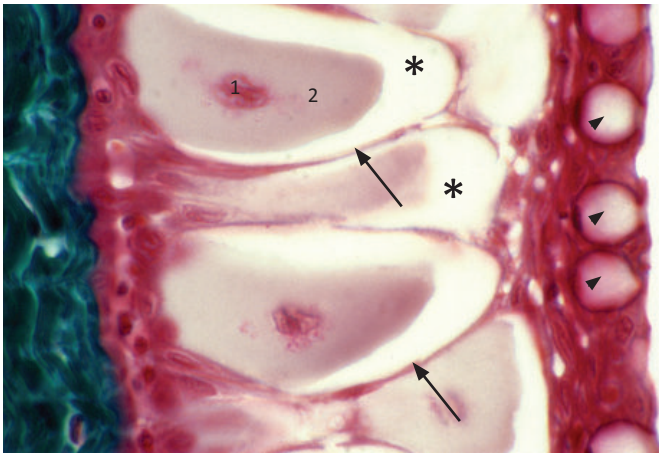


Fig. : 6.8 *Pangasius micronemus* (MT / IM)

The *Ostariophysi* (Siluriformes, Cypriniformes...) have one highly complex physiological feature in common which is absent in all other groups of teleostean fishes : the fright reaction elicited by alarm substance produced by special epidermal cells : the club cells (arrows). These large cells have a centrally located nucleus (1) and an eosinophilic cytoplasm (2). As they do not reach the surface of the integument, only injury of the skin can release the content of these cells into the water leading to the fright reaction of congeners. The arrowheads point to superficial mucous cells. In green, the dermis.

(*) : virtual space probably due to fixation.

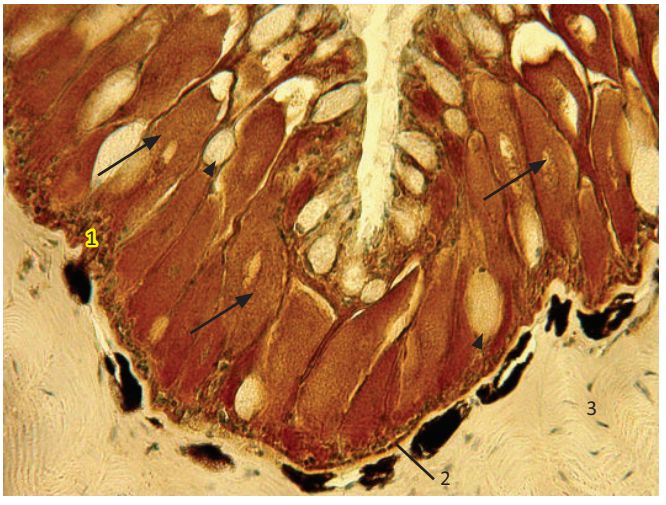


Fig. : 6.9 *Heteropneustes fossilis*

(α-HSP70 Ab / HM)

Head epidermis stained immunohistochemically with an antibody against a family of stress proteins (heat shock proteins, HSP 70). The alarm substance cells (club cells) are heavily stained (arrows) unlike the mucous cells which are negative (arrowheads). Note some basal filament-containing cells (1) and melanophores (black) of the dermis underneath the epidermis basement membrane (2).

3 : dermis

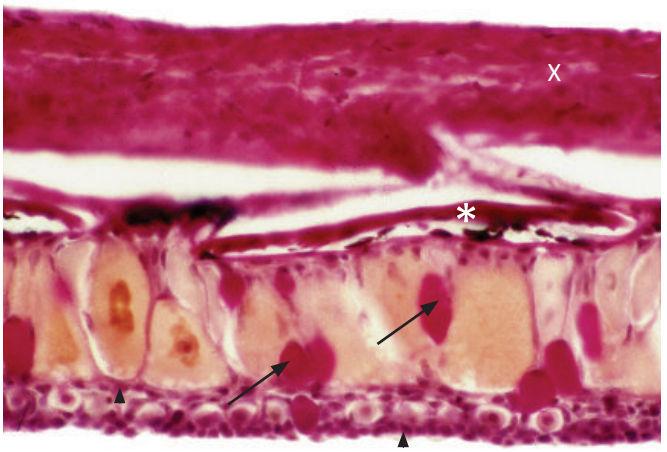


Fig. : 6.10 *Pangio kuhlii* (PAS-H-AUR / MM)

Epidermis. As the two former species, this one belongs to the *Ostariophysi* whose epidermis displays alarm cells (orange). Other cells found within the epidermis include mucous cells (arrows - magenta), and ordinary Malpighian cells (arrowheads). The micrograph also shows a little scale (∗) and the dermis (X) at the top.

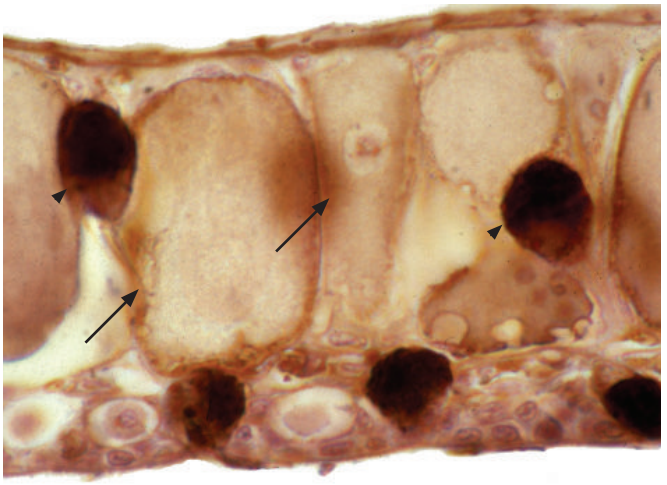


Fig. : 6.11 *Pangio kuhlii* (LEC DBA / HM)

This slide is a microphotograph of the epidermis stained with a lectin (DBA : *Dolichos biflorus* agglutinin) specific to N-acetylgalactosamine. Brown-black deposits attest to the presence of this sugar. The mucous cells (arrowheads) are heavily labelled whereas the club cells (arrows) are moderately reactive.

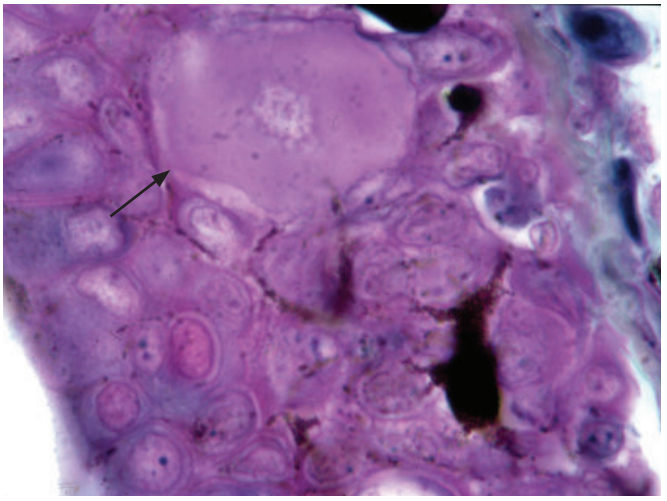


Fig. : 6.12 *Polypterus senegalus* (MT / HM)

In addition to large glandular epithelial cells (arrow) and ordinary Malpighian cells everywhere else, branched melanophores (brown-black) are illustrated. Note that the pigment cells can be found within the epidermis !

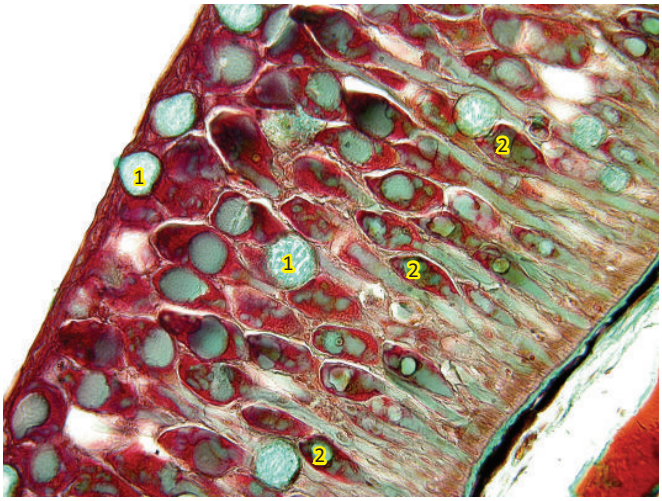


Fig. : 6.13 *Anguilla anguilla* (MT / HM)

The thickness of the epidermis varies with species, age, site and often stage of the reproductive cycle. It is usually thicker in species with no (see Figs 6.8 & 6.9) or negligible scale cover (e.g. the eel, see Fig. 6.23). Large and numerous unicellular gland cells (mucous cells - 1; serous cells with a secretory vacuole - 2) are obvious. The serous cells take up the largest part of the epithelium in this example.

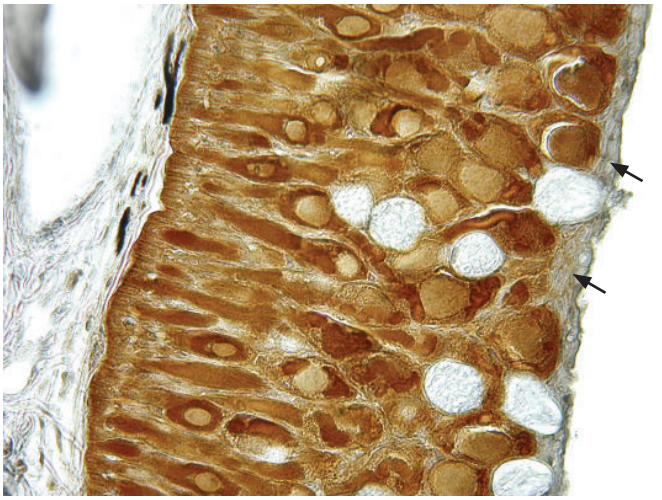


Fig. : 6.14 *Anguilla anguilla* (α-HSP70 Ab / HM)

The eel skin was treated with an antiserum directed against a family of stress proteins (heat shock proteins, HSP 70). All the epidermal cells, except the mucous cells (unstained) and the most superficial cells (arrows), are heavily stained as revealed by the brown color. The dermis (left) is unreactive.

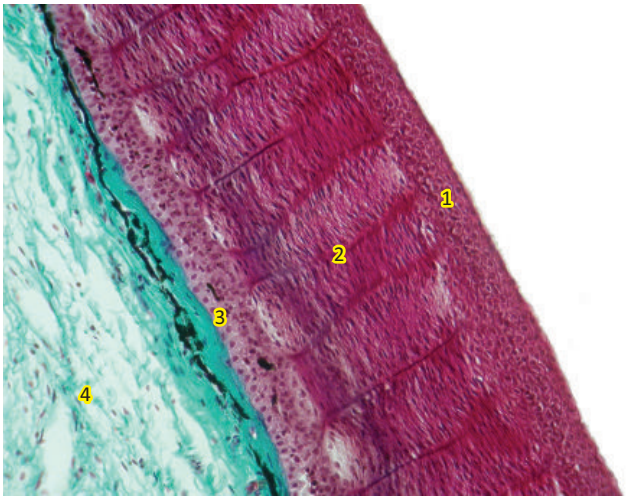


Fig. : 6.15 *Gnathonemus petersii* (MT / MM)

In mormyrids the epidermis is really exceptional. It is an electroreceptive epidermis consisting of three layers : the superficial polyhedral cells (1); the flat cells of the intermediate layer (2) and the basal polyhedral layer (3).

4 : *stratum spongiosum* of the dermis.

See also Figs 15.51 & 15.54.

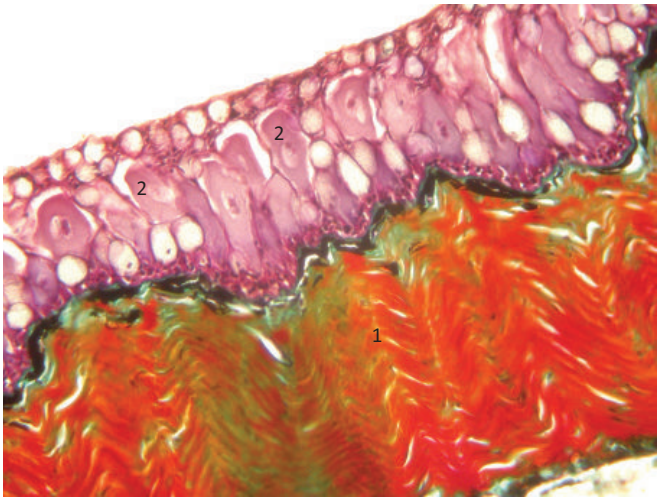


Fig. : 6.16 *Heteropneustes fossilis* (MT / MM)

The thickness of the dermis varies with species, position on the body and stage of life history. The relative thickness of the *stratum laxum* depends on whether or not there are scales and can even be absent as in this case. At the contrary, a *stratum compactum* (1) is always present and is important in locomotion. The dermis acts as a tendon in parallel with the muscles.

The epidermis contains large pink club (alarm) cells (2) and unstained mucous cells scattered troughout the epithelium.

Note : specimen kindly provided by G. Zaccone, Department of Animal Biology and Marine Ecology, Messina University, Italy.

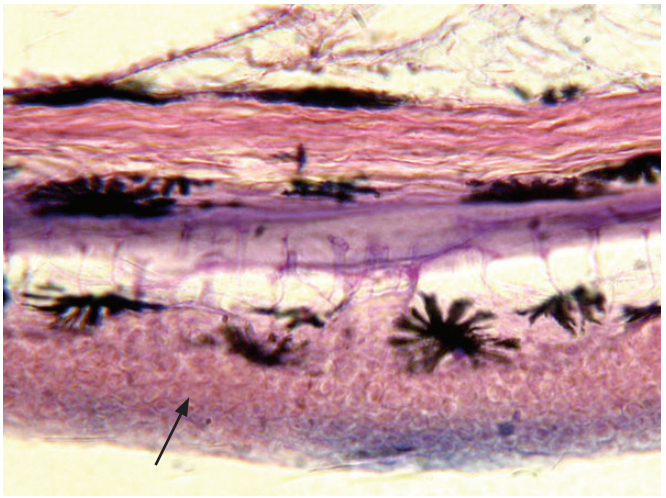


Fig. : 6.17 *Astatotilapia burtoni* (AB-H-E / MM)

Fishes may contain both dermal and epidermal melanophores (black) but the former are far more abundant in most species. Dermal melanophores are located just underneath the epidermis (arrow) in close apposition to the basement membrane.

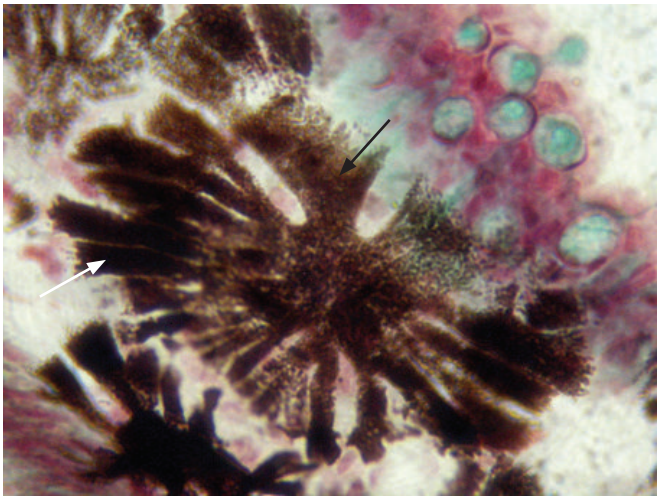
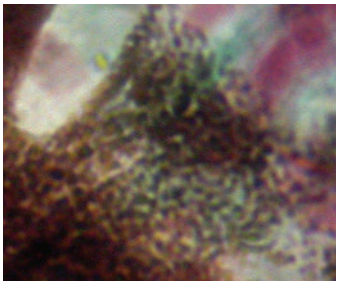


Fig. : 6.18 *Poecilia reticulata* (MT / IM)

Skin coloration in fishes depends on chromatophores or pigment cells. The melanophores are the most widely distributed group of chromatophores which absorb light rays of a wide range of wavelengths. They are mostly found in the dermis of the fish skin. The cell processes (arrows) give the cell an irregular outline and are filled with lots of brown-black widely dispersed melanin granules (see insert below).



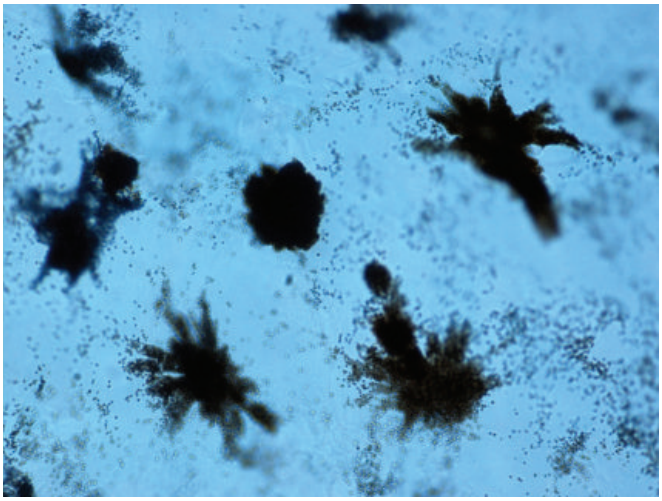


Fig. : 6.19 *Thalassoma pavo* (TB / HM)

Dermal melanophores photographed on isolated scales of this gorgeously coloured fish. Here these cells have a relatively small diameter (about 2500 cells / scale). Note numerous cell processes extending from the cell bodies. The central cell on the image shows aggregated melanin (with no processes).

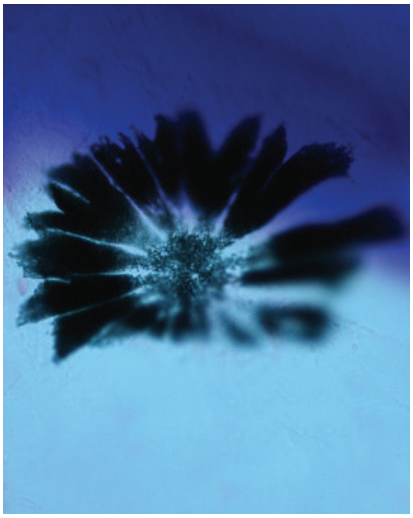


Fig. : 6.20 *Pelvicachromis pulcher* (AB-H / HM)

Chromatophores can populate many organs of the fish body (brain, eyes, skin...). The chromatophore shown here was located close to lower jaw acellular bone (purple-blue on top).

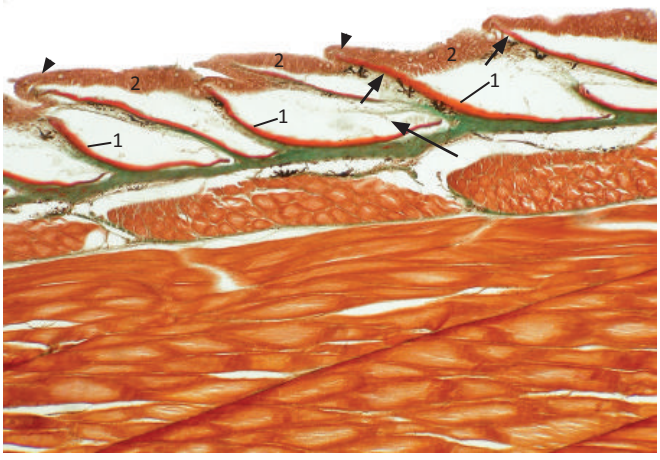


Fig. : 6.21 *Poecilia reticulata* (MT / MM)

General view of a skin sample taken from middorsal region of the trunk. Scales (1) originate in dermal scale-pockets and protrude posteriorly (short arrows) where they are covered by the epidermis (2). The epidermis is thinner (arrowheads) above the free portion of the scales The long arrow points to the *stratum laxum* of the dermis. *Stratum compactum* is stained green. Skeletal (epaxial) muscle cells fill the lower half part of the image. Take a chance on finding the chromatophores.

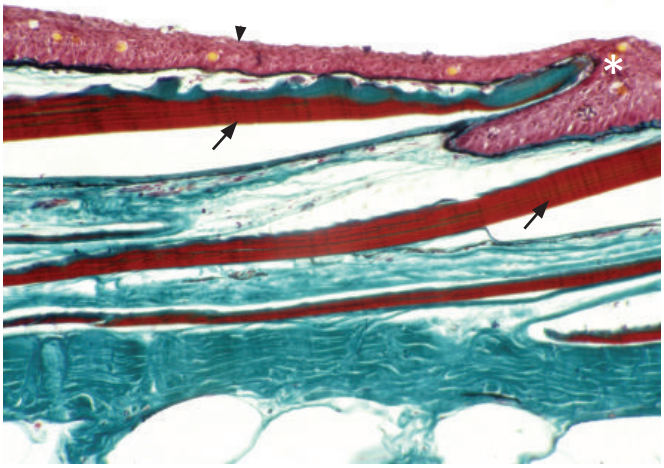


Fig. : 6.22 *Symphysodon aequifasciatus* (MT / MM)

Section showing the general structure of a scaled bony fish skin. Scales (arrows) embedded in the dermis, overlap one-another in an imbricated manner with free ends caudally pointed. The (∗) pinpoints a scale-pocket actually formed by a skin fold in which a scale is seated. The arrowhead points to the epidermis.

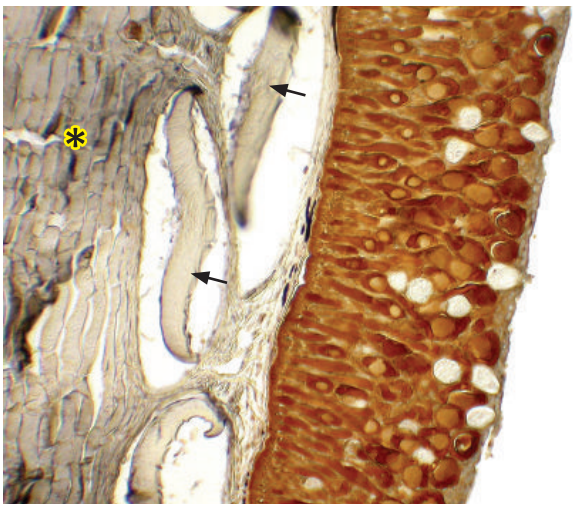


Fig. : 6.23 *Anguilla anguilla* (α-HSP70 Ab / MM)

Same slide as in Fig. 6.14 but at a lower power view. Many fish have no scales and some like the european eel have minute bony scales (arrows) barely visible to the naked eye. Scales and dermis (∗) are unreactive as compared to the epidermis (in brown except for the mucous cells).

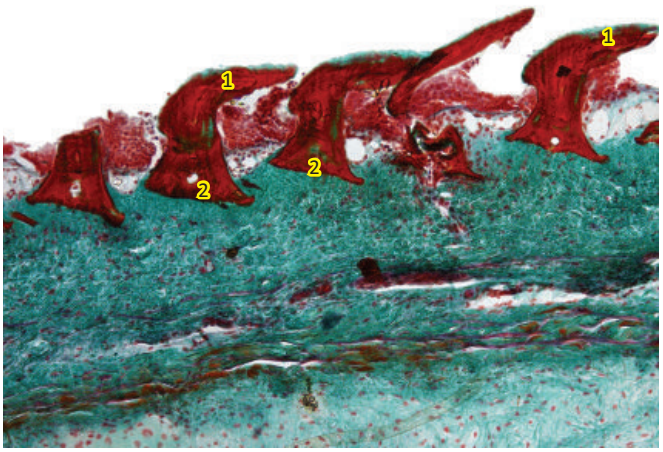


Fig. : 6.24 *Scyliorhinus canicula* (MT / MM)

Dogfish are protected by a rough skin covered by dermal bony denticles or placoid scales consisting of a backward-pointing spine (1) and a basal plate (2). These denticles bear a resemblance to teeth, and are larger on the dorsal surface of the body. They do not increase in size but are continuously replaced from underneath. Collagen in green.

Fig. : 6.25 *Scyliorhinus canicula* (MT / HM)

The placoid scales of living Chondrichthyans are isolated tooth-like denticles. Each of them contains a pulp cavity (partially visible - arrow) and consists of a dermal bony basal plate (1), a middle layer of dentine and a spine (2) covered with enamel. The scales penetrate the surface of the epidermis at the time of hatching.

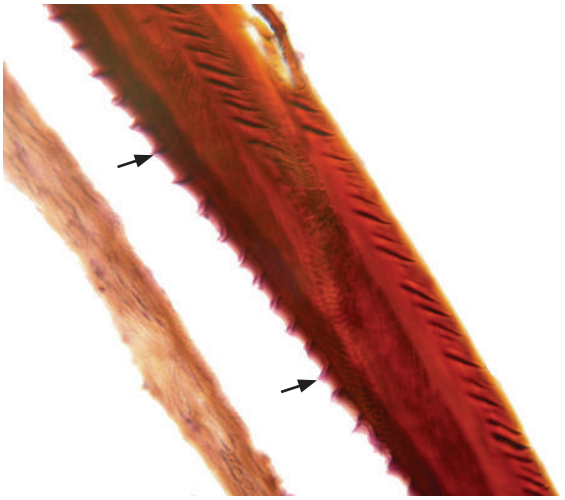


Fig. : 6.26 *Perca fluviatilis* (MT / HM)

Elasmoid (cycloid and ctenoid) scales are restricted to teleosts. They consist of an outer osseous part and an inner layer of parallel collagen fibers embedded in a proteinic extracellular matrix. The resulting bone is flexible and soft. This micrograph illustrates a fragment of ctenoid scale. The small stiff spines (*ctenii* - arrows) on its free posterior surface differentiate ctenoid from cycloid scales which lack such spines.

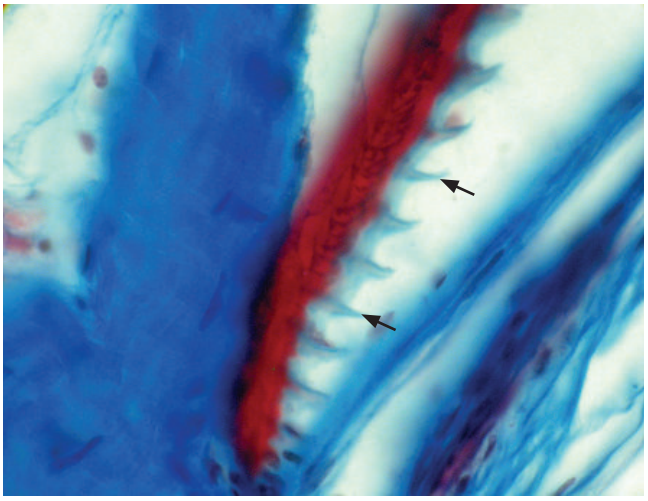


Fig. : 6.27 *Gnathonemus petersii* (MT / HM)

Fragment of ctenoid scale (red) showing clearly comb-like projections (*ctenii* - arrows) on the free exposed edges. Connective tissue stained blue.

7
Digestive system

The digestive system consists primarily of the digestive tract (gut), a long muscular-walled tube beginning at the mouth and terminating at the anus. From the oral cavity, the digestive tract extends through the body as the pharynx, esophagus, stomach and intestines. The other important components of the digestive system, namely the liver and the exocrine pancreas, will be described in the following chapter.

Oral cavity and pharynx

The oral cavity and pharynx (Figs 7.1 to 7.16) are lined by a stratified squamous epithelium containing abundant mucus-secreting cells (Figs 7.5 & 7.6) and often sensory cells forming taste buds (Figs 7.2 to 7.4 & 7.7). In a few species the pharynx epithelium is lined by a horny layer (Fig. 7.9). In most fishes, the tongue is poorly developed and reveals absence of specific muscles (Fig. 7.10). Often it is little more than connective tissue covered with an epithelium (Fig. 7.11) that contains many unicellular glands (Fig. 7.10). Cartilage is sometimes found particularly in Chondrichthyes.

Teeth (Figs 7.12 to 7.16) are generally homodont (all teeth with similar morphology), but many cases of heterodonty (dentition with different kinds of teeth) occur as in *Characidae*, *Sparidae*, *Blenniidae*… Most species have teeth that may be divided into three types, according to their position in the oral cavity. The jaw teeth are borne on the premaxilla and the maxilla bones above and on the dentary below. Oral teeth are borne by the vomer, palatine and ectopterygoid bones and sometimes by the tongue. The pharyngeal teeth represent the third type and develop from various sources; they are common in herbivores and molluscivores. The general form of teeth varies according to the fish feeding habits. They may be pointed, spherical, curved, dagger shaped, canine or molariform in shape. A few species of fishes are naturally toothless, e.g.: the sturgeons, seahorses and the pipefishes. The tooth consists of an enamel coating, a dentine layer and a pulp core.

General outline plan of the digestive tract

Compared to mammals, the teleost gastrointestinal tract is histologically simple and some fundamental points in the architecture of this tube are remarkably constant. The tube typically consists of four main layers (Figs 7.17 & 7.18):

1) a *mucosa*, lining the lumen of the tube and consisting of an inner epithelium, a middle *lamina propria* (a cellular connective tissue) and an outer *muscularis mucosae*;

2) a *submucosa*, a less cellular connective tissue layer with blood vessels, lymphatic tissue and nerve plexi. Submucosal glands are usually lacking in fishes;

3) a muscular coat which is often divided into an inner circular and an outer longitudinal layer; and

4) a *serosa* of connective tissue surrounded by the simple squamous peritoneal epithelium.

Esophagus

The esophagus (Figs 7.19 to 7.23) is short and thick-walled, the *muscularis* comprising interweaving striated muscle fibers that may extend as far as the stomach. The stratified cuboidal epithelium may be ciliated. Scattered through the various layers of the epithelium are single secretory cells (Fig. 7.20, 7.22 & 7.23) which release large amounts of neutral and acidic glycoconjugates which could have a role in the lubrication of the epithelium, in the interaction between *mucosa* and viruses and/or bacteria. In some fish, digestion has been observed to start in the esophagus and to go on in the stomach. Occasionally taste buds and chloride cells (Figs 7.22) are observed within the epithelium. The *mucosa* is thrown into longitudinal folds. The *serosa* contains prominent nerve fibers. Salivary glands are lacking, their place being seemingly taken in part by the uni-

cellular mucous cells. There are no glands in the connective tissue of the esophagus beneath the epithelium. Lymphoid tissue is frequently present in the *submucosa*. The *muscularis mucosae* usually consists of rather small bundles of longitudinally coursing smooth muscle cells. The *submucosa* contains both collagenous and elastic fibers. The muscular coat varies considerably in its degree of development. In many forms, the striated skeletal muscle passes very far caudally and serves as a muscular coat, both an inner circular and an outer longitudinal layer being present. Sturgeons and primitive teleosts are called physostomous fish because their swim bladder is connected to the esophagus by the pneumatic duct (Figs 7.25 & 7.26).

Stomach

The stomach (Figs 7.17, 7.27, 7.28, 7.31 & 7.34) of teleosts presents a variety of different shapes. In some teleosts, the entire gut appears as a tube of uniform diameter with no marked anatomical differences between the esophagus and stomach. In others, it appears as a round and muscular structure situated at the end of the esophagus. In a few others, the stomach can be divided into a cardiac and a pyloric part and possesses a blind sac of variable size, lying between the two.

The stomach itself is absent in many families (*Cyprinidae, Labridae, Gobiidae, Scaridae, Cyprinodontidae,* some *Poeciliidae* - Figs 7.29 & 7.30). When present, from the surface of the epithelium numerous gastric pits (crypts) sink down into the *mucosa* with the gastric glands opening at their bottom. The epithelium of the stomach surface and of the lining of the crypts consists of a single layer of high columnar, prismatic cells, the apical portion of which is clear and homogeneous in appearance with the H-E staining, but stains strongly with the PAS reaction (Fig. 7.28). These cells secrete a protective mucus. The glands in the stomach are of two distinct types : fundic and pyloric, the former extending over most of the *mucosa* of the body of the stomach, the latter being confined to the pyloric part. The fundic branched tubular glands possess but one type of cell. This gland cell contains acidophilic granules and produces both pepsin and hydrochloric acid (oxyntopeptidic cell). The pyloric glands are separated more widely from one another than are the fundic ones. They consist of shorter, less frequently branched tubules. Their epithelium closely resembles that of the surface of the stomach. The stroma of the *mucosa* contains numerous lymphocytes and eosinophilic granular cells. A *muscularis mucosae* is found and consists almost entirely of smooth muscle cells disposed longitudinally. The *submucosa* contains nerves, arteries, veins, and lymphatics and coarse eosinophilic granulocytes. The muscular coat is composed of inner circular and outer longitudinal coats of smooth muscle cells with sometimes an additionnal inner oblique layer.

Intestine

The segment of the digestive canal following the stomach is called the intestine (Figs 7.31 to 7.42). In contrast to mammals there is no marked distinction between small and large intestines in fishes.

Intestinal length is variable and is generally correlated with feeding habits; carnivorous species often have shorter intestines than herbivorous fish, and in these latter extensive coiling takes place. Some species of bony fishes have an intestine with a relatively smooth surface. Other have longitudinal folds or folds forming a rather complex pattern or network and still others have *villi*, very similar to those found in higher vertebrates. A *villus* (Figs 7.32 & 7.33) is a finger-like process of the *mucosa* which consists of an epithelial covering and a core of connective tissue containing blood and lymph capillaries. In fishes which possess *villi*, there may be considerable variation in their form between different species. The intestinal epithelium (Figs 7.32 & 7.33), of the simple or pseudostratified columnar type, is made up of cells possessing a well-marked striated border (*microvilli*) and goblet (mucus-secreting) cells. The former are both absorptive and secretory in function. Ciliated cells occurring among the ordinary prismatic cells of the intestine have been described in some teleosts. A clear dis-

tinction between the epithelium on the *villi* and that in the simple tubular invaginations from the surface is lacking in fishes, thus no true glands of LIEBERKUHN are present. However, such glands were described in Gadiformes. The *lamina propria* and *submucosa* of several species contain large numbers of wandering eosinophilic granular cells and variable quantities of lymphoid tissue. The role of the eosinophilic granular cells, which in some respects are analogous to mast cells and stain similarly, is becoming clearer. They contain antimicrobial peptides and their degranulation can increase the vascular permeability and promote neutrophil adhesion, suggesting that they are intimately involved in innate immunity and inflammation. The *muscularis mucosae* when present usually consists of a thin layer of smooth muscle cells, longitudinal in direction. The *submucosa* generally is composed of an loose connective tissue containing blood vessels and wandering cells (see above). In the majority of fishes, the muscular coat (smooth muscle) of the intestine has become well developed to insure peristaltic activity. The terminal section of the intestine is usually slightly widened into a rectum. Many species have a number of *diverticula* extending from the anterior part of the intestine close to the pylorus. These blind-ending structures are the pyloric caeca (Figs 7.34 to 7.37). They possess a multi-folded intestinal type epithelium and seem to be chiefly a device for increasing the area for the absorptive processes and the duration food stays in the intestine.

It must be borne in mind that in elasmobranchs (sharks, rays) Holosteans (gars, bowfins) and Chondrosteans (sturgeons) the *mucosa* and *submucosa* form a prominent spiral fold, called the spiral valve (Figs 7.38 to 7.40), throughout most of the intestine. This fold forces the food to spiral on its course through the intestine, rather than passing directly down its length, thus prolonging its contact with the intestinal walls. The rectum (Figs 7.41 & 7.42) may unload into a cloaca, but in higher fishes (as in mammals) the openings of the digestive system and of the urogenital system are separate (Fig. 7.42).

In elasmobranchs, a rectal gland opens into the rectum. Although this gland is not directly involved in digestion, it eliminates excess salt ingested during feeding (see chapter 11).

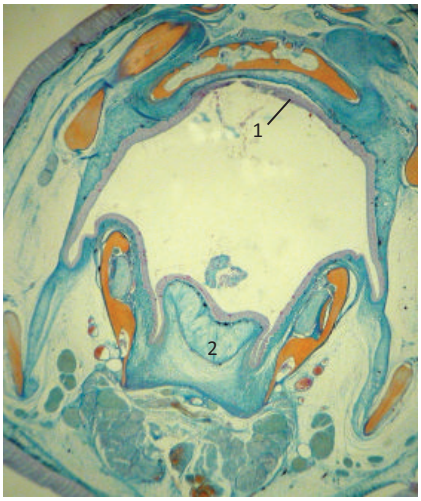


Fig. : 7.1 *Gnathonemus petersii* (FR-HB / LM)

General view of the buccal cavity. This most anterior part of the digestive tract is lined by a stratified epithelium (1) which contains numerous taste buds and covers the tongue (2). Connective tissue is stained in blue and jaw bone pieces are in orange.

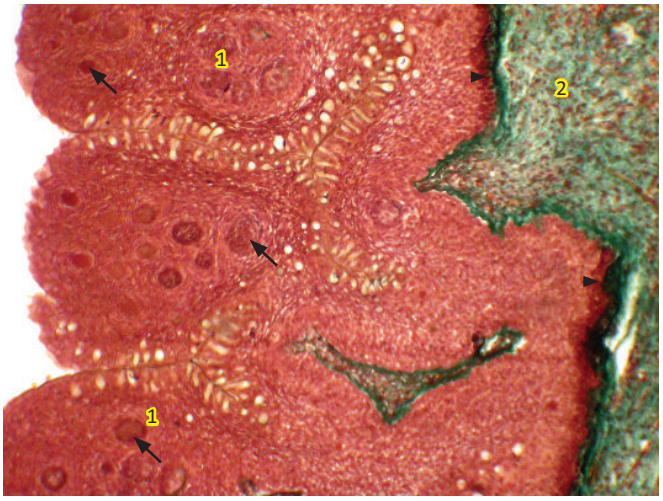


Fig. : 7.2 *Cyprinus carpio* (MT / MM)

Section of oral epithelium. The lining of the roof of the buccal cavity is made up of *mucosa* (1), *submucosa* (2) and *muscularis* (not visible). The *mucosa* comprises many layers of stratified polygonal epithelial cells. Taste buds (arrows) and a large number of mucous cells (unstained) are present in between the stratified cells. The connective tissue of the *submucosa* is stained pale green and the deep green basement membrane (arrowheads) is well-marked just beneath the epithelium.

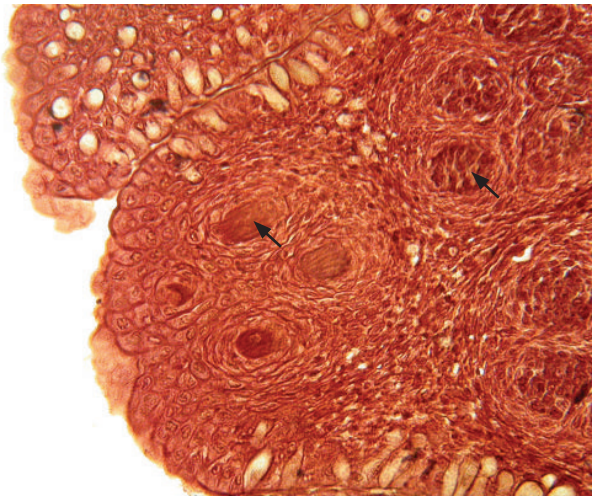


Fig. : 7.3 *Cyprinus carpio* (MT / HM)

Higher magnification of the previous document. This semi-tangential section through the *mucosa* of the buccal cavity shows numerous taste buds (arrows) and pale mucus-secreting cells. The other cells are ordinary cells constituting the stratified epithelium.

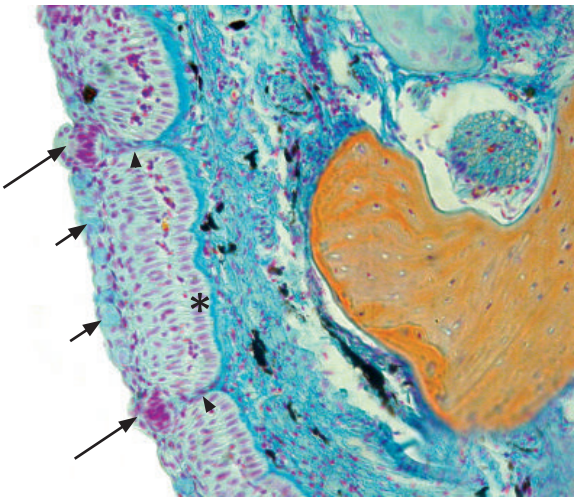


Fig. : 7.4 *Gnathonemus petersii* (FR-HB / MM)

Buccal cavity. This photomicrograph shows on the left the squamous stratified epithelium lying on the *submucosa* (in blue). This epithelium is plenty of mucous-secreting cells (pale blue – short arrows) and reveals two taste buds (long arrows). These are on long stalks (arrowheads) extending from the basement membrane and connective tissue below. A piece of bone (orange) and chromatophores (black) are also seen.

∗ : well-defined basal layer of the buccal epithelium.

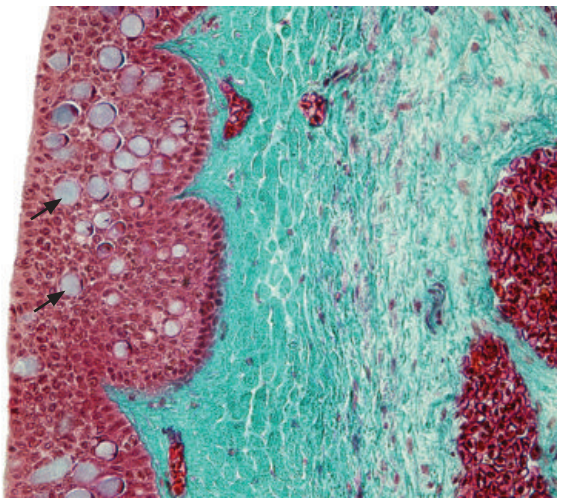


Fig. : 7.5 *Scyliorhinus canicula* (MT / MM)

The pharynx is lined with stratified epithelium. Unicellular mucous cells (arrows) are abundant and scattered among the ordinary epithelial cells. The dense connective tissue of the *submucosa* (turquoise) is thick and comprises homogeneous collagen fibers and some fibrocytes. On the right two veins filled with erythrocytes.

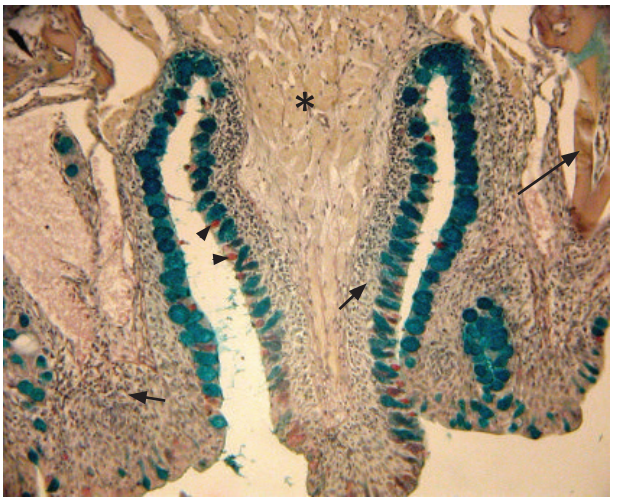


Fig. : 7.6 *Pelvicachromis pulcher* (AB-PAS-H / MM)

Posterior part of the pharynx. This micrograph demonstrates the abundance of AB+ mucus-secreting cells (in blue) lining two mucous glands. A few cells are stained only in red (PAS+ - arrowheads). Both AB and PAS reactions are classical techniques used in carbohydrate histochemistry for detection of mucous substances particularly. The other cells of the stratified epithelium are in grey (short arrows).

Skeletal muscle (∗) in transverse section and an immature tooth (long arrow) at the right corner are displayed.

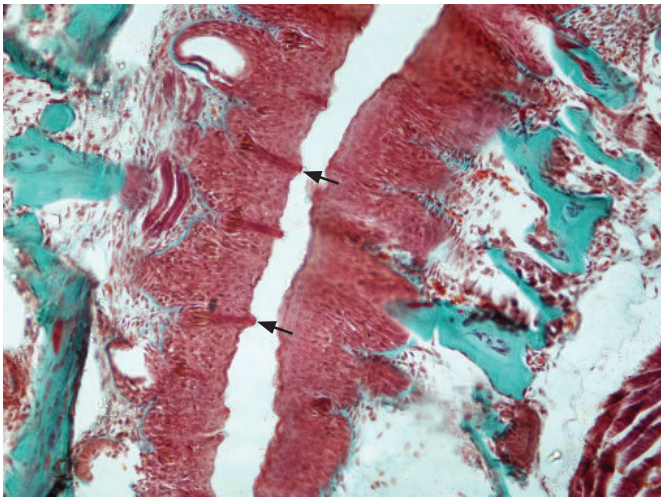


Fig. : 7.7 *Poecilia reticulata* (MT / MM)

The histological features of the pharynx are similar to those of the buccal cavity. Each of these regions has a stratified epithelium containing saccular mucus-secreting cells keeping moist these areas subjected to mechanical abrasion. In addition this picture emphasizes numerous elongated taste buds (arrows - see also Fig. 15.29). Jaw bones are in turquoise.

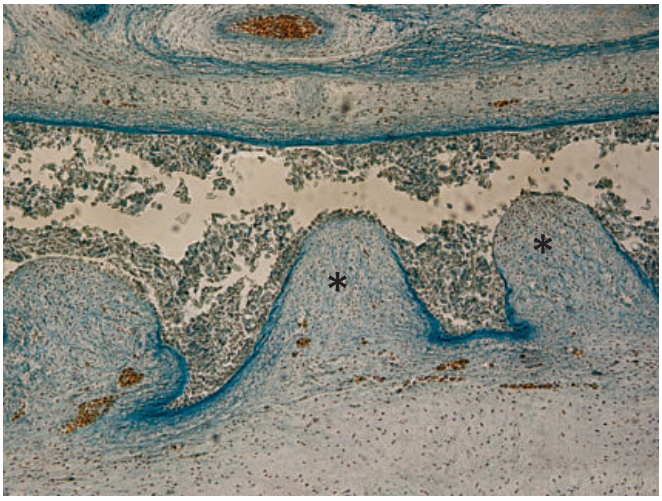


Fig. : 7.8 *Scyliorhinus canicula* (AB-H-OR / MM)

Pharynx. Some species have pharyngeal buds (∗) covered by a stratified epithelium and supported by a thick fibromuscular layer. These structures may help to swallow food by squeezing it to extract a maximum of water.

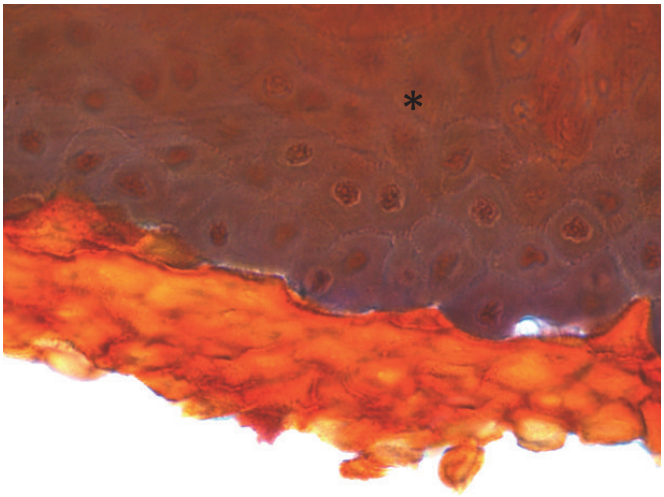


Fig. : 7.9 *Danio rerio* (MT / HM)

In some cyprinids the posterior stratified pharynx epithelium (∗) is covered with a «horny pad» (orange). Stomachless fish like the zebrafish usually possess four pairs of teeth (pharyngeal teeth) on their inferior pharyngeal bones. Food is triturated between these teeth and the horny epithelium.

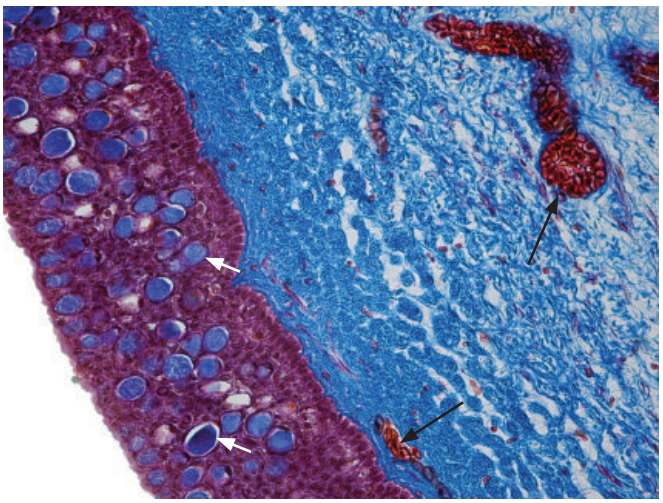


Fig. : 7.10 *Scyliorhinus canicula* (MT / MM)

«Tongue». Chondrichthyes lack a true flexible and muscular tongue and for processing prey they use a diversity of jaw muscles. They possess only a mucosal thickening at the anterior part of the floor of the bucco-pharyngeal cavity. The *mucosa* is thick and resembles that of the pharynx. Mucous cells (white arrows) are particularly abundant and nearly as numerous as the ordinary epithelial cells. The *stratum compactum* of the supporting connective tissue (in blue) is well-developed and contains various blood vessels (black arrows).

This tongue-like structure is also supported by a centrally located piece of hyaline cartilage (not visible – see Fig. 2.17).

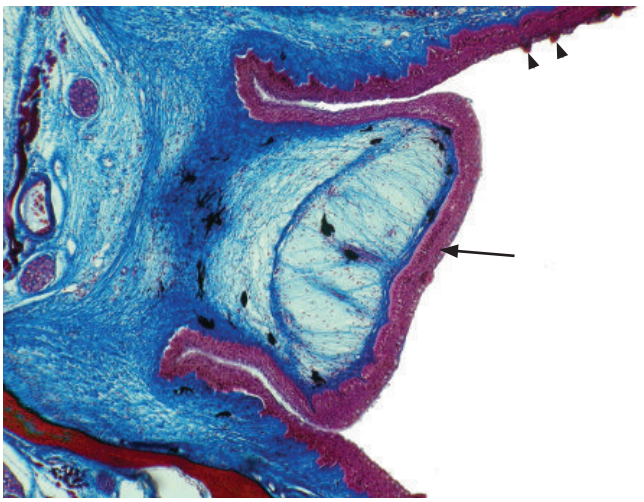


Fig. : 7.11 *Gnathonemus petersii* (MT / MM)

Tongue. Transverse section of the anterior part of the buccal cavity. The fish tongue usually appears as a mucosal thickening without muscular fibers. The tongue epithelium - a continuation of the oral epithelium - contains several layers of cells (arrow) within which taste buds and unicellular gland cells are distributed. The thick *lamina propria* is composed of loose and dense connective tissue (blue) which continues laterally and ventrally extending up to the ventral jaw muscles. Note the total absence of skeletal muscular tissue.

Chromatophores are stained in black. Piece of jaw bone in red at the lower left.

The arrowheads point to taste buds of the oral epithelium.

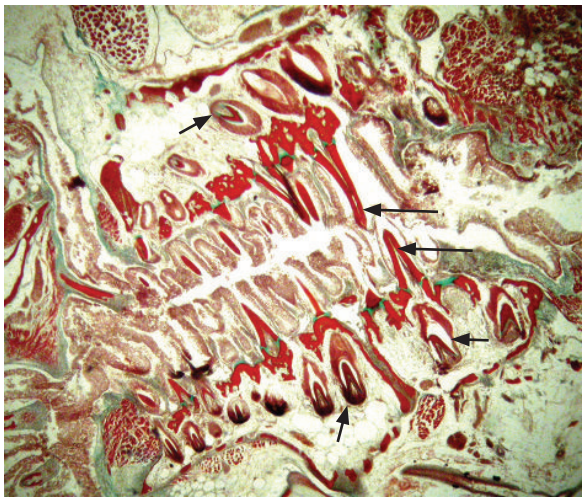


Fig. : 7.12 *Pelvicachromis pulcher* (MT / LM)

The five following documents (Figs 7.12 to 7.16) illustrate jaw cavity containing teeth in various stages of development. Fish teeth are generally homodonte but many examples of heterodonty occur. This general view shows several pointed mature (long arrows) and non-mature (short arrows) homodonte teeth.

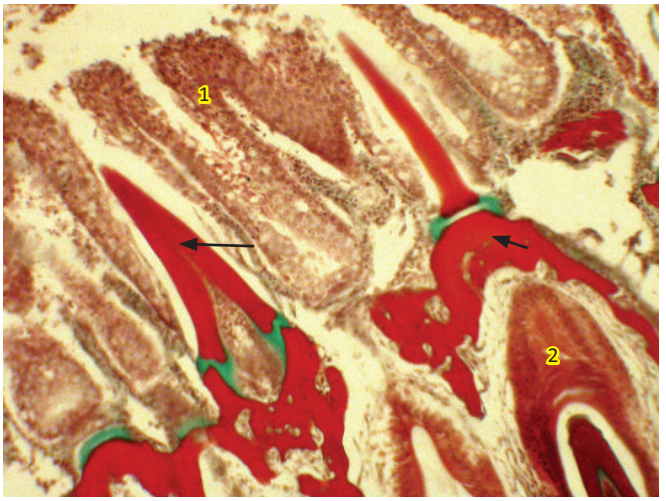


Fig. : 7.13 *Pelvicachromis pulcher* (MT / MM)

According to the feeding habits fish teeth can be pointed, spherical, curved, molariform ... but in most cases, like here, a typical tooth has a broad base (short arrow) and a pointed apex (long arrow) which is characteristic of this carnivorous cichlid.

1 : buccal stratified epithelium
2 : immature tooth

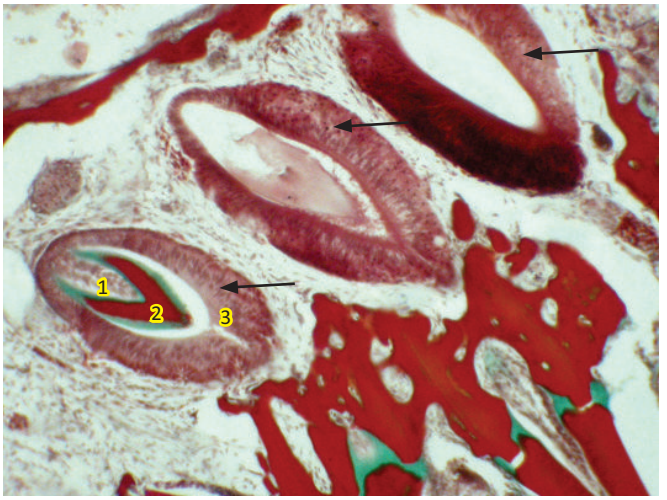


Fig. : 7.14 *Pelvicachromis pulcher* (MT / MM)

The teeth of most fish are polyphyodont i.e. they are continuously replaced. This picture illustrates three immature teeth (arrows) originating from the base of worn out ones. On the non-mature tooth located at the left one can recognize the pulp core (1), the dentine (2 - secreted by odontoblasts) and the inner epithelium (3) with ameloblasts secreting the enamel cap.

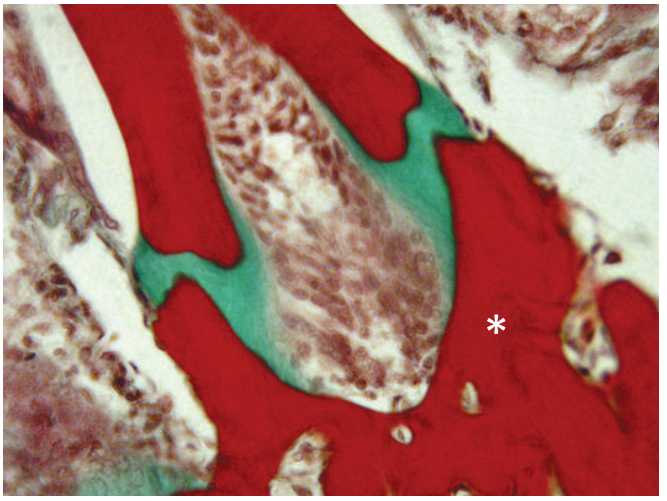


Fig. : 7.15 *Pelvicachromis pulcher* (MT / HM)

Fish teeth are mostly in depression and may be immovable or slightly to freely movable. This micrograph illustrates a semi-movable tooth whose base is fixed on underlying bone (*) by fibroelastic ligaments (in green) allowing both its forward and backward movement. Note the pulp core at the centre.

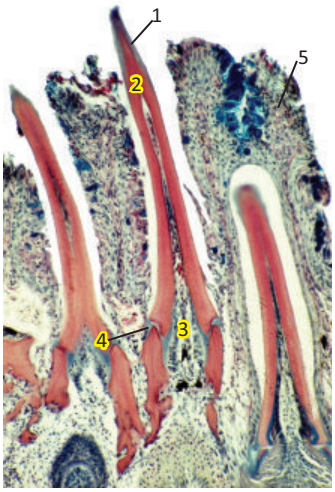


Fig. : 7.16 *Astatotilapia burtoni* (AB-PAS-H / MM)

Elongated and pointed teeth of a mainly carnivorous cichlid. Adult fish tooth usually consists of an enamel cap (1), a dentine layer (2) and a pulp core (3). The teeth here illustrated are more or less movable as it is testified by the presence of basal ligaments (4 – see previous image). The oral stratified epithelium (5) containing numerous mucous cells AB+ (blue) and/or PAS+ (magenta) is also displayed.

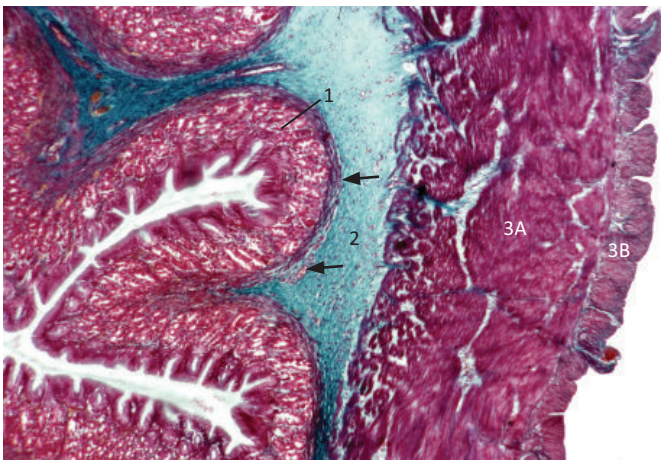


Fig. : 7.17 *Perca fluviatilis* (MT / MM)

Stomach. This image and the next one demonstrate the typical appearance of the gut wall cut transversely. Although each portion of the alimentary tract has its own characteristic feature, the digestive tract as a whole is composed of four layers :

- the *mucosa* (1) consisting of an epithelium, a thin connective *lamina propria*, and a smooth *muscularis mucosae* (arrows);
- the *submucosa* (2), a fairly dense connective layer binding the *mucosa* to the muscular wall;
- the *tunica muscularis*, with its typical arrangement of two perpendicular layers (inner circular – 3A and outer longitudinal – 3B) of smooth muscle;
- the *serosa* or *adventitia* lined by a simple squamous epithelium being supported by a thin connective layer (see Fig. 7.18).

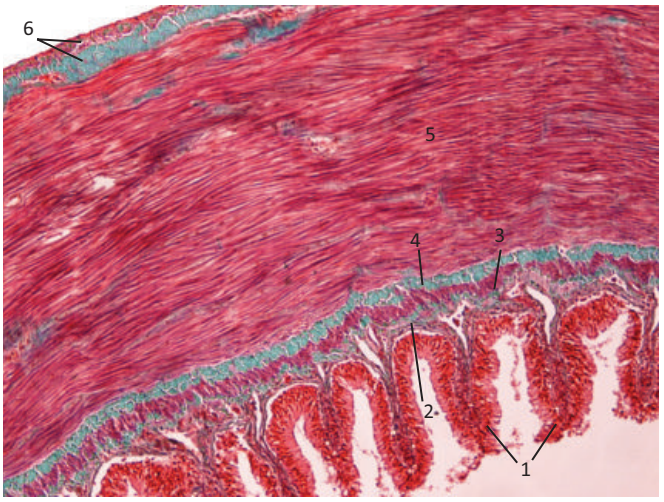


Fig. : 7.18 *Scyliorhinus canicula* (MT / MM)

Transverse section of the spiral valve of the intestine. Sometimes, as shown in this picture, a thin smooth muscle layer (*muscularis mucosae* - 3) is well-defined and clearly separates the *submucosa* (4) from the *lamina propria* (2).

1 : folds of the *mucosa*;

5 : thick layers of smooth muscle (*muscularis*); note that the outer longitudinal layer is not visible at this level;

6 : *serosa* (*mesothelium* + turquoise collagen)

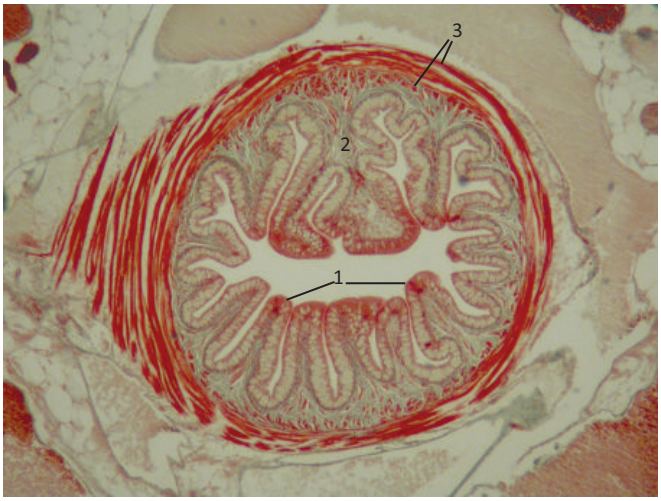


Fig. : 7.19 *Astatotilapia burtoni* (MT / MM)

Section of the esophagus. In this general view, the mucosal epithelial folds (1), *submucosa* (2) and *muscularis* (3) are shown.

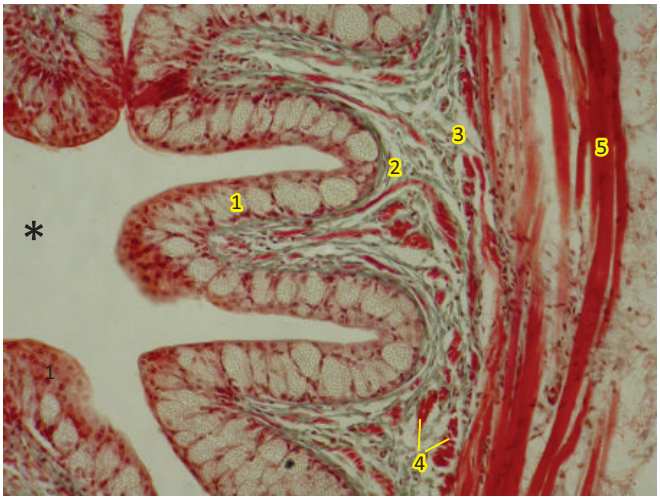


Fig. : 7.20 *Astatotilapia burtoni* (MT / HM)

Detail of the previous document showing esophageal wall. The lumen (∗) of the esophagus is lined by a protective stratified epithelium (1) containing a huge amount of goblet cells (= mucous cells, unstained). The mucus probably plays an important role in lubrication. The *lamina propria* (2) and the *submucosa* (3) are more or less separated by some *fasciculi* of the *muscularis mucosae* (4). Collagen is stained in green and the *muscularis* is present on the right (5).

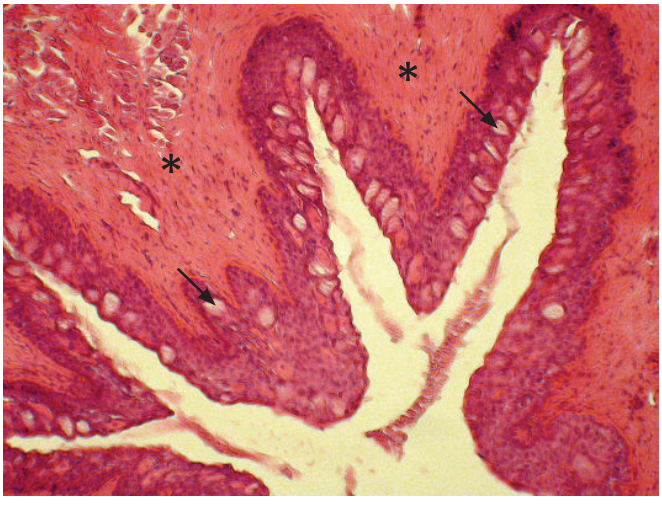


Fig. : 7.21 *Anguilla anguilla* (H-E / MM)

In a relaxed condition the esophageal lumen is irregular in transverse section but becomes distended with the peristaltic movement of the bolus. The principal components noted are the surface stratified epithelium containing numerous mucous cells (unstained - arrows) supported by a mixed *lamina propria-submucosal* layer (∗) considering the absence of a true *muscularis mucosae*.

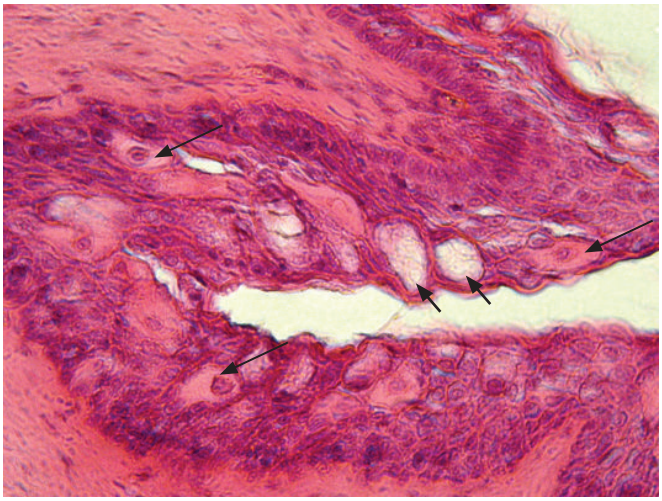


Fig. : 7.22 *Anguilla anguilla* (H-E / HM)

A view of a broad fold of the anterior esophagus showing mucous cells (short arrows). In euryhaline species like the European eel, large chloride cells (long arrows) can be found at this level of the digestive tract. These eosinophilic cells involved in osmoregulation (see chapters 10 & 11) are usually rounded with a large central nucleus («fried egg appearance»).

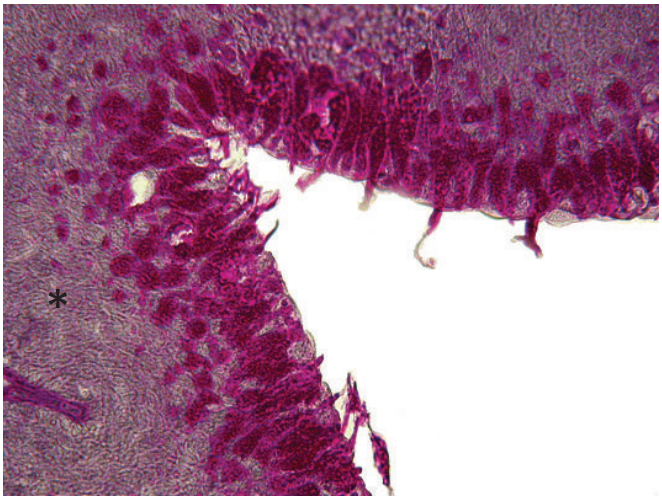


Fig. : 7.23 *Anguilla anguilla* (PAS-H / HM)

Posterior esophagus. The abundant mucous cells located close to the lumen produce large quantities of mucin which strongly stain magenta with the PAS reaction. Their number increases considerably in the middle and posterior parts of the esophagus. The other cells (∗) of the stratified epithelium are the ordinary epithelial cells found along the anterior digestive tract.

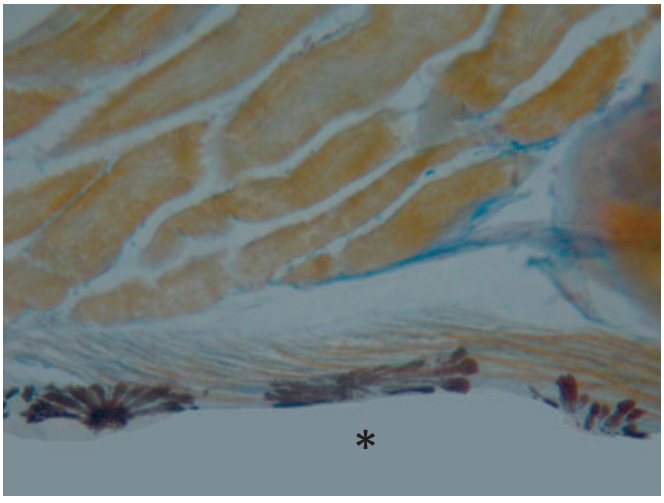


Fig. : 7.24 *Poecilia reticulata* (FR-HB / MM)

The *serosa* actually begins where the gut enters the peritoneal cavity (∗). This photomicrograph emphasizes the presence of chromatophores (in black) within the parietal layer (squamous epithelium) of the *peritoneum*. In orange, rhabdomyocytes of the dorsal musculature.

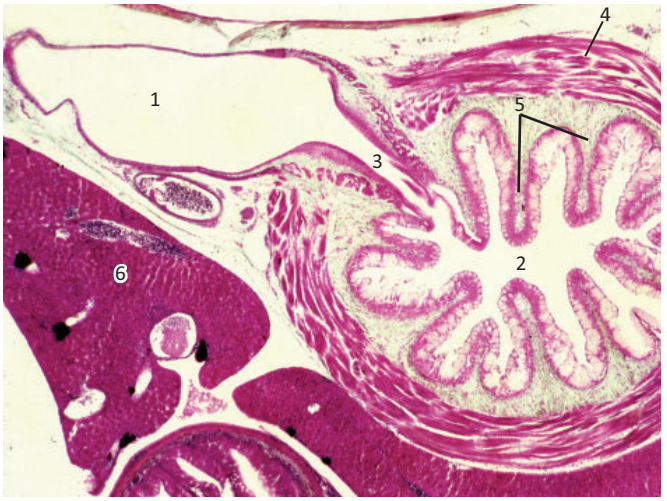


Fig. : 7.25 *Petrocephalus microphthalmus* (MT / LM)

This transverse section remarkably shows the connection between the gas bladder (1) and the esophagus (2). This gas bladder is said physostomous because it is connected to the foregut (esophagus) by the pneumatic duct (3). This type of bladder is characteristic of sturgeons and primitive teleosts. *Tunica muscularis* of the esophagus (4), folds of the esophageal *mucosa* (5) and liver parenchyma (6) are also seen.

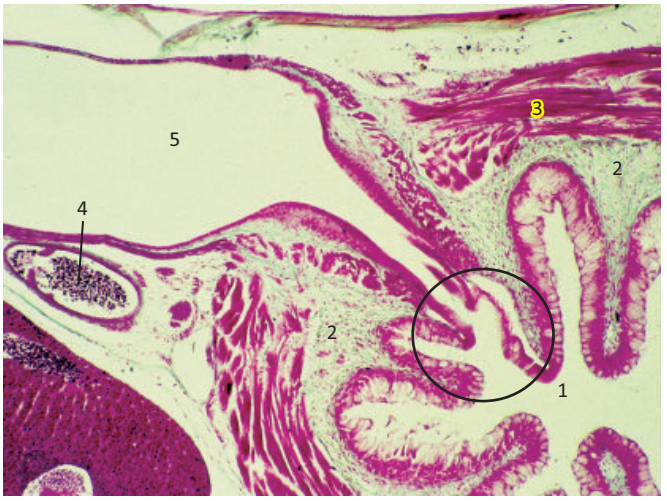


Fig. : 7.26 *Petrocephalus microphthalmus* (MT / MM)

Detail of the previous image. The circle clearly indicates the opening of the pneumatic duct into the esophagus (1). Note in pale green the collagen of the esophageal *submucosa* (2) surrounded by the two smooth muscular layers of the *muscularis* (3). A large vein with numerous blood cells is shown in 4.

5 : gas bladder.

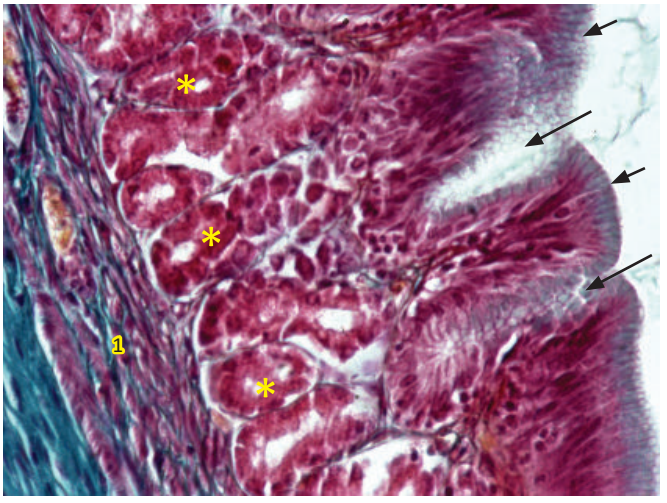


Fig. : 7.27 *Perca fluviatilis* (MT / MM)

Stomach wall in transverse section. This image shows the single-layered mucous epithelium (short arrows – mucus in grey-blue) lining this organ. Invaginations of the epithelium form gastric pits (long arrows) in which multiple tubular glands drain. These gastric glands (∗) are lined with a single type of secretory cell and extend throughout the sub-epithelial (*lamina propria*) connective tissue. A *muscularis mucosae* (1) is obvious and the collagen in the *submucosa* is stained turquoise.

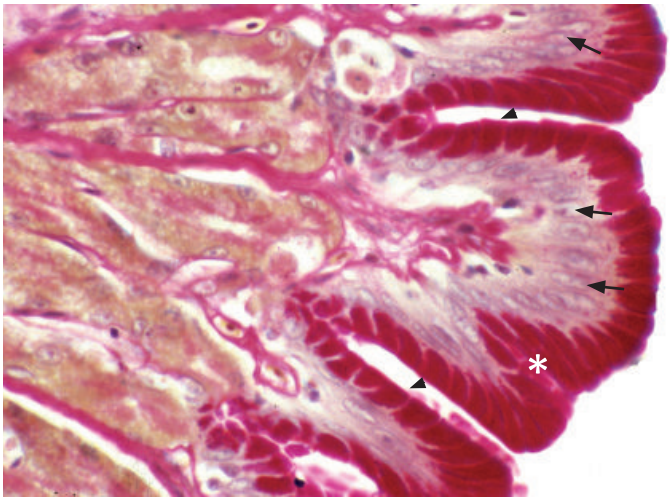


Fig. : 7.28 *Schilbe mystus* (PAS-H-AUR / HM)

This transverse section of the stomach wall demonstrates the typical appearance of the epithelium which lines the gastric lumen. This epithelium is formed by a single layer of elongated columnar mucus-secreting cells (arrows). As it is clearly shown, the mucus (hydrated glycoproteins - ∗) is very abundant and intensely stained with the PAS reaction. The mucus protects the stomach from self-digestion and fills the apical cytoplasm of all cells. On this picture, two gastric pits (arrowheads) are formed by the invaginations of the mucosal layer into the *lamina propria*. The gastric glands (to the left - orange) are present in such abundance that they almost occupy the entire mucosal layer beneath the superficial epithelium. They possess cells of one single type, called oxyntopeptidic cells which are never differentiated into parietal and peptic cells as in mammals.

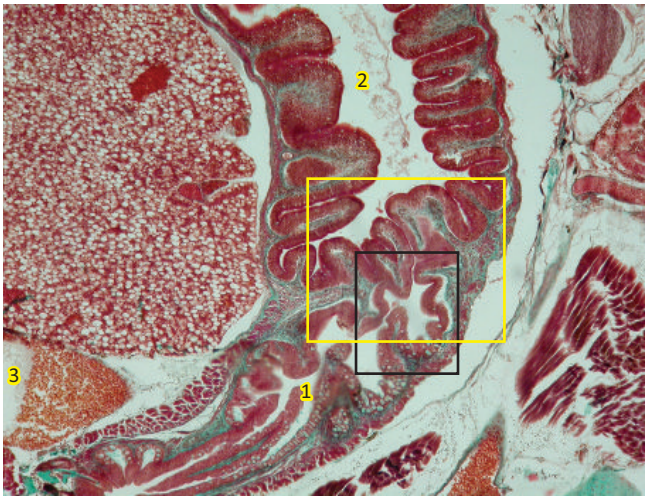


Fig. : 7.29 *Poecilia reticulata* (MT / LM)

Longitudinal section. Many fish like the guppy are stomachless : the intestine (2) directly connects to the pharynx (1) through a short esophagus (black square). The stratified squamous epithelium lining the esophagus is replaced by a single epithelium of intestinal type. On the left, hepatic tissue (white spotted) and a part of the *sinus venosus* (3 - erythrocytes in orange) are found.

Higher magnification of the yellow rectangle below.

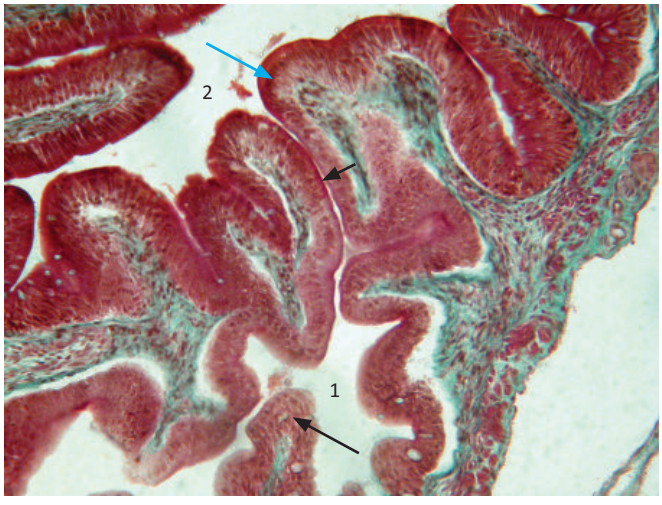


Fig. : 7.30 *Poecilia reticulata* (MT / MM)

Higher magnification of the yellow rectangle of the previous document showing the esophagus-intestine junction (narrow duct - short arrow). The esophageal stratified epithelium (long black arrow) transforms here into a simple absorptive epithelium (cyan arrow) mainly composed of columnar cells (enterocytes – see below). Some bundles of smooth muscle (red) are scattered in the green connective tissue.

(1) and (2) seat respectively in the lumen of the esophagus and of the intestine (duodenum).

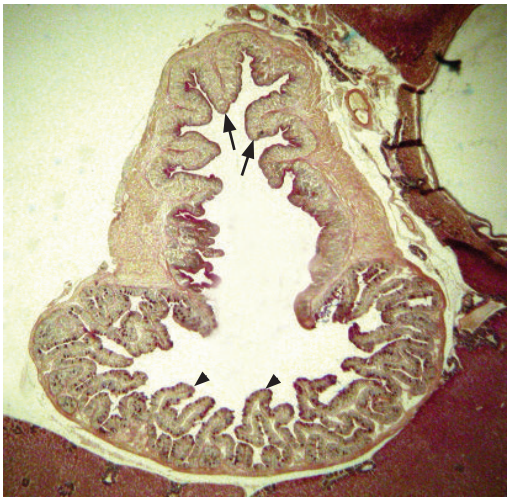


Fig. : 7.31 *Petrocephalus microphthalmus* (AB-PAS-H / LM)

This low magnification illustrates the gastro-intestinal junction cut longitudinally. The short and large folds of the posterior end of the stomach (arrows) are replaced by *villi*, finger-like projections (arrowheads) in the wider lumen.

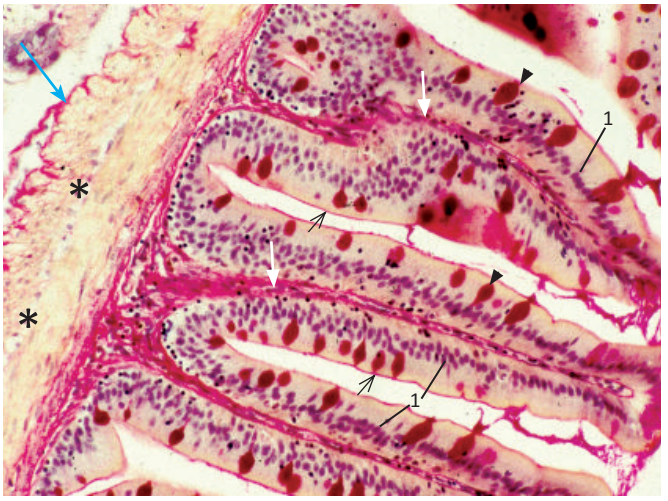


Fig. : 7.32 *Atherina boyeri* (PAS-H-AUR / MM)

Intestine. This micrograph displays three elongated *villi*, deep finger-like processes of the intestinal *mucosa* extending in the organ lumen. These expansions are lined by a simple columnar epithelium comprising mainly absorptive cells (enterocytes – 1) and mucus-secreting or goblet cells (arrowheads). The PAS reaction for neutral glycoconjugates produces an intense magenta stain in the mucous granules of goblet cells and emphasizes the brush border (thin arrows) of the enterocytes. The villous core (white arrows) is filled with connective tissue of the *lamina propria* containing blood and lymph capillaries. On the left the orange *tunica muscularis* (the two perpendicular layers are separated by enteric nerves ∗) is covered by the *serosa* (magenta – blue arrow).

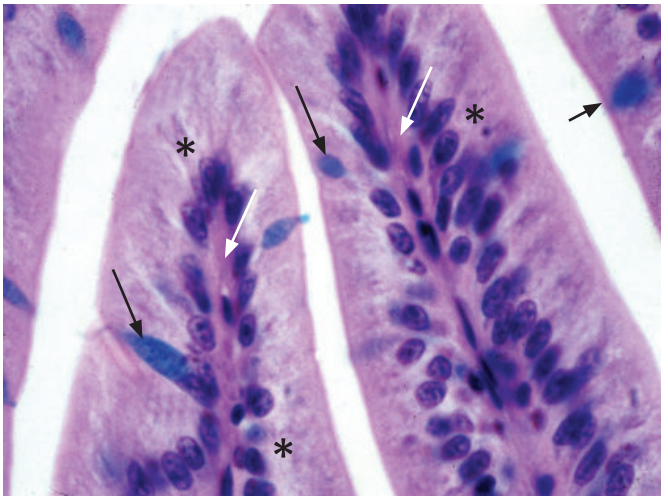


Fig. : 7.33 *Kryptopterus bicirrhis* (AB-H-E / HM)

Intestinal epithelium, distal ends of two *villi*. In this picture the columnar absorptive cells (enterocytes - ∗) are the most numerous. Their plasma membranes are not discernible, but their (dark purple) nuclei are easily visible and usually lie just below the centre of the cell. Thanks to the AB technique for acid mucosubstances, the goblet cells show a strong positive stain (blue - long arrows). The white arrows point to the villous core consisting of connective tissue of the *lamina propria*.

Short arrow show the brush border at the luminal surface of the enterocytes.

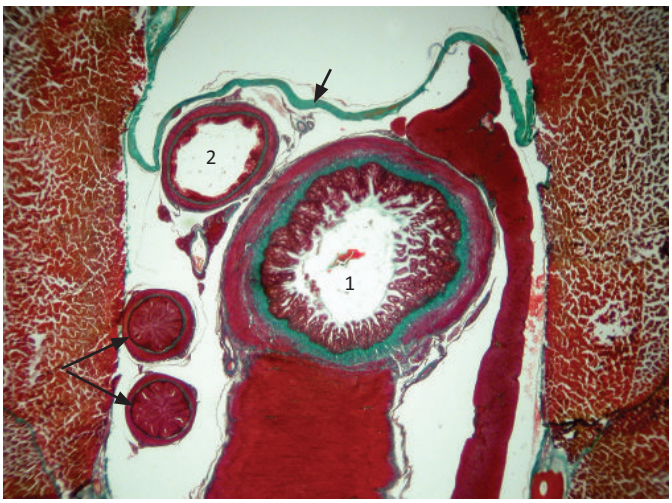


Fig. : 7.34 *Gnathonemus petersii* (MT / LM)

Transverse section at the abdominal level. This picture shows two pyloric *caeca* (long arrows) seated in the peritoneal cavity. These blind-ending structures increase the area of food absorption. The number of *caeca* varies from zero to several hundreds and the elephant nose fish has only two of them.

Note at the centre the small stomach (1) surrounded by green connective tissue and the gall bladder (2).

In mormyrids the abdominal cavity is quite curiously dorsoventrally divided in two parts by a connective *septum* («diaphragm» - short arrow).

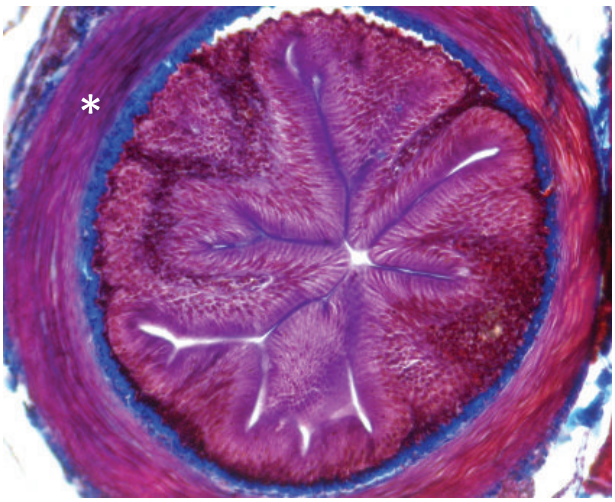


Fig. : 7.35 *Gnathonemus petersii* (MT / HM)

Cross section through one of the two short pyloric *caeca* of this species. The caecal *mucosa* is richly folded filling the biggest part of the lumen. The histological and histochemical features of the *caeca* remind the structure of the intestinal wall from which they issue.

The *submucosa* (blue) and the thick *muscularis* (∗) consisting mainly of a circular layer of leiomyocytes (see chapter 3) are easily recognized.

Remember that the number of *caeca* is not always constant even within a same teleost species.

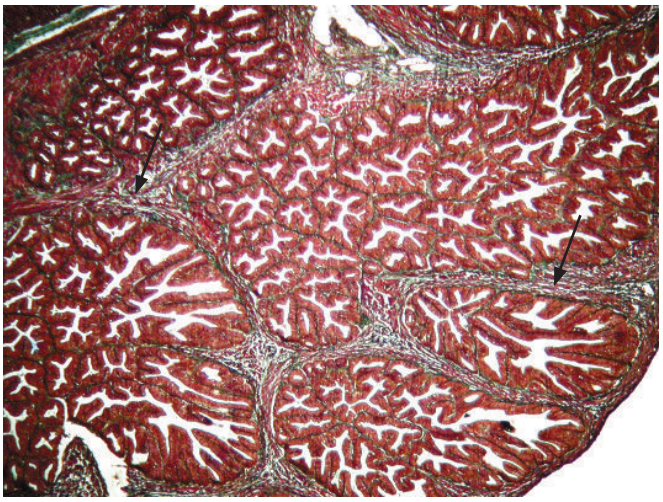


Fig. : 7.36 *Acipenser gueldenstaedtii* (MT / LM)

Transverse section among a lot of pyloric *caeca*. Along the proximal intestine of many fish species are blind-ending diverticula extending from the anterior part of the intestine close to the pylorus. These pyloric *caeca* have secretory and absorptive functions. In sturgeons each of these appendices is surrounded by a delicate connective tissue (see next figure) and the *caeca* are grouped together in clusters separated by thicker connective sheaths (arrows).

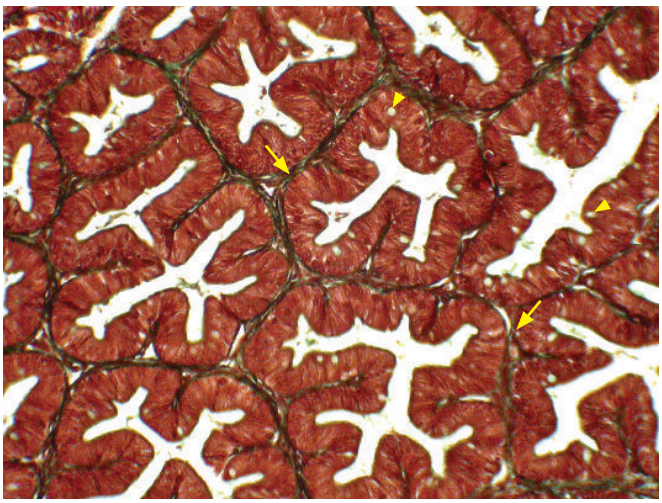


Fig. : 7.37 *Acipenser gueldenstaedtii* (MT / MM)

Pyloric *caeca* in transverse section. Considering the relatively short length of the adult gut, absorptive surface area is increased by the presence of the spiral valve (see below) and pyloric *caeca*. The richly folded caecal *mucosa* edges an irregular (unstained) lumen. The epithelial cells are typically columnar in shape (enterocyte-like cells) and some small goblet cells are also present (yellow arrowheads). A delicate connective tissue (arrows) surrounds each caecal portion.

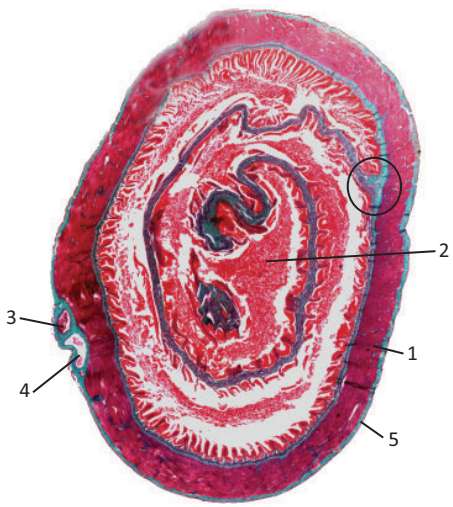


Fig. : 7.38 *Scyliorhinus canicula* (MT / LM)

Spiral valve (transverse section). In sharks (but also in sturgeons, lungfishes, bichirs, garpikes) the lower portion of the intestine is fitted with a spiral valve. This structure is a differentiation of the *ileum* and is internally coiled or twisted to increase the surface for digestion and absorption in fish with short intestine. One can see the place (circle) where the *submucosa* (in blue) covered by the *mucosa* begins to sweep into the (unstained) lumen to form the long spiral fold.

1 : *muscularis* of the *ileum* - 2 : food particles in the lumen – 3 : intestinal artery – 4 : intestinal vein – 5 : *serosa*.

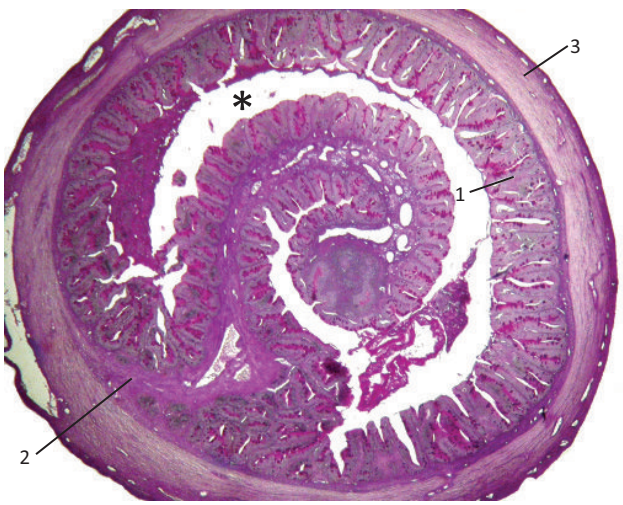


Fig. : 7. 39 *Acipenser gueldenstaedtii* (PAS-H / LM)

Transverse section through the spiral valve. The spiral valve of the sturgeon occupies the majority of the lumen (unstained space - ∗) of the lower intestine. Like sharks sturgeons compensate their relatively short intestine by having a spiral valve which slows down the digestible food for a better absorption. Folded *mucosa* (1), connective *submucosa* (2) and *muscularis* (3) are shown.

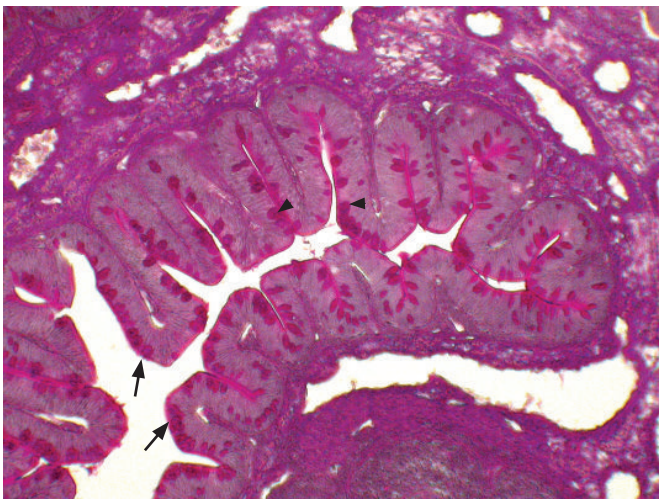


Fig. : 7.40 *Acipenser gueldenstaedtii* (PAS-H / MM)

As the spiral valve is derived from the *ileum*, there is no noticeable difference in the microscopic anatomy of their *mucosa*. The spiral valve shows a folded *mucosa* (arrow) with numerous goblet cells (magenta - arowheads) and supported by connective tissue.

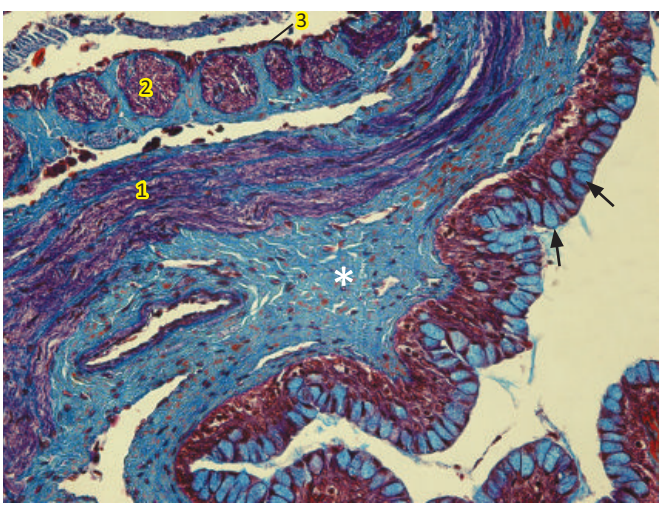


Fig. : 7.41 *Scyliorhinus canicula* (MT / MM)

Transverse section. In sharks, the hind end of the intestine runs into a short rectum before opening into the cloaca. The rectum is lined with a stratified epithelium housing numerous side-by-side mucous cells (arrows). The *mucosa* folds become more or less flat and the number of goblet cells increases considerably. The well-vascularized *lamina propria-submucosa* layer (∗ - note that the *muscularis mucosae* is very reduced) and the *muscularis* are very thick. This latter consists of usual inner circular (1) and outer longitudinal (2) layer of smooth muscle fibers lined by the *serosa* (3).

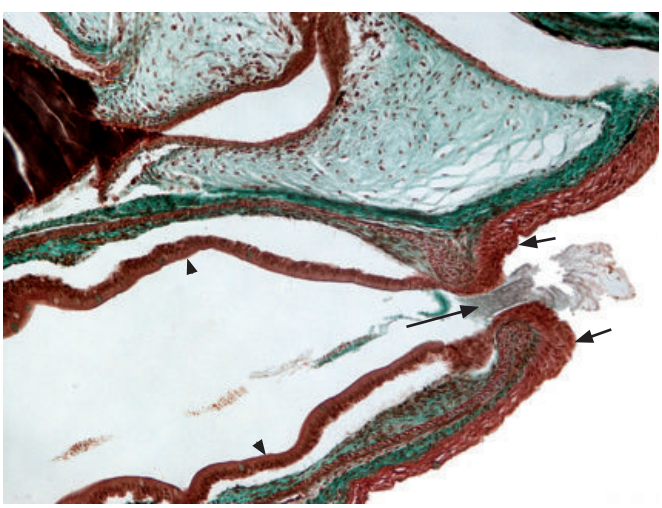


Fig. : 7.42 *Poecilia reticulata* (MT / MM)

Parasagittal section of the hind end of the digestive tract. The rectum is often difficult to distinguish from the intestine as well externally as internally. The intestinal structure simplifies in the distal intestine (rectum), where the *mucosa* flattens (arrowheads) and opens to the exterior through the anus (long arrow). The anus is lined on both sides by skin folds (short arrows) and situated immediately anterior to the genital pore (see also Fig. 14.9). Dense (deep green) and loose (pale green) connective tissues are fairly abundant.

8

Glands associated with the gastrointestinal tract

The major glands of the digestive system in fish are the liver and the pancreas.

Liver

The liver (Figs 8.1 to 8.8) is the largest of the extramural (outside the alimentary canal) organs. Fish liver serves functions similar to those in mammals. Its functions include assimilation of nutrients, production of bile, detoxification, maintenance of the body metabolic homeostasis that includes processing of carbohydrates, proteins, lipids and vitamins. The liver also plays a key role in the synthesis of plasma proteins, like albumin, fibrinogen, and complement factors. The histology of the liver varies among species, but there are general features that are found in the majority of species.

The parenchyma of the organ is contained within a thin capsule of fibroconnective tissue. The parenchyma itself is primarily composed of polyhedral hepatocytes typically with central nuclei. Glycogen deposits (Fig. 8.3) and fat storage (Figs 8.4 & 8.5) often dissolved during the routine histological process, produce considerable histological variability. The histology of fish liver differs from the mammalian in that there is far less tendency for disposition of the hepatocytes in lobules and the typical portal triads of the mammalian liver are rarely seen. Sinusoids (Fig. 8.2) are lined with endothelial cells forming a very thin cytoplasmic sheet. The nuclei of these cells are elongated and protrude into the sinusoidal lumen. The endothelium is fenestrated by small pores. Macrophagic KUPFFER cells are not found in teleosts, although cells capable of ingesting foreign particles were described among the hepatocytes of a few species (*Salmonidae*). Bile ducts (Figs 8.3, 8.6 to 8.8) also occur within the parenchyma of the liver. Originating between adjacent hepatocytes, bile *canaliculi* anastomose to produce ducts of increasing diameter. The ducts merge and almost always end (except some sharks and skates) in the gall bladder (Figs 8.9 & 8.10), lined by a pseudostratified epithelium (Fig. 8.11). The bile drains into the duodenum by the common bile duct. Smaller ducts within the liver are lined with a single layer of cuboidal epithelial cells. Larger ducts may incorporate connective tissue and a thin *muscularis*. The hepatic structure normally varies (and considerably) in direct relationship to gender, age, available food (especially with regard to glycogen and fat content), or temperature, and with endocrine influences strongly connected to the environmentally regulated breeding conditions.

Exocrine pancreas

There are two general basic types of fish livers : those that contain pancreatic tissue *versus* those that do not. Fish livers with exocrine pancreatic tissue are often called “hepatopancreas” (Figs 8.13 to 8.15). The pancreas is generally diffusely spread within the fat and mesenteries (Fig. 8.12) that connect the intestine, stomach, liver and gall bladder. It can also form a discrete organ like as in the Chondrichthyans (Figs 8.16 & 8.17). It is composed of exocrine and endocrine tissues. The exocrine pancreas consists of clusters of pyramidal cells mostly organised in acini as observed in mammals. The cells have a dark basophilic cytoplasm, distinct basal nuclei, and many large eosinophilic zymogen granules (Fig. 8.17) containing enzymes responsible for the digestion of proteins, carbohydrates, fats and nucleotides. Enzymes are delivered to the anterior intestine via pancreatic ductules (Fig. 8.18) which coalesce to form the main pancreatic duct. This latter opens, distinctly or after rejoining the common bile duct, into the proximal part of the intestine (duodenum). The pancreatic ductules and the main pancreatic duct are lined with cuboidal to columnar epithelium respectively (Fig. 8.18). For the endocrine pancreas, see chapter 13.

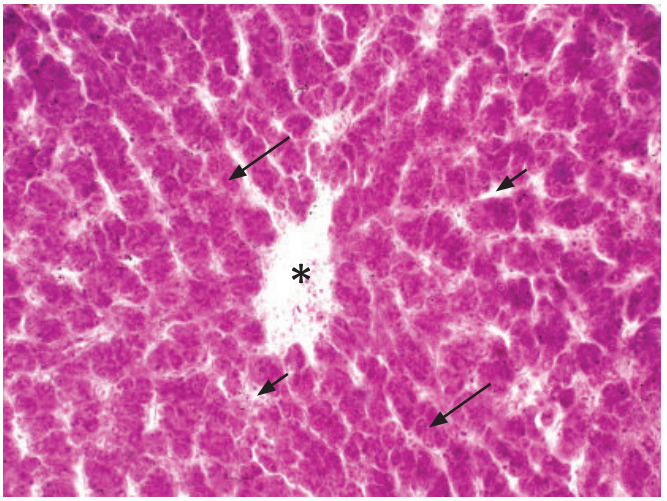


Fig. : 8.1 *Gnathonemus petersii* (H-E / MM)

Liver. The teleost liver resembles that of the mammals but lacks a lobular architecture with true portal tracts. The hepatocytes are however closely similar in function and appearance to those of mammals. This picture of fish liver tissue demonstrates the sponge-like appearance of the parenchyma which is primarily composed of polyhedral hepatocytes. The long arrows indicate cords of hepatocytes bathing in sinusoidal portal blood (sinusoids – short arrows). A vein (*) is located at the centre of the image.

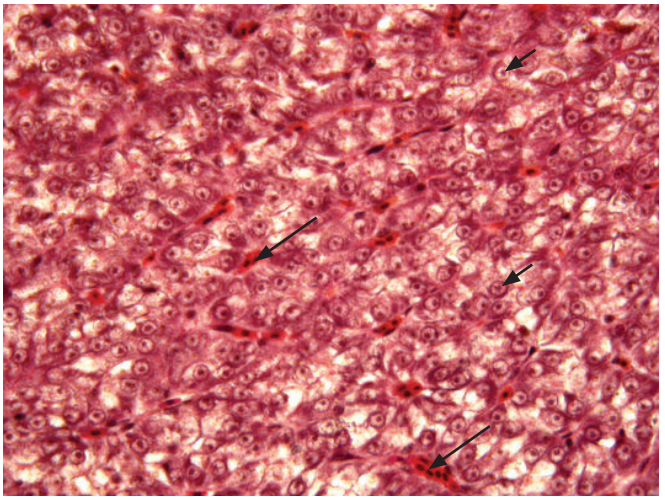


Fig. : 8.2 *Rutilus rutilus* (MT / HM)

Liver parenchyma. This photomicrograph shows cords of hepatocytes separated by sinusoids (long arrows) containing erythrocytes (orange). Hepatocytes are large cells with typically central nuclei showing prominent nucleoli (short arrows). Sinusoids are lined with endothelial cells whose nuclei are elongated and protrude into the sinusoidal lumen. The endothelium is fenestrated by small pores.

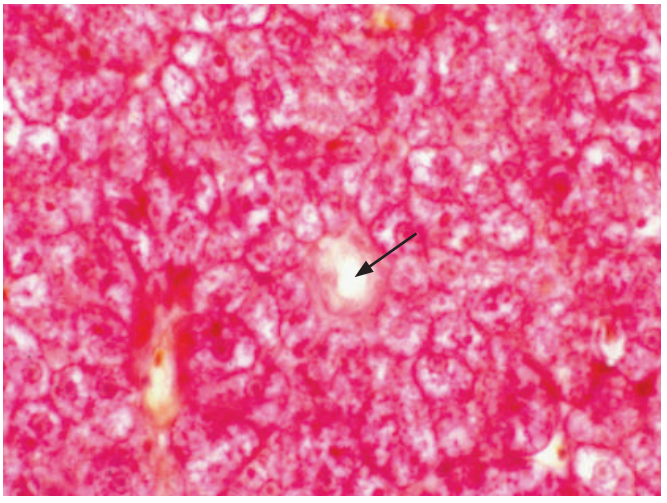


Fig. : 8.3 *Pangasius micronemus* (PAS-H-OR / HM)

Liver parenchyma. One of the liver's most metabolic function is storage of glycogen. At this high magnification, one can see that the hepatocytes are strongly stained in magenta by the PAS method; this reaction reveals the presence of polysaccharides and consequently glycogen.

Bile is actively secreted by hepatocytes into minute *canaliculi* barely visible (except with specific enzyme histochemical methods) at the light microscope level. Bile *canaliculi* are delimited by plasma membranes of adjacent hepatocytes. They merge to form intrahepatic ducts (arrow). These latter are of higher diameter and scattered throughout the liver parenchyma.

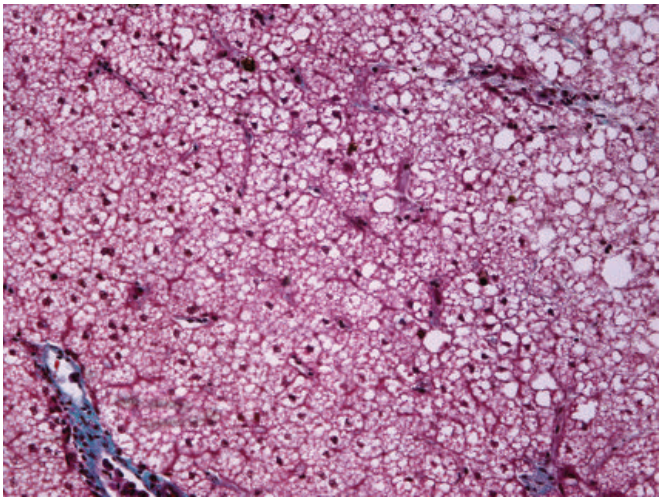


Fig. : 8.4 *Scyliorhinus canicula* (MT / MM)

Liver. Most sharks partially adjust their buoyancy by swimming constantly, using their caudal and pectoral fins. However, the liver which stores an enormous amount of oil is necessary to achieve neutral buoyancy. This photomicrograph shows part of the parenchyma of dogfish liver. The unstained areas largely represent cytoplasmic lipid droplets removed during histological preparation. Numerous dark spots are small melanomacrophage *centers*.

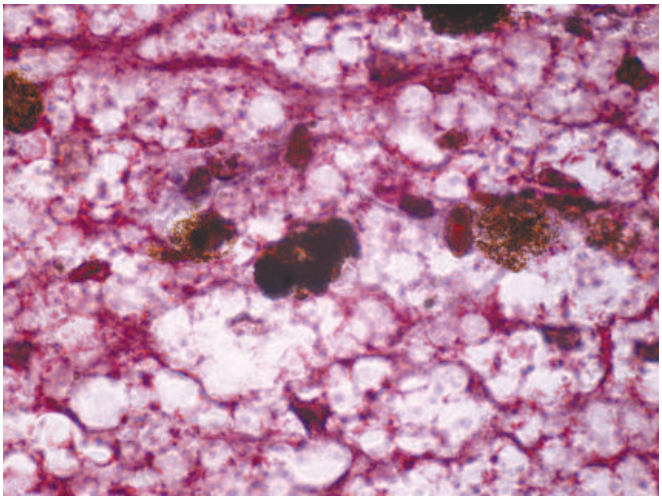


Fig. : 8.5 *Scyliorhinus canicula* (MT / HM)

Section of the dogfish liver. Note the swollen hepatocytes with (unstained) dissolved unilocular fat and oil globules. The liver of sharks may occupy more than 80 percent of the abdominal cavity. In some species this organ represents 25 percent of the total weight and can produce over a thousand liters of oil (basking and whale sharks). Dark-brown melanomacrophage centers are seen.

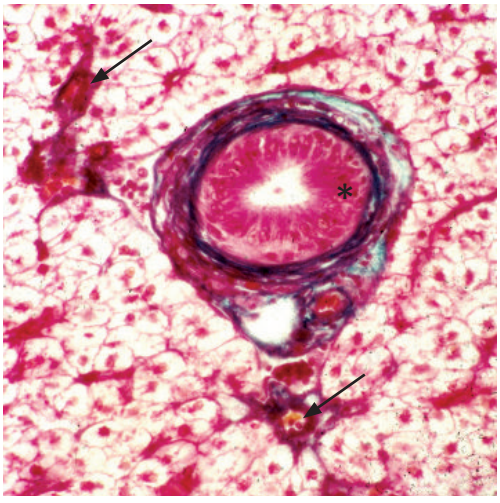


Fig. : 8.6 *Cyprinus carpio* (MT / HM)

In the centre of the image one can see a transverse section of a bile duct lined by a simple prismatic (or columnar) epithelium (∗) and a coat of connective tissue. Originating between adjacent hepatocytes, bile *canaliculi* anastomose to produce ducts of increasing diameter surrounded by increasing fibromuscular layers. Sinusoids containing blood cells are clearly seen (arrows). The vacuolar aspect of the large polyhedral hepatocytes is explained by their rich dissolved glycogen content.

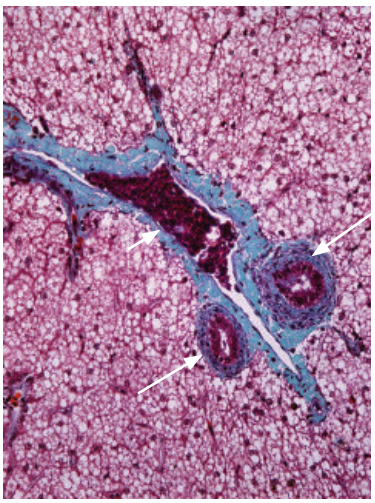


Fig. : 8.7 *Scyliorhinus canicula* (MT / MM)

In addition to vacuolar hepatocytes the centre of the photomicrograph displays two fairly large bile ducts (long arrows) and a large vein (short arrow) surrounded by connective tissue (blue). Despite the presence of these structures (vein and bile ducts) this can not be compared to the typical portal triad of the mammalian liver.

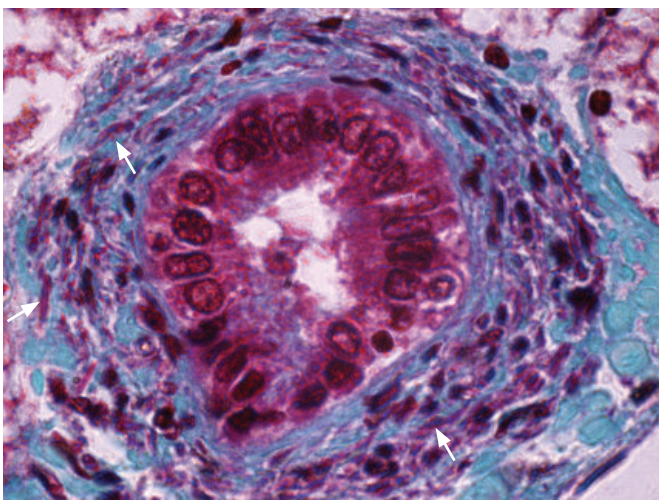


Fig. : 8.8 *Scyliorhinus canicula* (MT / HM)

Another major function of the liver with regard to digestion is the production of bile. Bile is involved in emulsification of dietary lipids and thus facilitates the action of pancreatic lipases. A large bile duct is illustrated in this photomicrograph. The prismatic epithelium (dark pink) is well demonstrated as well as the cell nuclei. The connective coat contains smooth muscle cells (leiomyocytes - arrow) bathing in blue collagen.

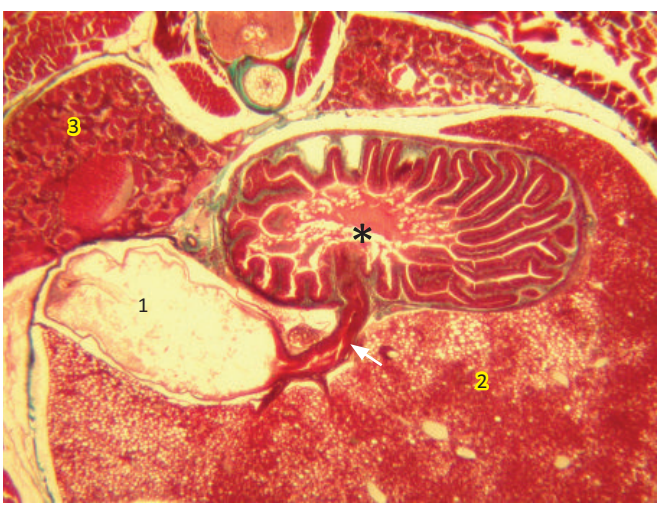


Fig. : 8.9 *Poecilia reticulata* (MT / LM)

This photomicrograph shows the gall bladder (1) located between the liver (2) and the kidney (3). Bile is concentrated and stored in the gall bladder. This hollow sac drains by a large bile duct (white arrow) into the first part of the intestine (duodenum - ∗) where pancreatic enzymes are active.

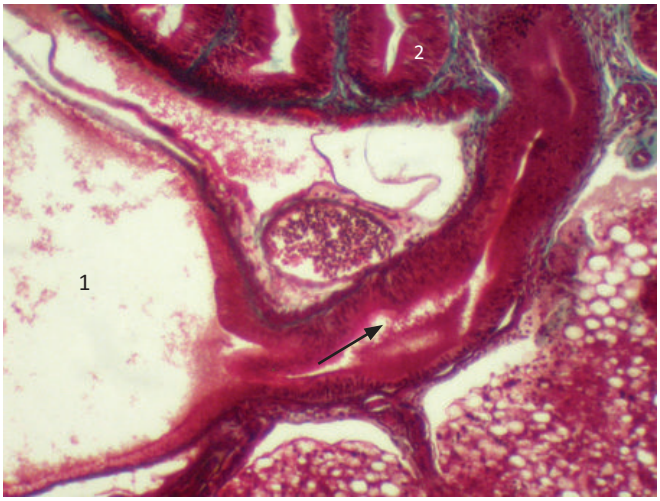


Fig. : 8.10 *Poecilia reticulata* (MT / MM)

Enlargement of the previous picture emphasizing the large bile duct (arrow) connecting the gall bladder (1) to the intestine (2). In many fish the main bile duct and the pancreatic duct merge before entering the duodenum.

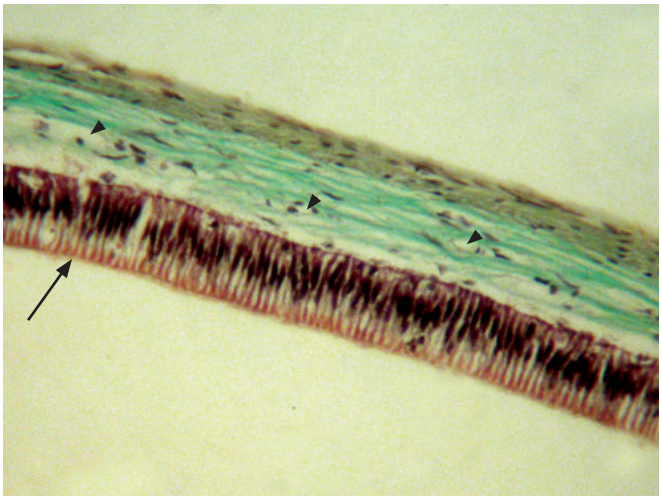


Fig. : 8.11 *Rutilus rutilus* (MT / MM)

Gall bladder. The histology of the gall bladder wall consists of a simple columnar epithelium (arrow) supported by an underlying fibrovascular *lamina propria* (green). The epithelial cells are very tall and possess elongated nuclei usually basally located. These lining cells concentrate bile, water of the lumen being passed into the capillaries (arrowheads) of the connective tissue.

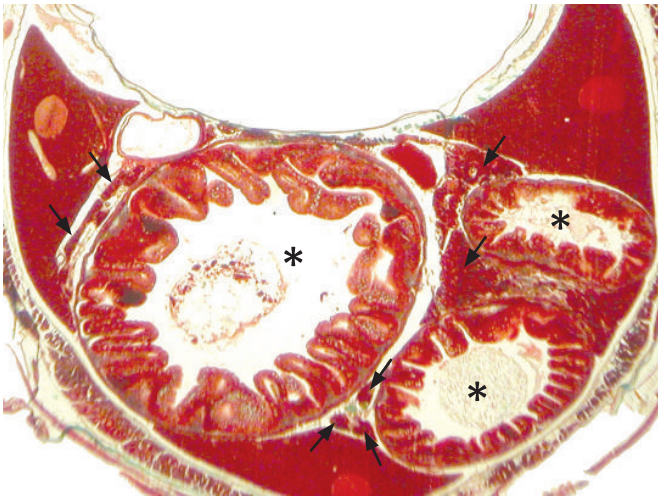


Fig. : 8.12 *Danio rerio* (MT / LM)

Pancreas. In many fish the pancreas is generally diffusely spread within the fat and mesenteries anchoring the different organs (liver, intestine...) of the abdominal cavity. This general view (see also Fig. Q in the introduction) shows such a pancreas. It occurs as diffuse masses (arrows) suspended in mesenteries and surrounding most of the intestine (∗). The pancreas is the second major gland of the digestive system and is composed of exocrine (see below) and endocrine (see chapter 13) tissues.

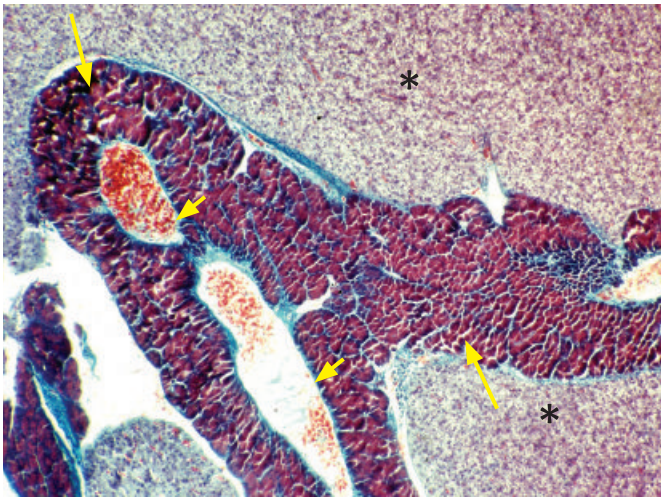


Fig. : 8.13 *Cyprinus carpio* (MT / MM)

Hepatopancreas. Many fish livers contain spread exocrine pancreatic tissue and are therefore called «hepatopancreas». The image shows hepatic parenchyma (∗) and a longitudinal section of a pancreatic-venous tract composed of a portal afferent vein (short arrows – erythrocytes in orange) and the exocrine pancreas cover (long arrows). The striking feature about this arrangement are the «islands» of pancreatic masses scattered throughout the hepatic tissue.

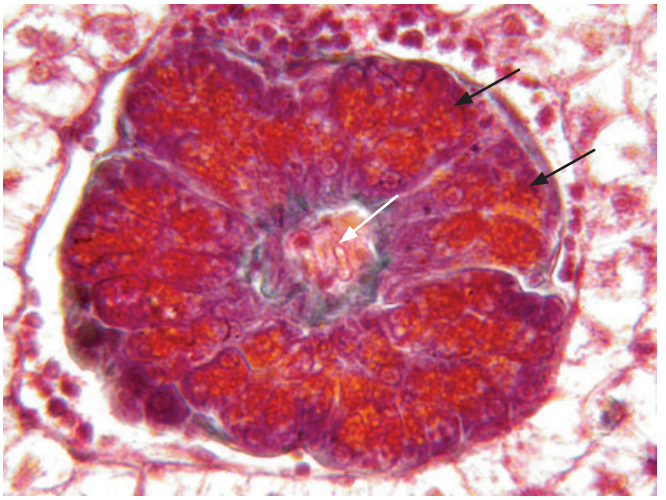


Fig. : 8.14 *Cyprinus carpio* (MT / HM)

Hepatopancreas. Picture highlighting a small cross-sectioned pancreatic-venous tract. In carp pancreatic tissue is found within the liver along branches of the portal vein. The components of this complex are a small afferent vein (white arrow) surrounded by about ten pyramidal pancreatic cells (black arrows). Note poorly stained hepatocytes all around the periphery of this complex.

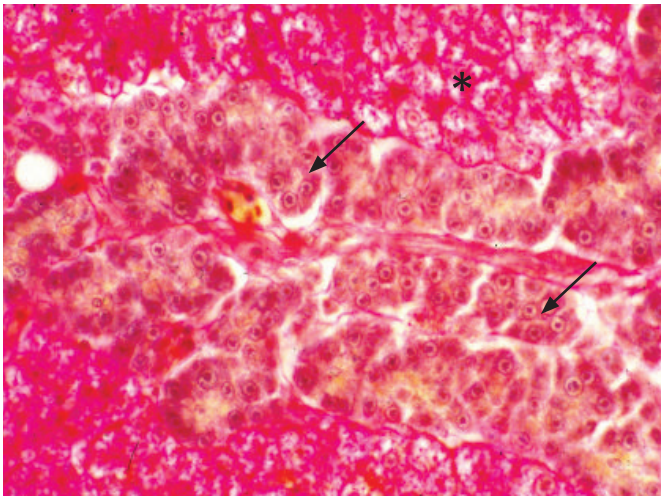


Fig. : 8.15 *Pangasius micronemus* (PAS-H-OR / HM)

This photomicrograph shows numerous clusters (arrows) of glandular exocrine pancreatic cells surrounded by hepatocytes with glycogen storage (PAS +, magenta - ∗).

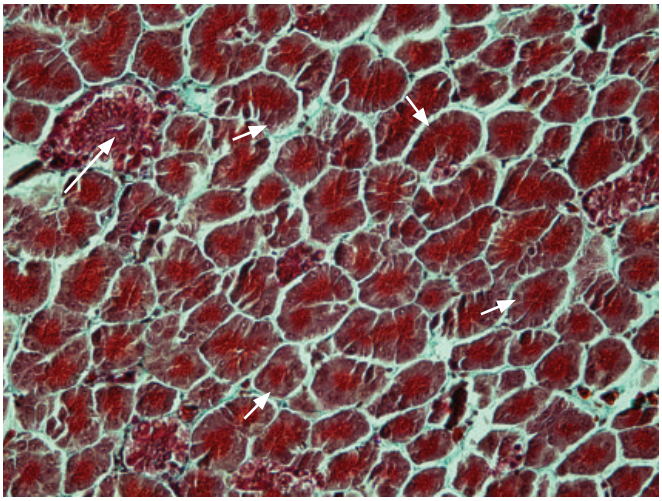


Fig. : 8.16 *Scyliorhinus canicula* (MT / MM)

Section through the dogfish pancreas. In elasmobranchs the pancreas is a discrete organ lying between the stomach and the intestine. It is composed of two unequally sized lobes largely made up of numerous masses of exocrine *acini* (short arrows) which secrete digestive enzymes. The secretion leaves the pancreas via pancreatic duct(ule)s : such a duct is seen in the upper left field (long arrow). These acinar units are separated from each other by connective tissue.

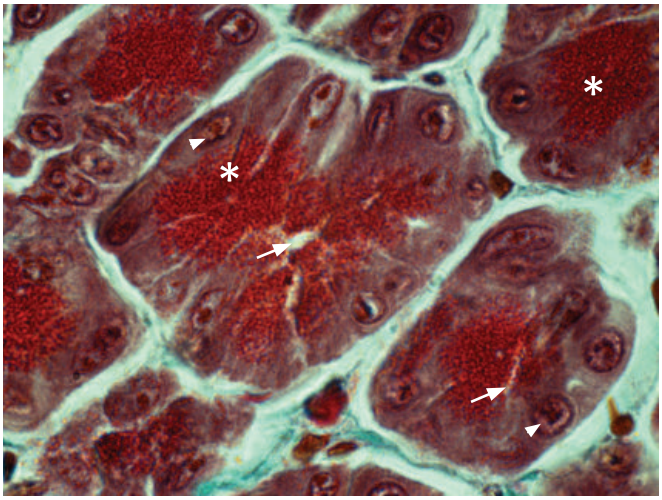


Fig. : 8.17 *Scyliorhinus canicula* (MT / HM)

Dogfish pancreas. *Acini* are enzyme-secreting units of the exocrine pancreas. Each *acinus* (a beautiful one takes up the image centre) is an ovoid-elliptical cluster of pyramid-shaped secretory cells (acinar cells) surrounding a common minute luminal space (arrows). The fish pancreatic cells are very similar to their mammalian counterparts. A prominent feature of the acinar cells is the presence in their apical regions of aggregated bright eosinophilic zymogen granules (red staining - ∗) facing the narrow lumen.

Remind that a zymogen or proenzyme is an inactive enzyme precursor. The rounded or flattened cell nuclei (arrowheads) are located basally. The interacinar supporting tissue (green) is thin.

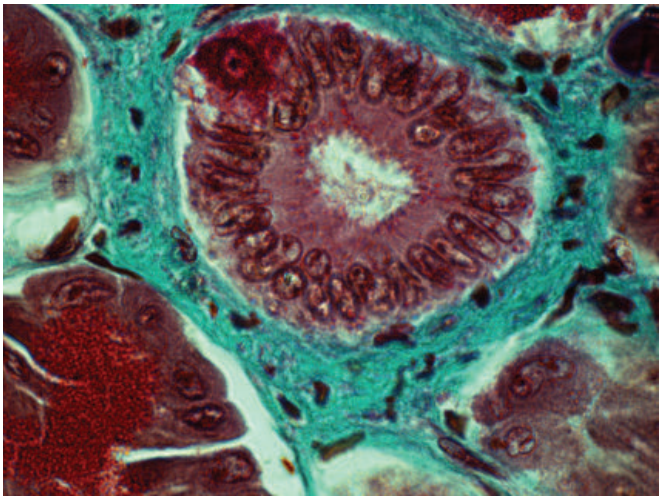


Fig. : 8.18 *Scyliorhonus canicula* (MT / HM)

Exocrine pancreas. Pancreatic *acini* drain into a branched system of variously sized ducts. In this picture one can see a medium-sized duct surrounded by a sheath of supporting tissue composed of collagen (green) and fibrocytes. In comparison with acinar cell nuclei those of the duct cells are elongated and right angled with the basal cell membranes. Note also the wider lumen in the excretory ductule.

9

Swim and gas bladders

Many bony fishes possess a gas bladder, a single elongated sac dorsally located to the digestive tract. Gas bladders are filled with air that enters via the pneumatic duct or with gas (O_2, CO_2, N_2) secreted into the bladder from the blood. As gas bladders are mainly used to control the buoyancy of the fish, they are often called swim bladders (less appropriate term). Occasionally they may be heavily vascularized to participate in supplementary respiration and called respiratory gas - or air - bladders. The internal vascular walls of respiratory gas bladders are subdivided into many partitions that increase the surface area available for external respiration exchange. The gas bladders are not necessary for life as they are absent in many fishes (*Blennidae, Pleuronectidae, Thunnidae, Scombridae,* some *Scorpenidae,* Chondrichthyes...). This organ may be one, two or three chambered. Its presence or absence can reflect the behavior of fishes.

Swim bladders come in two kinds : physostomous and physoclistous. Physostomous swim bladders are connected to the foregut (esophagus) by a duct called the pneumatic duct (Figs 7.25 & 7.26). This type of bladder is characteristic of sturgeons and primitive teleosts (*Anguillidae, Cyprinidae*...). In physoclist fishes the pneumatic duct is lost during embryonic development. This type of swim bladder is found only in advanced teleosts. (*Percidae, Gadidae, Balistidae, Tetraodontidae*...). Typically, the filling and the emptying of the gas bladder are respectively made by a secretory section (the gas gland - Figs 9.1 to 9.3) and a resorbing section (the oval), but the actual anatomy of these sections varies from species to species. In the European eel (*Anguilla anguilla* - Figs 9.4 to 9.9), the pneumatic duct develops into the resorbing section of the bladder, which can be separated from the secretory section by a sphincter. In cod or perch, the oval, can be separated from the rest of the organ by muscular activity and is designed to allow the resorption of gases.

The swim bladder wall consists of several layers. The outer layer, consisting mainly of fibromuscular tissue, is called the *tunica externa.* The *submucosa* may be impregnated with guanine crystals, which render the swim bladder wall impermeable to gases. In some fishes large amounts of membranous material is present which typically is arranged in a bilayer structure. Below the *submucosa*, smooth muscle cells are present, termed the *muscularis mucosae*. The *tunica interna* comprises a cuboidal secretory epithelium. Anteriorly this epithelium is modified into the gas gland, which is made up of folded columnar epithelium heavily vascularized by long loops of densely packed capillaries. This type of structure is called a *rete mirabile* (Figs 9.7 to 9.9). This *rete* in association with the gas gland allows gas secretion into the bladder. The production of gas in the bladder is made possible among others by the countercurrent arrangement of the arterial and venous capillaries of the *rete mirabile*.

Although hydrostasis is the primary function of gas-filled organs in the vast majority of present-day bony fishes, these structures sometimes perform a respiratory function.

A phylogenetically diverse array of fishes breathe air through their air-bladder. Air-breathing through the use of an air-bladder is restricted to those fishes that have retained a connection between the esophagus and the bladder. This is the case in diverse primitive teleosts, for example, the bony tongue fishes *(Osteoglossoidei*), the tarpons *(Megalopidae*), the gars (*Lepidosteidae*), the bowfin (*Amiidae*), the aba (*Gymnarchidae*), the knifefishes (*Notopteridae*) and the trahiras (*Erythrinidae*). Functional lungs are found among relatively primitive fishes. There are few examples, among preteleosts : the bichirs *(Polypteridae*) and the lungfishes (Dipnoi).

Most bottom-dwelling teleosts (*Pleuronectidae, Blennidae, Cottidae*) and deep-sea species

(Stomiiformes, Aulopiformes, Myctophiformes) whose protective and food-gathering mechanisms depend on their staying at the bottom of the sea do not have a gas bladder. It is also usually lost or greatly reduced in certain other fishes whose functioning would be impeded by a large bubble of gas, for instance, in freshwater species that live in turbulent streams and in the most rapidly swimming marine fishes such as mackerels (*Scombridae*), tunas and pelagic sharks.

In addition the gas bladder has evolved other functions. It facilitates auditory reception in several teleosts (*Ostariophysi*) by means of tiny Weberian ossicles. In others it is involved in sound production (*Balistidae, Triglidae, Batrachoididae*…).

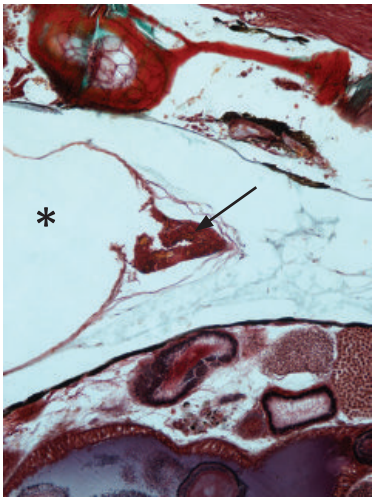


Fig. : 9.1 *Poecilia reticulata* (MT / LM)

Gas bladder. A characteristic of most actinopterygian fishes is the presence of a gas bladder, an elongate sac arising from the anterior part (esophagus) of the digestive tract. It contains a varying mixture of gases (N_2, O_2, CO_2). Many fish are physostomous and have a pneumatic duct (see Figs 7.25 & 7.26). In physoclists (Perciformes and others advanced teleosts including the guppy) gas bladders are completely closed, separated from the gut through loss of the pneumatic duct.

This photomicrograph shows a part of the simple saccular gas bladder (∗) and its anteriorly located gas gland (arrow) supplied by arterial and venous capillaries (*rete mirabile* – see Figs 9.7 to 9.9). Note portions of male gonad at the lower side of the picture.

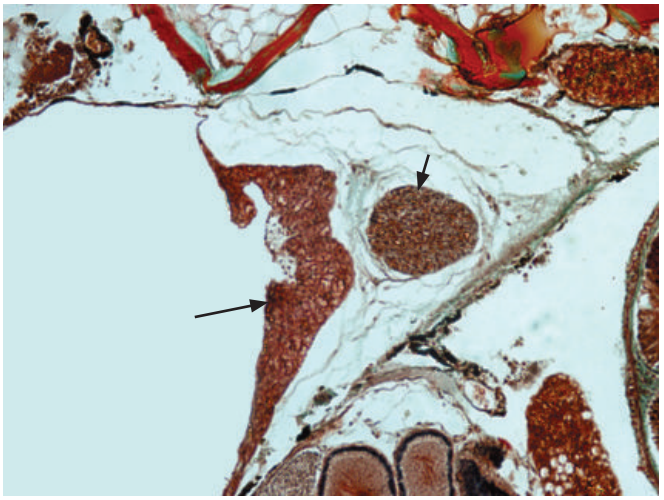


Fig. : 9.2 *Poecilia reticulata* (MT / MM)

Gas bladder. The gas bladder appears as a dorsal outgrowth extending along the abdominal cavity (*cf.* Figs of the introduction), below the dorsal aorta and the vertebral column (vertebra in red). The gas gland (long arrow) and a portion of the *rete mirabile* (short arrow) are illustrated ; together they are sometimes called the «red body» ensuring gas secretion into the gas bladder.

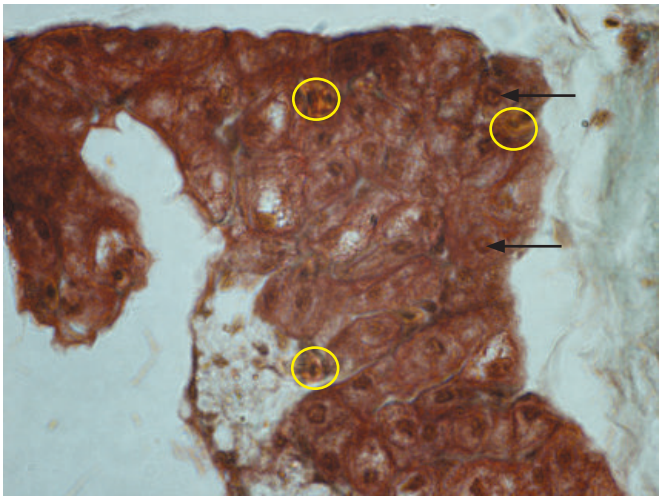


Fig. : 9.3 *Poecilia reticulata* (MT / HM)

Gas gland. The filling of the gas bladder is typically ensured by the gas gland in combination with the *rete mirabile*. The gas gland is a modification of the inner lining (*tunica interna*) ; it is composed of a highly specialized and vascularized epithelium whose cells produce lactic acid and CO_2. The induced acidification allows gas diffusion from afferent arterial capillaries into the bladder. This high magnification shows gas gland cells (arrows) with capillaries (circles - red blood cells stained by orange-G) in between.

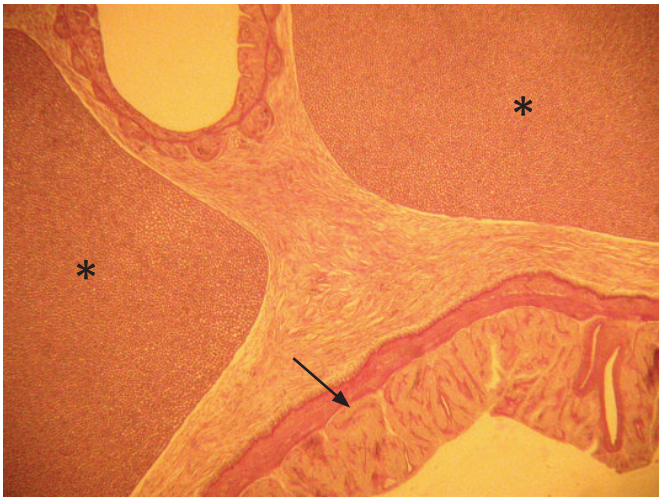


Fig. : 9.4 *Anguilla anguilla* (H-E / LM)

General view of the gas bladder. The eel is a physostomous fish with a persisting pneumatic duct. This type of swim bladder is also characteristic of sturgeons and primitive teleosts. In the eel, an enlarged part of the pneumatic duct develops into a resorbing section, equivalent of the oval. This section illustrates part of the gas bladder wall (arrow) and its bipolar *rete mirabile* (∗), a clump of parallel arterial and venous capillaries found on the outside of the gas bladder.

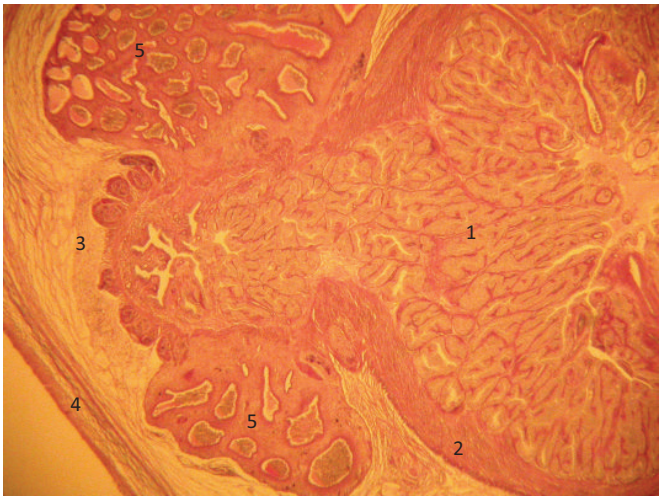


Fig. : 9.5 *Anguilla anguilla* (H-E / MM)

Gas bladder. The gas bladder wall usually consists of three layers and reminds that of the esophagus : the *tunica externa*, the *submucosa* or middle layer and the *tunica interna*. The outer layer consists mainly of elastic fibromuscular tissue often linked to myomeres. The supporting connective *submucosa* may be impregnated with guanine crystals. Close to the *submucosa*, smooth muscle cells are present and form the *muscularis mucosae.* The *tunica interna* comprises a cuboidal or pseudostratified secretory epithelium. Anteriorly the latter is modified into the gas gland (see Figs 9.1 to 9.3).

This photomicrograph displays numerous epithelial folds of the gas gland (1), the *muscularis mucosae* (2), the *submucosa* (3), the *tunica externa* containing some smooth muscle (4) and numerous blood vessels (5).

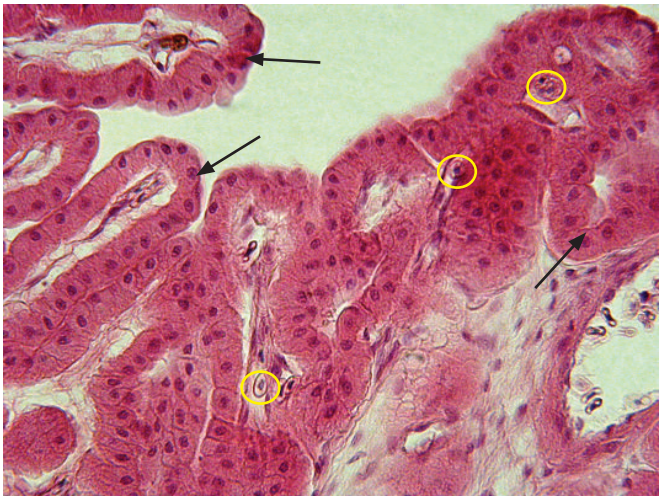


Fig. : 9.6 *Anguilla anguilla* (H-E / HM)

A portion of the gas gland is magnified. The gas gland cells form a cuboidal/columnar folded epithelium (arrows). Like the epithelial cells of the *tunica interna*, gas gland cells produce surfactant which is released at their apical membranes. The epithelium is heavily vascularized by long loops of densely packed capillaries (circles) belonging to the *rete mirabile*.

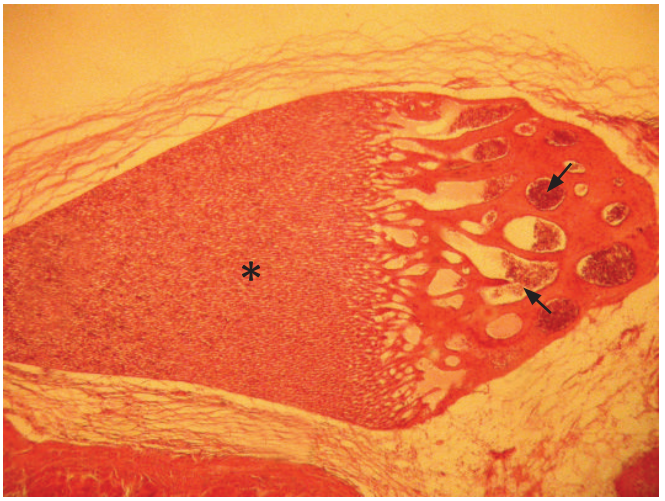


Fig. : 9.7 *Anguilla anguilla* (H-E / LM)

Longitudinal section of the *rete mirabile* of an eel. The main blood vessel entering the anterior part of the gas bladder breaks up into smaller branches (arrows) which subdivide into a multitude of capillaries (∗). The *rete mirabile* is a dense bundle of parallel arterial and venous capillaries arranged side by side and supplying the gas gland with blood.

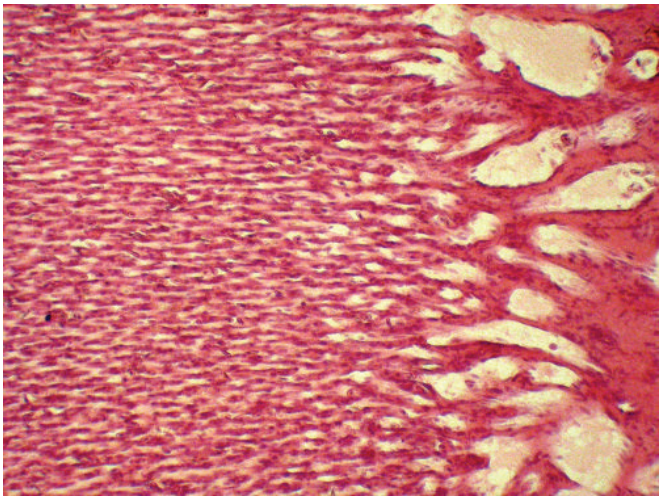


Fig. : 9.8 *Anguilla anguilla* (H-E / MM)

A portion of previous document magnified. The *rete mirabile* is a wonderful complex structure. It consists of a countercurrent arrangement of arterial and venous capillaries which do not communicate until they reach the gas gland. See also Fig. 4.23.

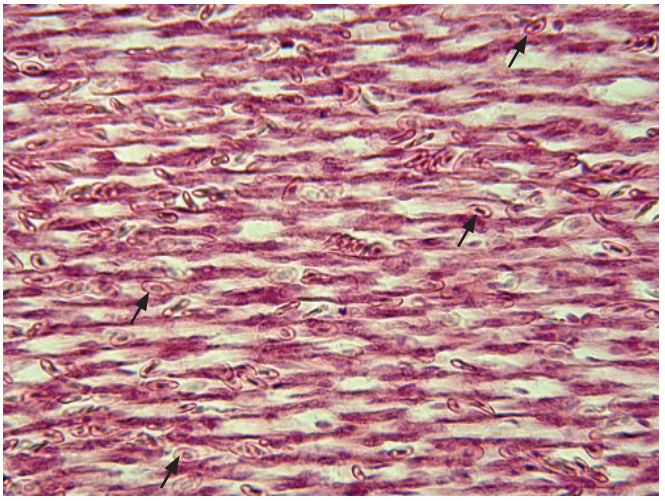


Fig. : 9.9 *Anguilla anguilla* (H-E / HM)

The blood capillaries in the *rete mirabile* are numerous and so closely arranged as to leave no interspaces for any supporting tissue. Arterial and venous capillaries are not distinguishable on this light microscopy image: only the electron microscope can differentiate the thicker arterial wall and the more irregular venous shape. The pattern of arrangement of the blood capillaries in this mass and the abundance of blood cells (arrows showing erythrocytes) give a fascinating appearance in a longitudinal section.

10
Respiratory system

Most teleosts use gills as the main respiratory surface, although accessory respiratory structures also occur (e.g. skin, pharyngeal and buccal diverticula, intestine,...) particularly in those species adapted to spend periods of their life out of water.

Elasmobranchs have five pairs of gills. Muscular interbranchial *septa* (Fig. 10. 1) separate gill slits. Each *septum* bears branchial arch, gill rays, one afferent blood-vessel and paired efferent blood-vessels. Chondrichthyes have a spiracle, a vestigial gill cleft just behind the eye and in relation with the gill cavity. It is used as a water passageway specially in rays and bottom dwellers. In pelagic species it is reduced or absent.

Most fish (teleost) have four pairs of gill arches extending from the floor to the roof of the buccal cavity. Each of the four pairs is supported by a cartilaginous and/or bony skeleton with associated striated abductor and adductor muscles (Fig. 3.6) facilitating movement of gills to favourable respiratory positions. The gills are covered and protected by an *operculum* (Figs 10.17 & 10.18). Unlike the Chondrichthyes, the *septa* of teleosts are very reduced : the *lamellae* are free and this arrangement provides a larger respiratory surface.

Each gill arch bears a number of gill filaments or holobranchs, each of which is made up of two halves, called hemibranchs. Each hemibranch bears many fine subdivisions called gill *lamellae* (Figs 10.2 to 10.12). The purpose of these structures is to provide a large surface area that supports respiratory and excretory functions. The efficiency of exchange, which in the case of oxygen is roughly 50-80%, is largely a function of the countercurrent exchange between blood and water. Gill filaments have a central cartilaginous support, afferent and efferent arterioles and other anastomosing vessels comprising the central venous *sinus*. They are covered with a thin epithelium contiguous with the covering of gill arches and the oral *mucosa* of the buccal cavity. Each *lamella* is best regarded as a thin envelope of cells lying on a basement membrane, the two surfaces of which are supported by pillar cells. Spaces between pillar cells, called *lacunae*, connect afferent and efferent arterioles. The contractile pillar cells control the lacunar diameter thus regulating blood flow.

Chloride cells (Fig. 10.12) surrounded by flattened pavement cells can be observed at the junction between the primary and secondary *lamellae* (see chapter 11).

Mucous cells (Figs 10.9 to 10.11) are a prominent feature of the gill epithelium. The biological importance of the mucus-rich interface between fish and their aqueous environment spans functions as diverse as ionoregulation, mechanical and immunological protection. Other cells found within the filamental interstitium include melanocytes, lymphocytes, macrophages and neuroepithelial cells (containing 5-hydroxytryptamine).

The inner surfaces of the gill arches carry one or more rows of stiff strainers called gill rakers. They serve to sort and aggregate particulate food material and to position larger food items before the food is passed into the esophagus and then into the stomach or intestine. The rakers tend to be long, slender, and tightly packed in planktivorous fishes and particle feeders (such as anchovies, herrings, alewife and certain scombrids). They are shorter, thicker and more widely spaced in fishes that feed on larger prey. The rakers illustrated here (*Cyprinus carpio* - Figs 10.13 to 10.16), are relatively short. Histologically, each gill raker is composed of an osseous or cartilaginous *lamella* supporting the pharyngeal pluristratified epithelium and connective tissue.

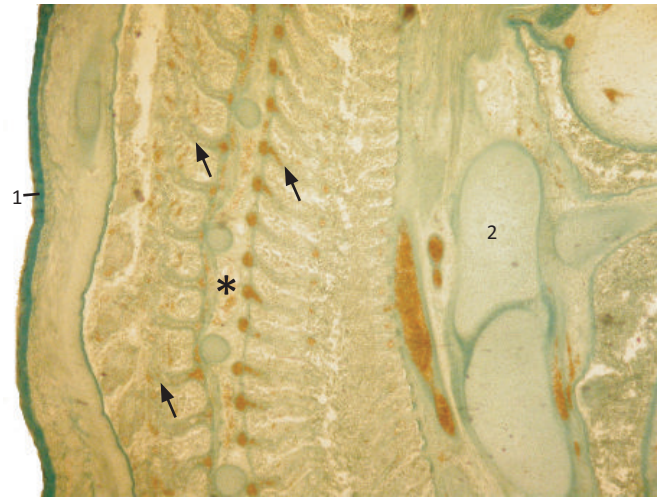


Fig. : 10.1 *Scyliorhinus canicula* (MT / MM)

Gills. Elasmobranchs present well-visible gill slits on both sides of the head in sharks, or underneath in skates and rays. Most chondrichthyean species have five pairs of slits, but six or seven pairs occur in Hexanchiformes (frilled and cow sharks). Gill slits are external openings leading to the orobranchial cavity and allowing water to exit after passing over the gill *lamellae*. Two gill slits are separated by a long and muscular interbranchial *septum* (∗) which supports the gill filaments (arrows). Skin is seen in 1 (dermis in green) and branchial cartilage in 2. Erythrocytes are stained in orange.

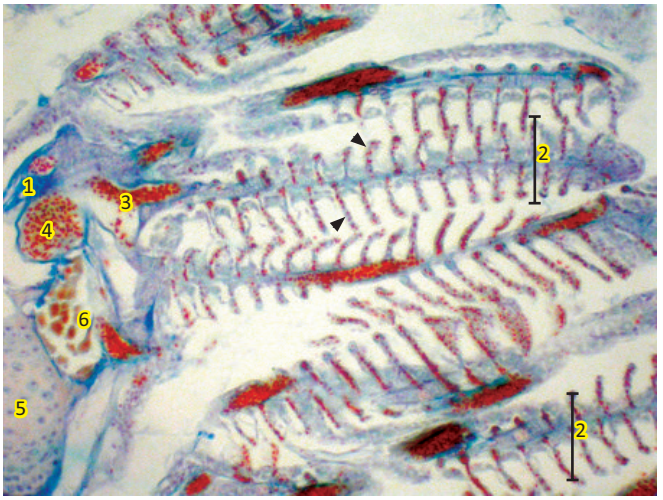


Fig. : 10.2 *Poecilia reticulata* (MT / LM)

Gills. The gills of the Osteichthyes are quite similar to those of the cartilaginous fish except for two major differences : the Osteichthyes always have an *operculum* (see Figs L, M, N, O, P , 10.17 & 10.18) and the gill *septa* are very reduced (aseptal gills). Their *lamellae* are free in the opercular cavity. Osseous fish have four pairs of cartilaginous or bony gill arches (gill arch in cross section – 1) onto which two rows of tens of gill filaments (2) are attached. Each filament is well vascularized (afferent - 3 and efferent blood vessels – 4) and bears many platelike secondary *lamellae* (arrowheads). In 5, gill arch cartilage and in 6 branchial muscles.

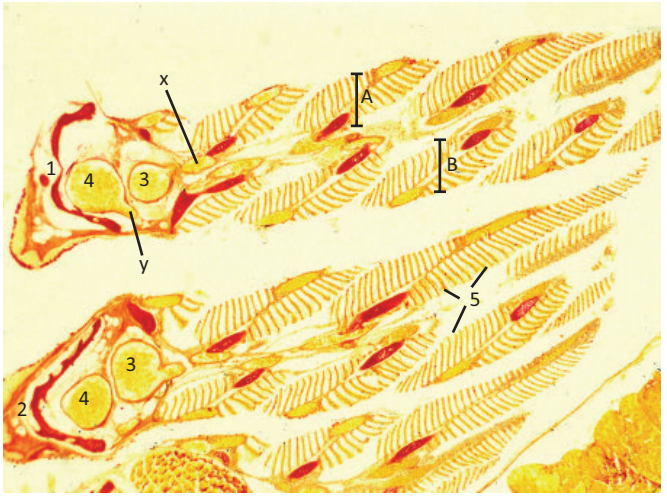


Fig. : 10.3 *Distichodus sexfasciatus* (PAS-H-AUR / LM)

Gills. This photomicrograph shows section through two bony gill arches (1,2). Each gill arch supports a double row of filaments (A, B) called a holobranch. Each filament is supplied by afferent (3 – deoxygenated blood) and efferent (4 - oxygenated blood) arteries. One can see the numerous gill *lamellae* (5) which are the actual respiratory surfaces.

x : deoxygenated blood going to the *lamellae*.
y : oxygenated blood coming from the *lamellae*.
In bright red : skeletal tissue (bone and cartilage).

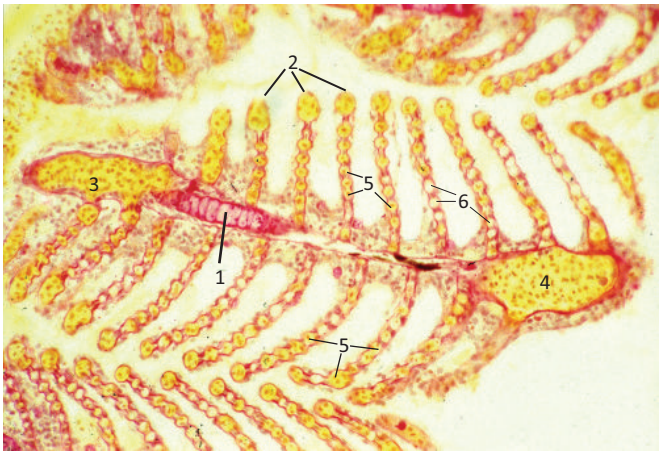


Fig. : 10.4 *Pangio kuhlii* (PAS-H-AUR / MM)

Transverse section through a gill filament (primary *lamella*). The well vascularized gill filaments are supported by cartilaginous tissue (magenta - 1) and bear numerous flattened secondary *lamellae* (2) which constitute the respiratory membrane.

Erythrocytes (bright yellow) are present everywhere as well in the afferent (3) and efferent (4) arterial vessels as in the capillaries (5) between them. 6 indicates *lacunae* (capillary lumens).

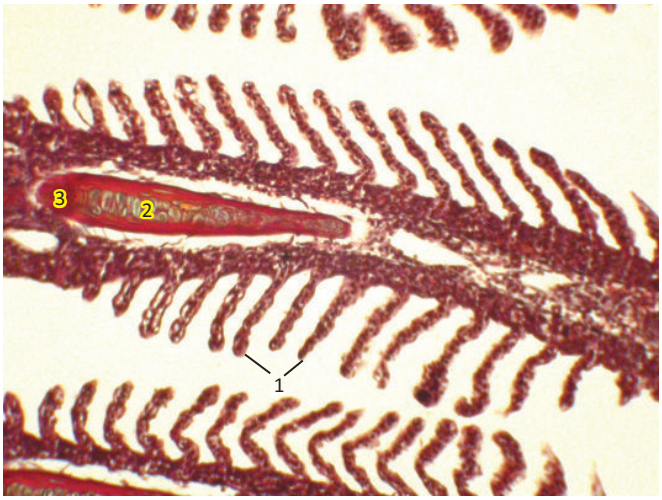


Fig. : 10.5 *Oncorhynchus mykiss* (MT / MM)

Sagittal section through a gill filament. Numerous parallel threadlike secondary *lamellae* are obvious (1) and arranged nearly at right angles to the filament. The cartilaginous skeleton, which supports the primary *lamella*, is also evident (chondrocytes – 2 ; extracellular matrix – 3).

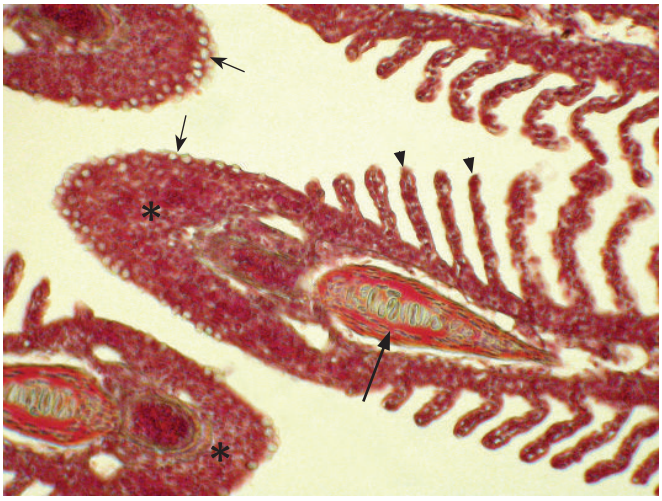


Fig. : 10.6 *Oncorhynchus mykiss* (MT / MM)

Gills. This picture shows the epithelium covering the distal ends (∗) of primary *lamellae*. This epithelium is continuous with the covering of gill arches and often rich in mucus-secreting cells (unstained – thin arrows). The arrowheads point to secondary *lamellae* and the long arrow indicates the skeleton supporting the primary *lamella* (gill filament).

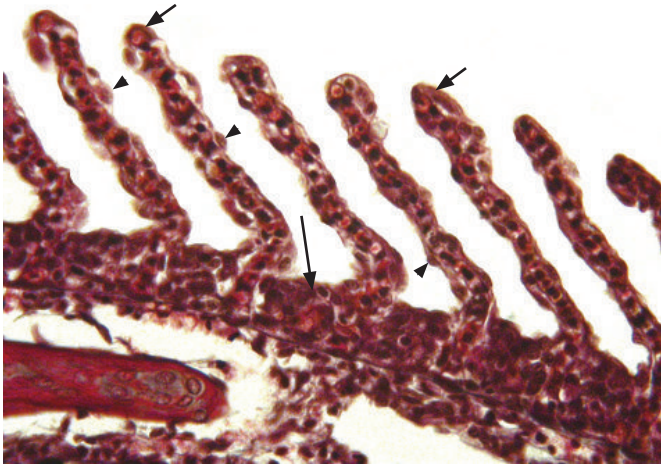


Fig. : 10.7 *Oncorhynchus mykiss* (MT / HM)

Gills. Portion of a sagittal section of a rainbow trout gill. Secondary *lamellae* (short arrows) are oriented perpendicular to the gill filament (primary *lamella*). Each primary *lamella* is covered by a stratified epithelium (long arrow) which contains several cell types including ordinary and pavement epithelial cells, lymphocytes, macrophages, mucous cells, chloride cells, undifferentiated cells... The fundamental histological structure of the secondary *lamellae* is similar in most fishes. The thin epithelium covering the secondary *lamellae* is low and consists of a single or double layer of cells (arrowheads) lying on a basement membrane supported by pillar cells (difficult to distinguish here - see Fig. 10.12).

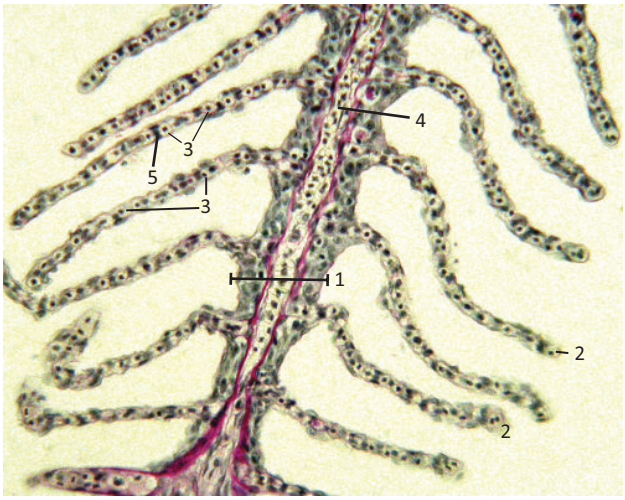


Fig. : 10.8 *Pimelodus pictus* (PAS-H / MM)

Gill filament, sagittal section through venous *sinus*. 1 : gill filament or primary *lamella* – 2 : secondary *lamellae* with central capillary network – 3 : erythrocytes within capillary lumen of secondary *lamellae* – 4 : erythrocytes (oxygenated blood) in the central venous *sinus* of the primary *lamella* - 5 : pillar cell.

The direction of blood flow from afferent to efferent arterioles is opposite to the direction of water flow over the *lamellae* (countercurrent exchange). This arrangement allows very efficient oxygen exchange (up to 80 %).

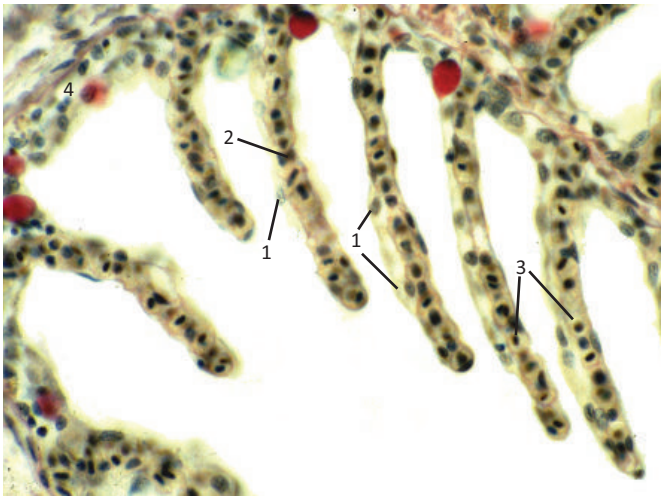


Fig. : 10.9 *Pelvicachromis pulcher* (PAS-H / HM)

Part of gill filament, sagittal section. The histological structure of the secondary *lamellae* is displayed. These *lamellae* are lined with a single or double layer of squamous epithelium (1) and narrow supporting pillar cells (2) which enclose capillary blood channels (*lacunae* filled with erythrocytes - 3) connecting afferent and efferent arterioles. The contractile pillar cells regulate blood flow.

The primary or filamental epithelium (4) is thick compared to the respiratory or lamellar epithelium and contains some mucous cells (PAS+, magenta).

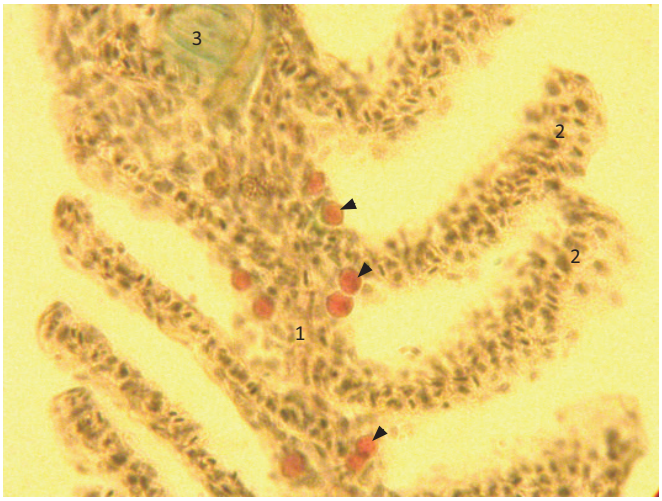


Fig. : 10.10 *Pelvicachromis pulcher* (AB-PAS-H / HM)

Gills. The vacuolated mucus-secreting cells (PAS+, arrowheads) are located principally within the epithelium of the primary *lamellae* near the base of secondary *lamellae*. The mucous cells produce a thin mucous coating which protects against bacterial infection and abrasion.

1 : gill filament or primary *lamella* – 2 : secondary *lamellae* – 3 : cartilaginous support

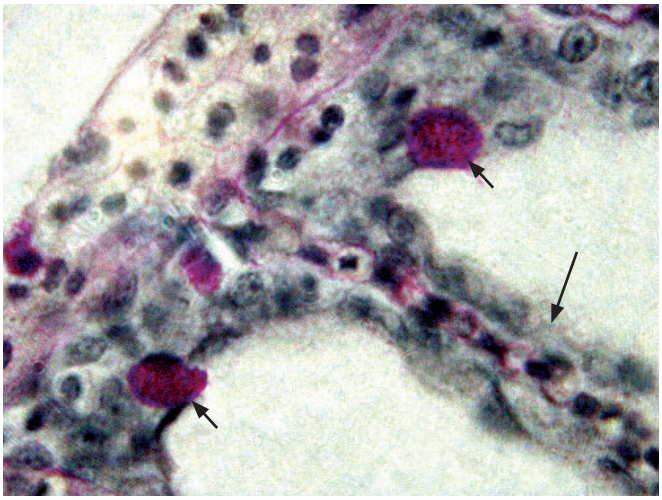


Fig. : 10.11 *Pimelodus pictus* (PAS-H / IM)

Gills. This photomicrograph shows mucous cells (magenta – short arrows) located within a primary *lamella* near the base of a secondary *lamella* (long arrow). Note the difference in thickness of the two epithelia. A few connective fibers are stained in magenta.

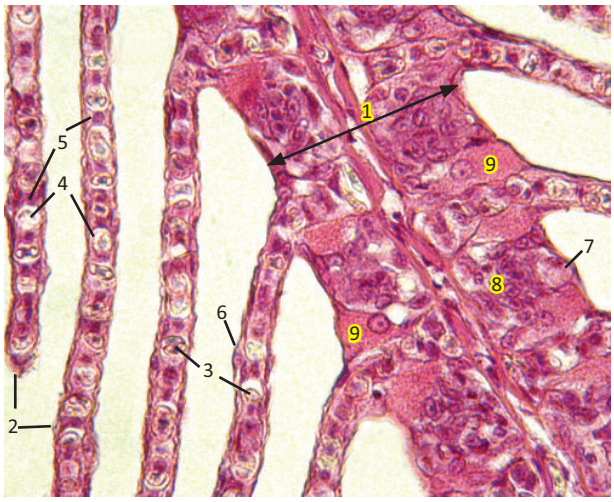


Fig. : 10.12 *Anguilla anguilla* (H-E / HM)

Gills. Sagittal section through a primary and some secondary *lamellae* whose histological fine structure can be seen.

1 : gill filament or primary *lamella* – 2 : secondary *lamellae* with central capillary network – 3 : erythrocytes within capillary lumen of secondary *lamellae* – 4 : *lacuna* (capillary lumen) – 5 : pillar cells (spool -shaped) – 6 : epithelial cells (respiratory epithelium) – 7 : mucous cell – 8 : undifferentiated cells – 9 : chloride cells (with rounded nuclei showing a prominent nucleolus).

Chloride cells, sometimes called ionocytes, are usually located along the bases of the secondary gill *lamellae*. These cells are surrounded by pavement epithelial cells and are involved in maintaining the osmotic balance. Their granular eosinophilia is due to the abundance of mitochondria and an extensive membranous tubular system (see also chapter 11 and Fig. 11.30).

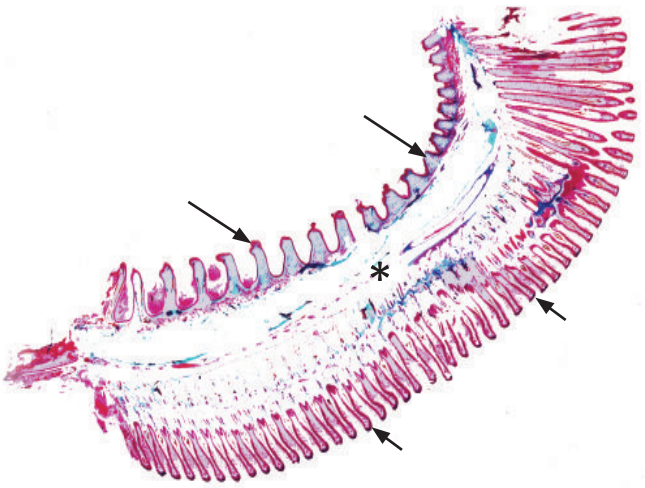


Fig. : 10.13 *Cyprinus carpio* (MT / LM)

Gill rakers. This very low magnification (8x) of a teleost aseptal gill displays in the same document the gill *lamellae* (short arrows) and the gill rakers (long arrows). Gill rakers are variously shaped bony or cartilaginous projections which point forward and inward from the gill (or branchial) arch (∗). The common carp possesses about twenty relatively short gill rakers.

The shape and number of these structures are a good indication of the diet of the fish. They are thinner, longer and more numerous in species eating small prey ; the plankton feeders have the longest and a number that can exceed 150 per arch.

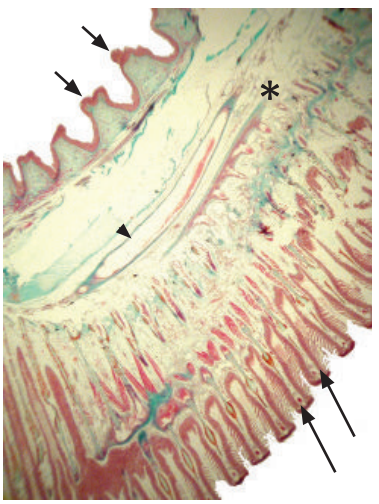


Fig. : 10.14 *Cyprinus carpio* (MT / LM)

Branchial arch (∗) with both gill rakers and gill filaments. On their pharyngeal (inner) margins, gill arches carry one or more rows of comb-like structures termed gill rakers (short arrows). Gill rakers serve to sort and aggregate particulate food material and they prevent the passage of solid substances which could damage the fragile gill filaments (long arrows). A large blood vessel (arrowhead) is also visible.

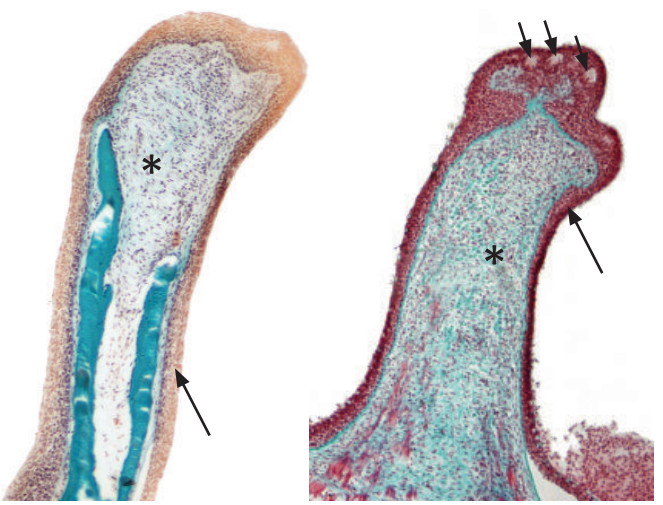


Fig. : 10.15 *Cyprinus carpio* (MT / MM)

Two gill rakers cut at different levels. These comb-like projections on the inner edge of the gill arches filter solid material from the water and serve to retain food particles in the buccal cavity. The gill rakers are lined with the mucous pharyngeal stratified epithelium (long arrows) supported by loose connective tissue (∗). On the left one the section passes through two supporting bony pieces (turquoise). The distal end of the right gill raker contains some epithelial taste buds (short arrows – see also Fig. 1.7).

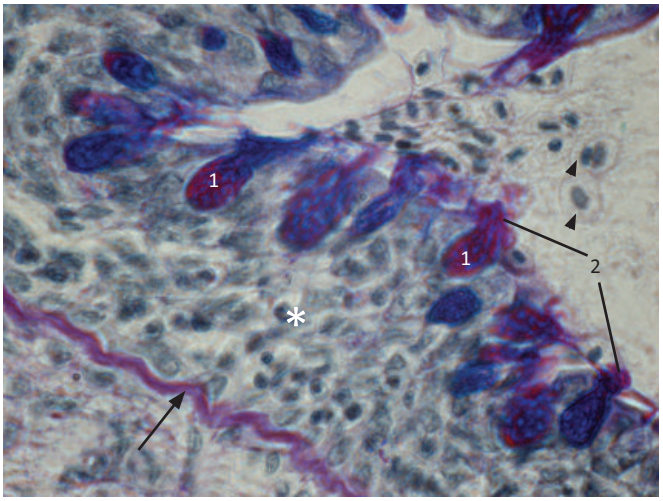


Fig. : 10.16 *Cyprinus carpio* (AB-PAS-H / HM)

Gill raker epithelium. Gill rakers are covered by a relatively thick stratified epithelium (∗) quite well endowed with mucus-secreting cells (deep red-purple – 1). The mucus released by exocytosis (2) assists in trapping small food particles which are directed to the esophagus. The arrow points to the clearly visible basement membrane.

The presence of erythrocytes (arrowheads) is due to dissection.

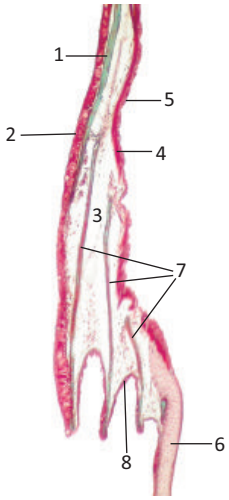


Fig. : 10.17 *Danio rerio* (MT / LM)

Operculum and branchiostegals. On each side of the head of bony fishes the chamber housing the gills is protected by an *operculum*. This protective flap is a bony plate (green) lined with the skin and supported by a loose connective tissue. The *operculum* is composed of four bones and its morphology varies greatly between species.

1 : bony *operculum* – 2 : skin – 3 : loose connective tissue – 4 : striated muscle – 5 : epithelium continuous with the skin – 6 : ventral cartilaginous support – 7 : three branchiostegals – 8 : branchiostegal or gill membrane.

Branchiostegals (or branchiostegal rays) are long and pointed dermal bones (or cartilages) that support the branchiostegal membrane (with some mucous cells and sometimes inappropriately called the gill membrane) below the *operculum*. Their number varies from none to up to 50. In cyprinids like the zebrafish there are three branchiostegals (7).

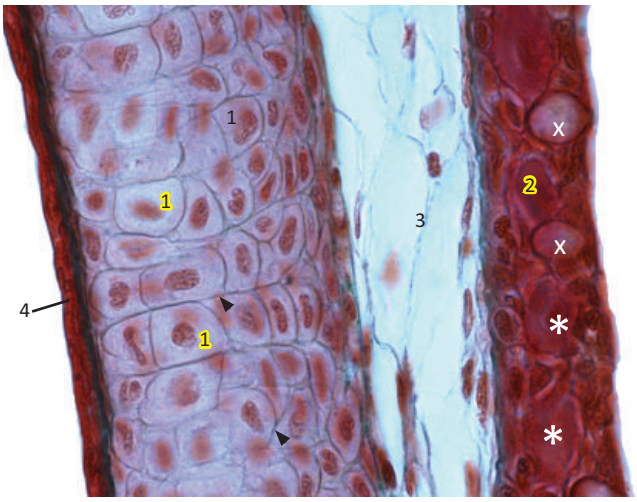


Fig. : 10.18 *Danio rerio* (MT / HM)

Section of the free posterior edge of a zebrafish *operculum*. In most fish, the rear edge of the *operculum* approximately indicates the limit between head and body. In *Danio rerio*, the ventroposterior part of the *operculum* shows tissue similar to hyaline-cell cartilage containing close packed cells (1) separated by scarce extracellular matrix (arrowheads).
2 : epidermis with mucous (x) and club (∗) cells ; 3 : loose connective tissue of the dermis; 4 : thin epithelium. Note that the latter is continuous with the skin epithelium. Together they surround the *operculum*. Remind that unlike bony fish, sharks and rays do not have gill covers.

11

Excretion and osmoregulation

The kidney of teleost fish is a mixed organ comprising hematopoietic, phagocytic, endocrine and excretory elements. The first three functions are all dealt with elsewhere and this section will be confined to consideration of the excretory element.

Excretory kidney

The entire kidney of adult fishes is a mesonephros. The functions of excretion and osmoregulation are closely related and are performed by both gills and kidneys in fishes. Although the gills are chiefly respiratory organs, they are also important as excretory and osmoregulatory devices; the majority of the nitrogenous waste products of a fish is eliminated by excretion across the gills. Kidneys play the most important part in maintaining the water-salt balance.

The kidneys vary greatly between different species of fish, both grossly and histologically. Often (partially or totally) fused (*Clupeidae, Salmonidae, Anguillidae, Cyprinidae*, …) they lie in a retroperitoneal location just ventral to the vertebral column, and can extend from the head region to the posterior abdomen as one organ, or they can have distinct head and trunk regions.

In teleosts the anterior part of the kidney (head kidney) often functions as a complex hematopoietic tissue, which also contains chromaffin and adrenocortical endocrine elements; few renal tubules are observed. The posterior kidney (Figs 11.1 to 11.13, 11.18, 11.22 to 11.27) contains more renal tubules with a lesser amount of interstitial hematopoietic and lymphoid tissue and thus functions as a osmoregulatory and to some extent excretory organ. The primary task of a freshwater fish kidney is to produce copious dilute urine to counteract the passive influx of water across the gills and integument. By contrast, saltwater fish need to conserve fluid and this is achieved through modifications in the histology of nephrons.

A typical freshwater nephron consists of cytologically distinct regions : a *glomerulus*, neck segment, proximal, intermediate and distal tubules. The latter connect to small collecting tubules which join larger collecting ducts that empty into ureters. Fluid loss is limited in saltwater fish by reducing the size and number of *glomeruli* (Fig. 11.22), and in some species (such as toadfish, goosefish and syngnathids) by eliminating *glomeruli* altogether.

The *glomeruli* (Figs 11.2 to 11.4, 11.6 & 11.22) of different species of teleosts vary greatly in number and size of the capillary tuft. The parietal epithelium is flattened and BOWMAN's space is rather small.
As with the *glomerulus*, the morphology of the individual segments varies greatly from one species to the next. The neck region (Fig. 11.6) is continuous with the parietal and visceral epithelia of BOWMAN's capsule and shows a narrow lumen surrounded by ciliated cuboidal/columnar epithelial cells. The cytoplasm of these cells stains slightly basophilic. This segment is usually short, and opens into a wider proximal tubule (Figs 11.6, 11.8, 11.11 & 11.13). Proximal tubular epithelial cells are columnar with a prominent brush border (*microvilli*), abundant apical vacuoles, mitochondria and basal nucleus. In some fish the first part of the proximal tubule P1 stains faintly compared to the more intensely eosinophilic second part P2. This second part is also characterized by a less developed brush border. The intermediate segment has a narrow lumen surrounded by cuboidal cells that often have *cilia*, which help move the filtrate along the nephron. The distal segment (Fig. 11.10 & 11.13) is lined with large, relatively clear columnar epithelial cells lacking the brush border seen in the proximal tubules. Lectinology can be an excellent method for differentiating proximal and distal segments (Figs 11.12 & 11.13). Collecting tubules and ducts (Figs 11.9, 11.14 & 11.15) are located throughout the kidney. Their columnar epithelium is lightly eosinophilic with basal nu-

clei and no brush border. Successive collecting tubule segments increase in diameter with their epithelium becoming pseudostratified and possessing mucous cells (Fig. 11.14). Large collecting ducts incorporate layers of smooth muscle and connective tissue. The ureters (Figs 11.16 to 11.19) open directly to the outside by a urinary pore or end into the bladder (Figs 11.19 to 11.21) as in *Cyprinidae*. The bladder, lined by a pseudostratified epithelium (urothelium), contains smooth muscle, nerves and connective tissue in its wall. The bladder can be only a simple dilation of the ureters or a true saccular organ (*Barbus, Mystus*…) emptying outside by an urogenital pore.

In marine fishes, the *glomeruli* are smaller and the intermediate segment is absent. The requirement here is to slow down the movement of fluid so that there is time for the maximum amount of passive diffusion of water back into the blood. The distal segment is also often absent. Notwithstanding the classification of elasmobranch fish as a group of lower vertebrates, renal organization is highly complicated in marine elasmobranchs (Figs 11.23 to 11.27), such as *Scyliorhinus sp*.
Indeed, renal tissue is zonated, and composed of numerous long coiled nephrons. A rather large renal corpuscle, the neck, or first tubular portion, is lined by cuboidal cells which bear flagella. Following the neck is the large dorsal canal which is provided with a brush border and is the longest segment of the whole renal tubule. It is generally called the proximal convoluted tubule. A small dorsal canal follows the large one and its cells are small.

The most striking feature of the elasmobranch kidney, is that 95% of the urea in the glomerular filtrate is reabsorbed in the dorsal tubule, by a countercurrent system. The initial thinner part of the distal tubule is applied to the proximal tubule, making up lateral bundles (Figs 11.24,11.26 & 11.27) in the kidney. The end result of this folding of the tubule back on itself is that a double countercurrent system of five parallel tubular segments is formed, effectively resorbing urea and other organic nitrogenous solutes in the glomerular filtrate as it passes down the tubule.

Chloride cells

Key structures in ionic exchange across the gills in teleost fish are the chloride cells (Figs 10.12 & 11.28), which are implicated in pumping salt inwards (Ca^{2+} and Cl^-) in fresh water and outwards (Cl^-) in seawater. They are located principally at the junction between the primary and secondary *lamellae*. These cells stain strongly with eosin and are richly endowed with mitochondria intricately laced with a tubular network of smooth endoplasmic *reticulum*. The chloride cells of the freshwater and marine species seem to be of different nature. They lack in Chondrichtyes which have a rectal gland (see below).

Rectal gland of elasmobranchs

Unlike those of mammals, elasmobranch kidneys are incapable of producing a urine more concentrated than their blood. These cartilaginous fishes have an extrarenal organ, the rectal salt gland (11.29 to 11.33), which is located in the caudal region of the animal. This organ terminates in a duct that opens immediatly before the junction of the intestine with the rectum, and thus drains into the cloaca. The rectal gland is supplied by the rectal gland artery, the posterior mesenteric artery, and is drained by the large dorsal intestinal vein. The gland has a complex structure. It is composed of many, simple and branched, secretory tubules. These drain into the central canal which in turn drains into the duct of the gland. This arrangement facilitates the secretion of salt into the rectum of the animal. The gland is divided into lobules. Lobules are separated by interlobular *septa* that consist of connective tissue and blood vessels. Each lobule is composed of radially organized secretory tubules that run into the *center* of the gland. Tubules are made up of homogeneous cuboidal cells. The flow in secretory tubules constitutes a countercurrent arrangement. Thanks to this system, the epithelial tissue gland can secrete concentrated solutions of sodium chloride using transmembrane proteins as channels.

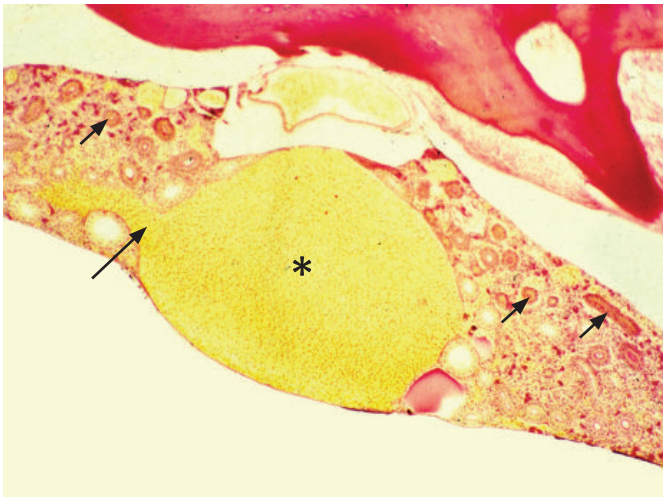


Fig. : 11.1 *Pangio kuhlii* (PAS-H-AUR / MM)

General view of a mesonephric kidney. Fish kidneys are complex organs comprising hematopoietic, phagocytic, endocrine and excretory elements. They are diffuse elongated structures lying above the gas bladder and just beneath the vertebral column. In this transverse section most of the parenchyma is filled with tubules (short arrows) and hematopoietic tissue. Renal arteries from the dorsal aorta supply the kidney and blood is carried away by two posterior cardinal veins. The long arrow indicates the place where a vein drains into the large cardinal vein (∗). At the top of the picture bone tissue (red) of a vertebra is found.

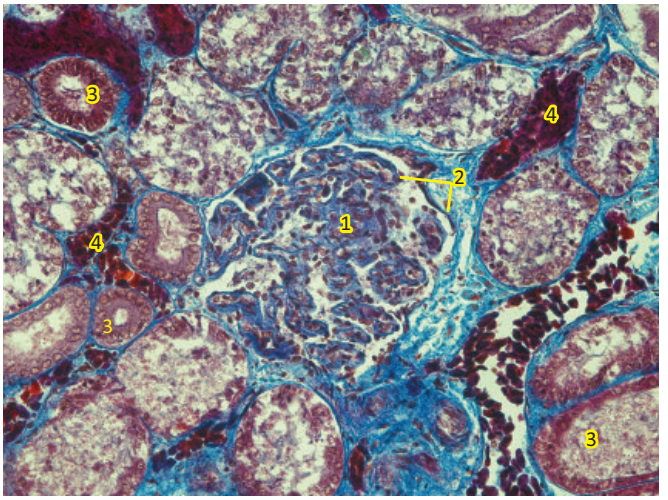


Fig. : 11.2 *Scyliorhinus canicula* (MT / MM)

Dogfish kidney. The photomicrograph shows at the centre a large renal corpuscle containing a well-developed *glomerulus* (1). Its cavity is lined by single layer of flat cells (2). The corpuscle is surrounded by connective tissue (blue), tubules (3) of various diameters and blood vessels (4).

The dogfish renal corpuscles are interposed between *sinus* zone and bundle zone (see Fig. 11.24). The mesonephric corpuscles of the anamniot vertebrates are very similar in appearance to metanephric ones, even to those of higher mammals.

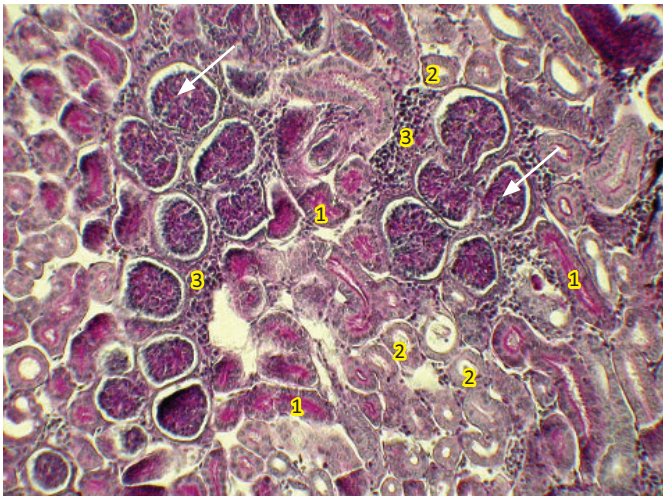


Fig. : 11.3 *Acipenser gueldenstaedtii* (PAS-H / LM)

Sturgeon kidney. A fish nephron typically consists of a renal corpuscle and a renal tubule. The former is the combination of the *glomerulus* and the Bowman's capsule ; the renal tubule has different histological zones such as neck segment, proximal, intermediate and distal (absent in marine species) tubules. This section shows several renal corpuscles (arrows) curiously grouped in this species. Nephron tubules of various diameters take up a great part of the micrograph. Even at this low magnification proximal tubules (1) can be distinguished from the other tubules (2) by their PAS+ brush border. In this posterior renal part hematopoietic tissue (3) is fairly scarce.

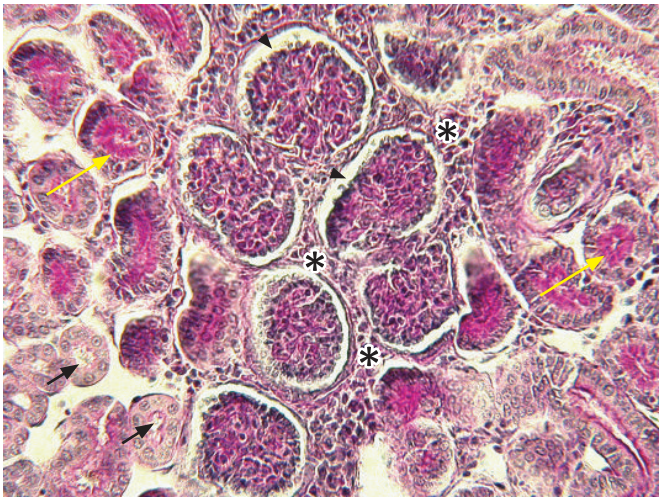


Fig. : 11.4 *Acipenser gueldenstaedtii* (PAS-H / MM)

Six grouped renal corpuscles are illustrated at the centre of the photomicrograph with few hematopoietic tissue in between (∗). In most fish the corpuscles are fairly scattered through the renal parenchyma. Proximal and distal tubules are also shown. The former possess an apical brush border of *microvilli* clearly PAS+ (long arrows) whereas the brush border of the distal segment (short arrows) is very reduced or absent. Bowman's space (arrowheads) is the name given at the space between the parietal and the visceral (podocytes) layers of the Bowman's capsule (see Fig. 11.6).

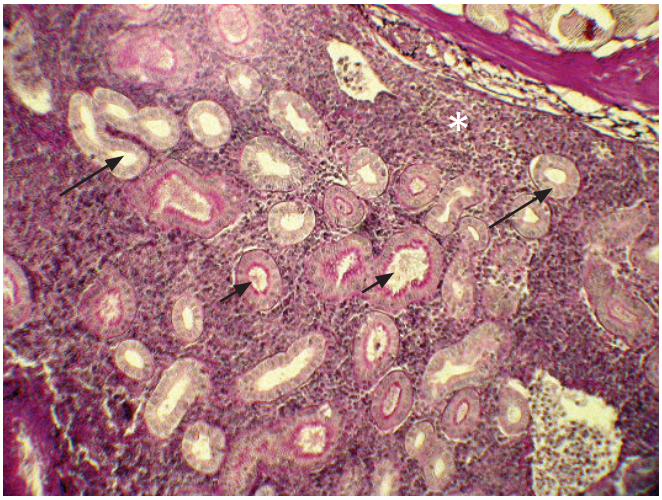


Fig. : 11.5 *Anguilla anguilla* (PAS-H / LM)

Eel kidney. Numerous proximal (short arrows) and distal (long arrows) tubules encircled by hematopoietic tissue (∗) are seen. Remind that kidney is regarded as being a major immune organ in fish (see chapter 5).

The proximal segments are identified by the PAS+ brush border.

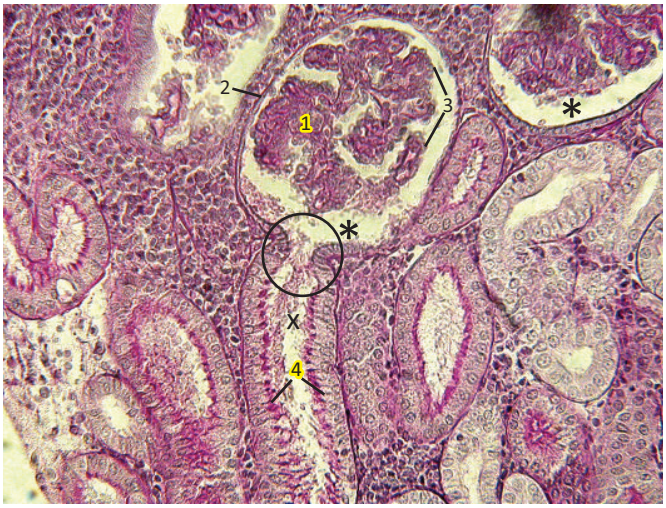


Fig. : 11.6 *Anguilla anguilla* (PAS-H / MM)

Eel kidney. The upper midfield shows a well-visible renal corpuscle with its well-vascularized *glomerulus* (1) and its Bowman's capsule. The latter is composed of an outer single layer (parietal layer – 2) and an inner layer of epithelial cells (podocytes – 3) in intimate contact with the *glomerulus*. Bowman's space (= space between the two layers - ∗) is continuous with the lumen (x) of the neck region. The epithelium of the neck segment is simple columnar and characterized by ciliated cells (*cilia* and basal corpuscles PAS+ - 4). The continuity of the renal corpuscle with renal tubule marks the urinary pole (circle).

Tightly packed proximal and distal tubules are also displayed. The brush borders of the former are PAS+ (magenta).

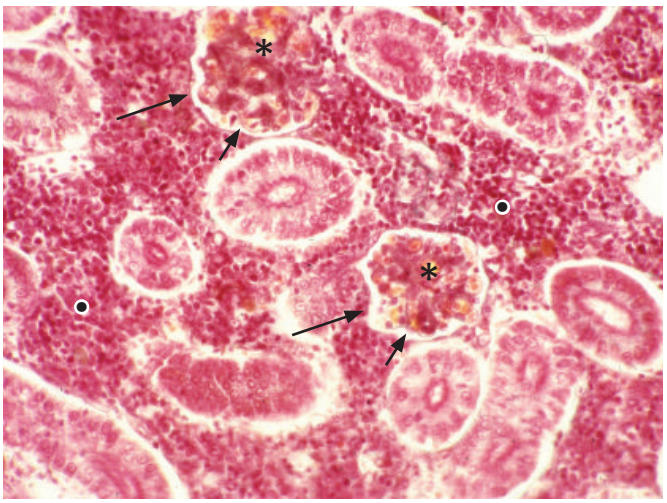


Fig. : 11.7 *Cyprinus carpio* (MT / MM)

Kidney. As carps are freshwater fish, their kidneys possess fairly large, well-vascularized *glomeruli* and long coiled proximal and distal tubules. This section shows two large rounded renal corpuscle (long arrows). The *glomeruli* (∗) are surrounded by Bowman's spaces (unstained - short arrows). *Glomeruli* consist of a network of capillaries (erythrocytes in orange) and the numerous nuclei inside are mainly those of podocytes and capillary endothelial cells. One can also see nephron tubules of various sizes and shapes among which lie hematopoietic areas (●).

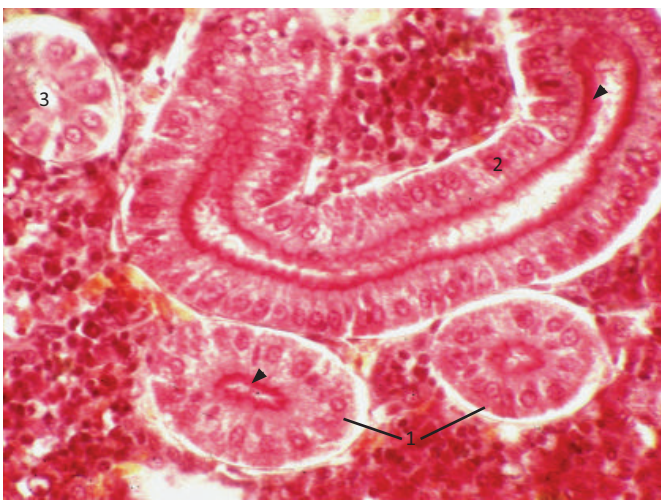


Fig. : 11.8 *Cyprinus carpio* (MT / HM)

Kidney. Two transverse sections (1) and one longitudinal section (2) of proximal convoluted tubules are illustrated. These tubules are easily identified by the dark-stained brush borders (arrowheads). The brush border enhances reabsorption of fluid and solutes from the lumen through or between the cuboidal epithelial cells and into capillaries. In addition, a distal tubule in transverse section is also noticed (3). The rest of the micrograph is filled with hematopoietic tissue.

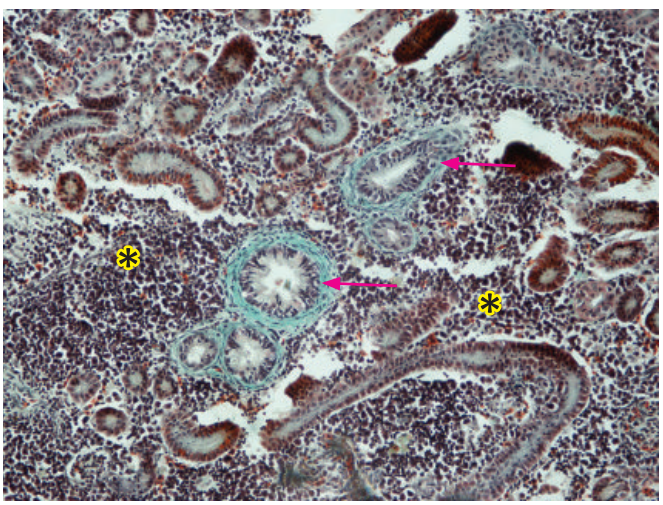


Fig. : 11.9 *Rutilus rutilus* (MT / MM)

Kidney. Sections of proximal and distal convoluted tubules of various diameters are demonstrated. The centre of the picture exhibits transverse sections through collecting tubules (arrows). They are histologically distinct from other segments as they are constructed of tall columnar epithelial cells enclosing wide lumens and surrounded by a thick sheath of connective tissue (turquoise). Nests of hematopoietic tissue are obvious (∗).

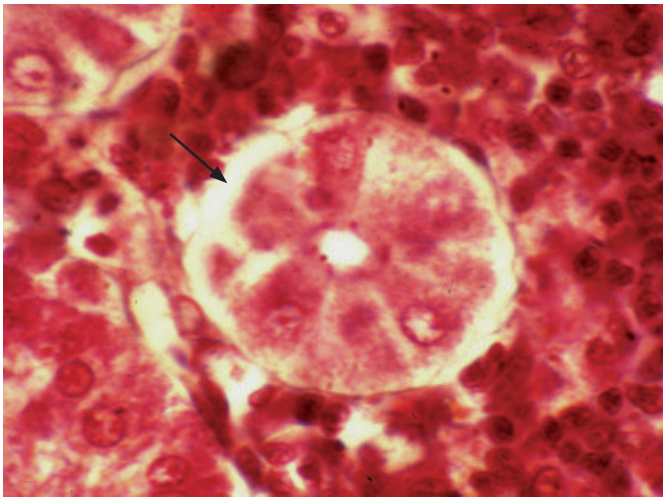


Fig. : 11.10 *Cyprinus carpio* (MT / HM)

Transverse section of a distal convoluted tubule. These tubules can usually be distinguished from proximal ones by the absence of a brush border and therefore by a more defined lumen. These tubules lead to the collecting tubules which merge to form collecting ducts. The distal tubule is lacking most of the time in marine teleosts. As usual in kidney tubules (except in some collecting ducts) the plasma membranes are not visible. The arrow indicates an artificial space (artefact) probably caused by the section preparation.

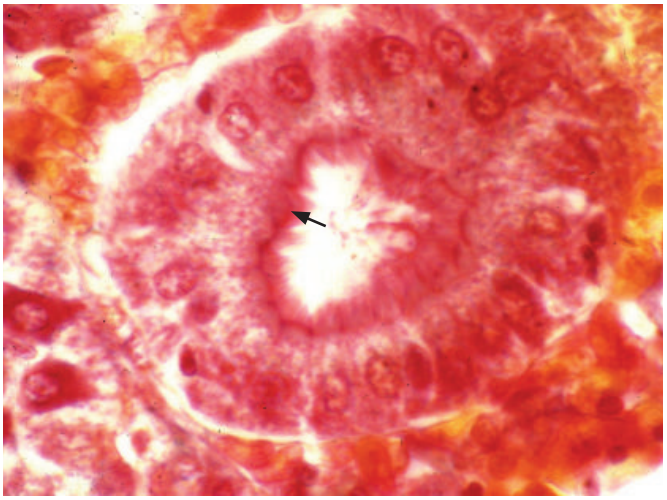


Fig. : 11.11 *Cyprinus carpio* (MT / HM)

Transverse section of a proximal convoluted tubule. In the proximal tubule, as in each part of the renal tubule, the epithelial cells form a simple cuboidal / columnar epithelium. The most characteristic feature of proximal tubule is the constant presence of *microvilli* constituting the brush border (arrow) protruding into the lumen. The epithelial cells of the proximal tubule are often very stained because of the large number of mitochondria closely related to basal plasma membrane folds. In many fish the proximal tubule is divided into two segments P1 and P2. P1 segment has longer *microvilli* (thus a taller brush border) and stains faintly compared to the more intensely eosinophilic second part P2. Erythrocytes stained orange.

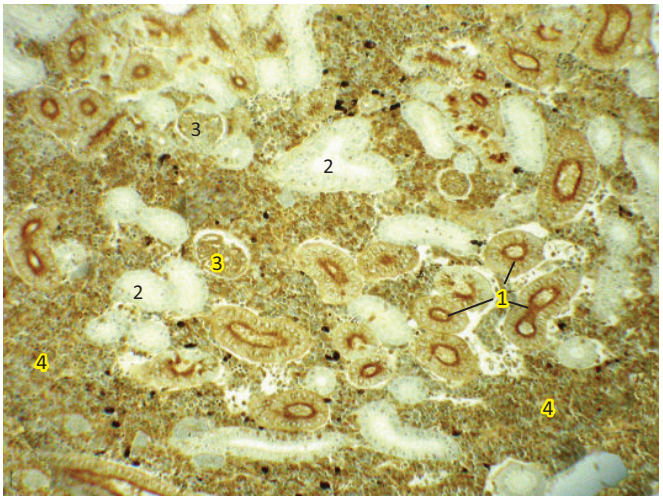


Fig. : 11.12 *Oncorhynchus mykiss* (LEC WGA / LM)

General view of kidney parenchyma. This slide is a photomicrograph of several proximal and distal tubules stained with a lectin (WGA : Wheat Germ Agglutinin) which selectively binds to N-acetylglucosamine (GlcNAc) and sialic acid (both staining brown). The brush borders of the proximal tubules are heavily stained (1) whereas the distal segments (2) are unreactive. 3 : points to renal corpuscles, slightly reactive. 4 : hematopoietic tissue.

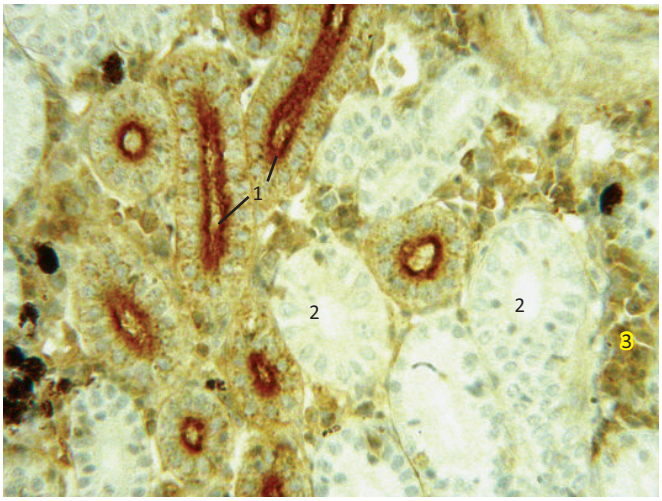


Fig. : 11.13 *Oncorhynchus mykiss* (LEC WGA / HM)

Kidney. Lectins can bind to appropriate carbohydrates and histochemical methods involving the use of lectins have much in common with immunohistochemical techniques. In this particular case, lectins are excellent probes for differentiating proximal and distal segments.

This micrograph reveals lectin histochemical localization of complex sugar moieties (stained in brown color) in the trout kidney. Lectin reactive signal was detected on the brush border (1) of the proximal tubules but not in the distal tubules (2) which lack a brush border. Melanomacrophage centers (black) and hematopoietic tissue (3) can be seen.

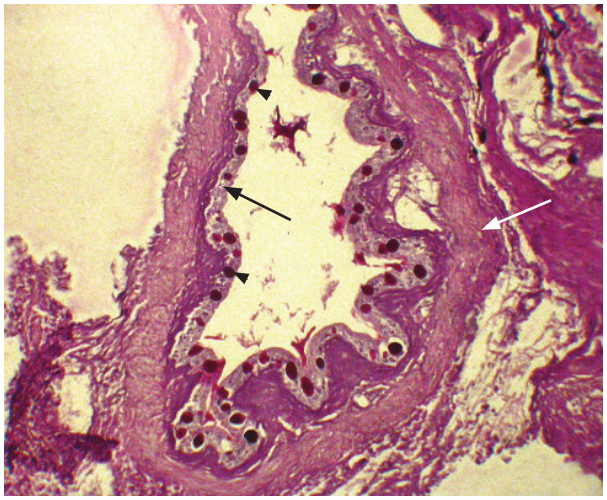


Fig. : 11.14 *Anguilla anguilla* (PAS-H / MM)

Collecting duct of the eel kidney. Large collecting ducts are lined with a mucous pseudostratified epithelium (black arrow) and several layers of smooth muscle cells (leiomyocytes – white arrow). The PAS reaction produces an intense magenta stain in mucous epithelial cells (arrowheads).

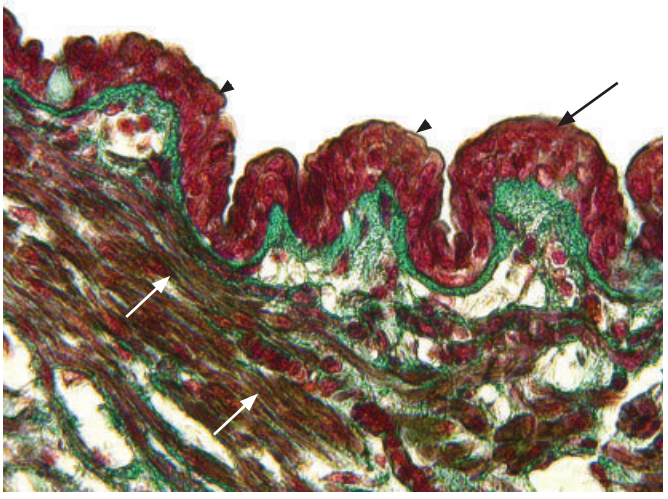


Fig. : 11.15 *Acipenser gueldenstaedtii* (MT / MM)

Collecting duct. The wall consists of a transitional pseudostratified epithelium (black arrow) resting on connective tissue (*lamina propria* in green) and on several layers of smooth muscle (white arrows). The epithelium is highly folded and the surface outline shows dome-shaped cells with thickened plasma membranes (arrowheads).

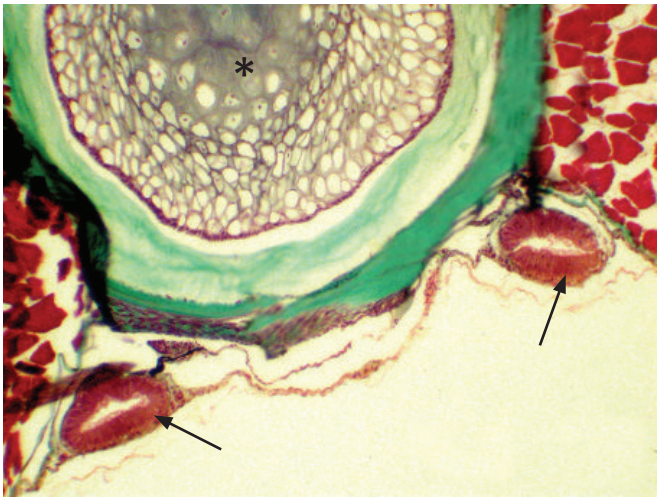


Fig. : 11.16 *Xiphophorus helleri* (MT / MM)

Transverse section through the posterior abdominal cavity of a xipho. The photomicrograph shows the (two) ureters (arrows), large ducts conducting urine from the kidneys. In some fish they fuse to form a common duct opening directly outside whereas in others they distend in a urinary bladder. Each ureter duct is lined with a tall columnar epithelium. The notochord (∗) surrounded by vertebral bone (mint green) occupies the upper part of the picture.

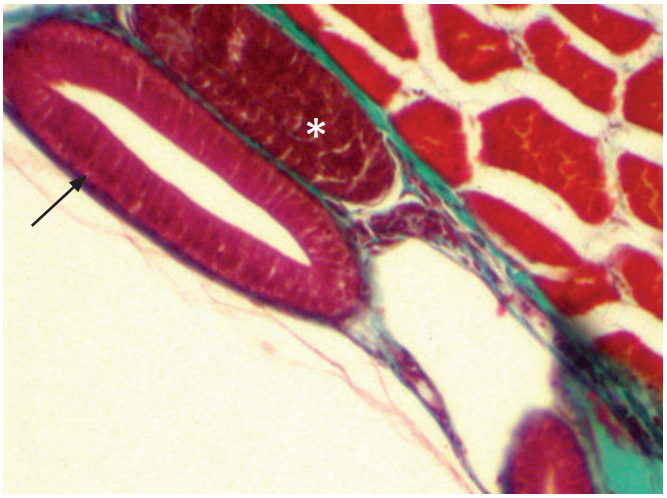


Fig. : 11.17 *Xiphophorus helleri* (MT / HM)

Ureter in transverse section. Higher magnification of a similar section showing one ureter and its tall columnar epithelium (arrow). Note a large blood vessel (∗) above the duct and obliquely sectioned rhabdomyocytes (red polyhedra) of the epaxial musculature on the upper right.

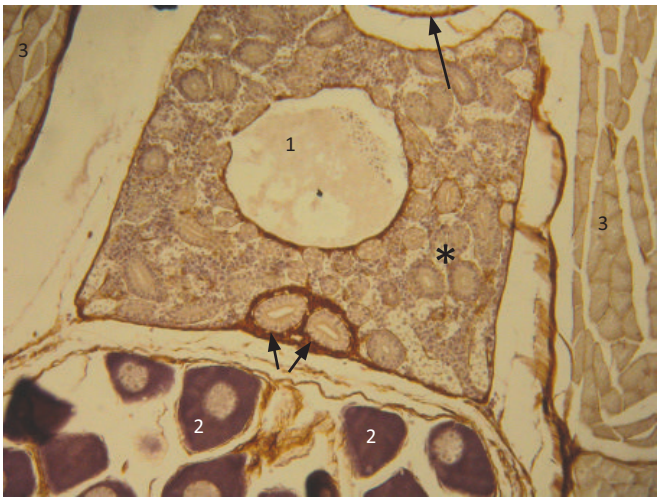


Fig. : 11.18 *Pangio kuhlii* (LEC SJA / MM)

Cross section of posterior kidney. As *Cyprinidae, Cobitidae* have partially fused kidneys in their posterior parts. Kidneys appear at this level as a single mass (∗) essentially composed of renal tubules. Ureters (short arrows) are ventrally located and arranged side by side (compare with Fig. 11.16).

SJA is a N-acetylgalactosamine (Gal-NAc)-specific lectin isolated from the bark of the legume tree *Sophora japonica*. Gal-NAc is strongly expressed (deep brown) in the connective tissue, mainly the one surrounding ureters ; proximal as well as distal tubules are unstained. One can also see the caudal vein (1) in the centre of renal tissue, some dark previtellogenic oocytes (2 - see chapter 14) and abdominal musculature (3). Long arrow points to the dorsal aorta.

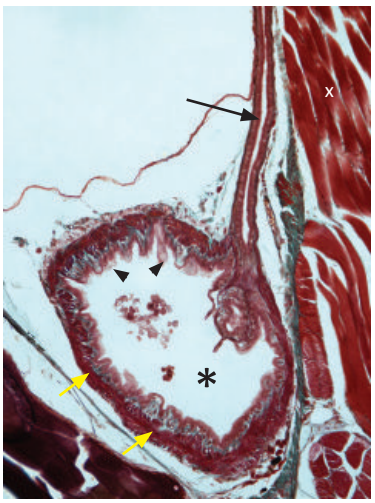


Fig. : 11.19 *Poecilia reticulata* (MT / MM)

Urinary bladder. Longitudinal section of one of the ureters (black arrow) running into the urinary bladder (∗). In many bony fishes, enlargement of the urinary ducts serve as bladder. An empty bladder exhibits mucosal folds (arrowheads) that disappear when the bladder is distended (filled with urine). The wall of this hollow sac contains dense smooth muscle bundles (yellow arrows). Note near these structures the presence of skeletal muscle fibers (red - x) of the gonopod musculature. At the lower left corner a portion of male gonad (dark) is visible.

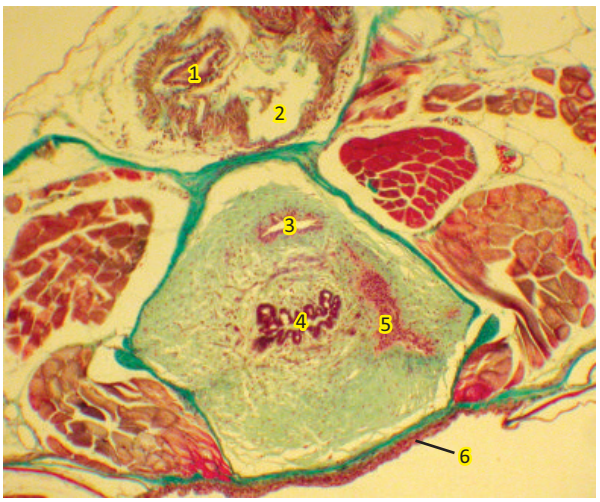


Fig. : 11.20 *Poecilia reticulata* (MT / MM)

Slightly oblique transverse section of the abdominal cavity at the urogenital level. This micrograph shows one ureter (1) ending in the urinary bladder (2), urethra (3), spermiduct (4) and a part of the epithelial wall (5) of the urogenital *sinus* (see next figure). Dense (mint green) and loose (pale green) connective tissue, abdominal rhabdomyocytes (red) and ventral epidermis (6) are also present.

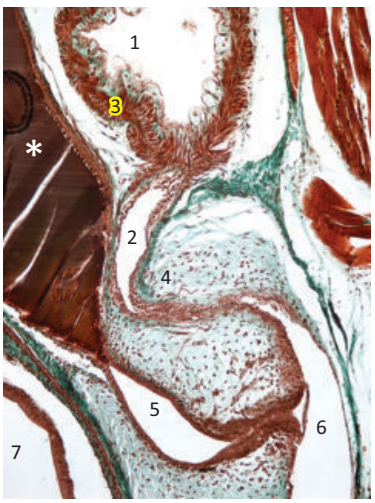


Fig. : 11.21 *Poecilia reticulata* (MT / MM)

Section close to the sagittal plane at the urogenital level. This micrograph reveals the anatomical arrangement of urogenital ducts in this fish. Urine exits the bladder (1) via the urethra (2). The lumen of the bladder is lined by a pseudostratified epithelium prolongated by a thinner and fairly cuboidal/squamous urethra epithelium. Smooth muscle layers (3) surrounding the urinary bladder are quite abruptly replaced by connective tissue (4) around the urethra. This image allows to distinguish three other ducts i.e. the spermiduct (5) coming from the testis (∗), the urogenital *sinus* (6) and the rectum (7). The urogenital *sinus* is lined by a thin squamous epithelium and opens to the outside world behind the anus (spermatozoa pass through the *gonopodium*).

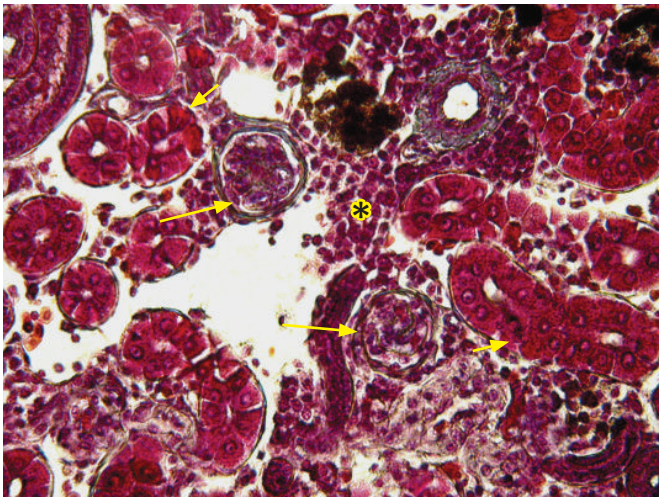


Fig. : 11.22 *Trisopterus luscus* (MT / MM)

Kidney of pouting. Marine fish lose water by osmosis through the gills and the body surfaces. To maintain their homeostasis, they pump excess salts away through the gills and drink a lot of water. The degree of development of the nephrons can be correlated with environment. In many marine teleosts, which are not subjected to osmotic inflow of water, *glomeruli* are small (long arrows) or secondarily absent (*Syngnathidae, Batrachoididae*) and the distal tubules are often missing. The short arrows indicate some proximal tubules. In addition, melanomacrophage centers (brown black) are also visible among hematopoietic cells (∗).

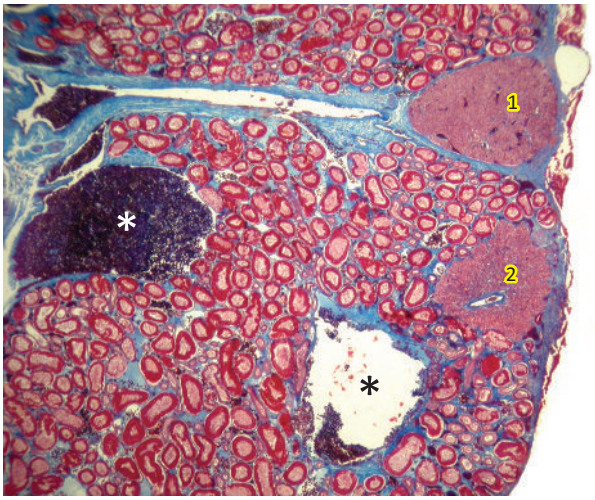


Fig. : 11.23 *Scyliorhinus canicula* (MT / LM)

Dogfish excretory kidney. The paired elongated kidneys are located retroperitoneally near the vertebral column. The anterior ribbon-like parts are separated whereas caudal parts form a single mass. The elasmobranch kidney is remarkable for its unique structure and is divided in two main histological zones : the *sinus* zone and the bundle zone (not visible here – see Figs 11.24, 11.26 and 11.27).

This low magnification presents a section through the *sinus* zone displaying numerous individual cross-sectioned tubules. The latter are of two types : large (proximal) and thin (late distal) tubules. Large venous sinuses (∗ - a filled one and an empty one) are evident among the tubules. Steroidogenic tissue of the interrenal tissue (1) and chromaffin tissue (2) are clearly visible (see chapter 13). Collagen is stained in blue.

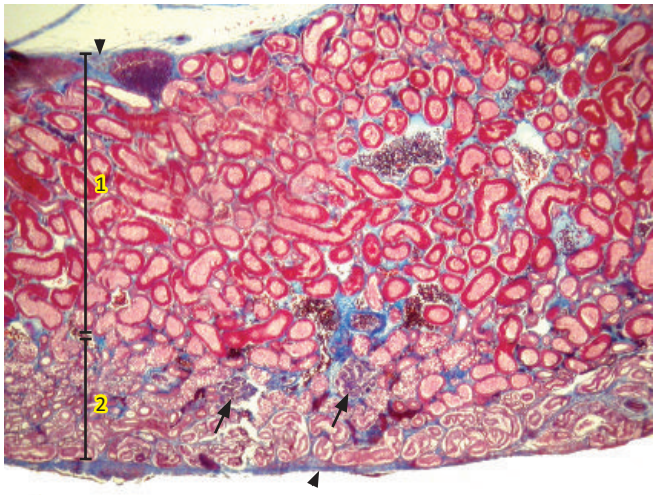


Fig. : 11.24 *Scyliorhinus canicula* (MT / LM)

Dogfish excretory kidney. The parenchyma consists of multiple segmental lobules separated into two zones : the *sinus* zone (1) with individual tubules and the bundle zone (2) with tubular bundles. Each single nephron forms two convoluted tubules in the *sinus* zone and two hairpin loops in the bundle zone. The boundary between these two areas is marked by large renal corpuscles with prominent *glomeruli* (arrows). The kidney is covered with a connective layer (arrowheads) of varying thickness and more or less dense according to its localization.

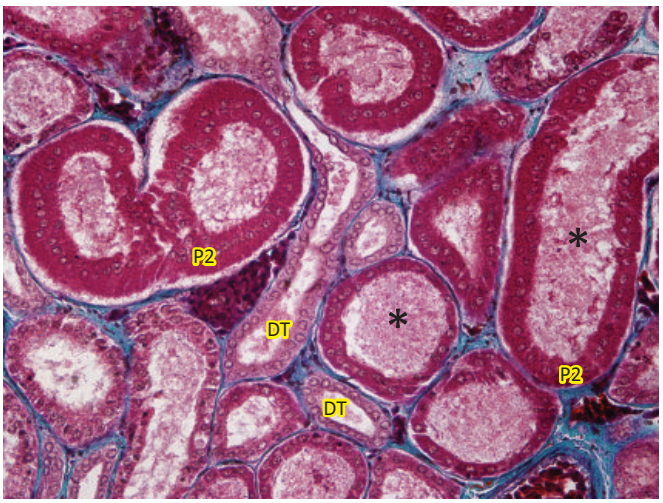


Fig. : 11.25 *Scyliorhinus canicula* (MT /HM)

Dogfish excretory kidney. A complete nephron comprises : the renal corpuscle, the ciliated neck segment, the first (P1) and the second (P2) portions of the proximal tubule, the intermediate segment, the early and late distal tubule and the collecting duct.

This photomicrograph presents a portion of the *sinus* zone showing the two types of convoluted tubules (thick parts - P2 and thinner parts or late distal tubules - DT). The proximal tubules are characterized by high columnar cells with centrally located nuclei and provided with a brush border. Their lumen contents are interpreted as vesicular material (∗). The thin tubules differ from the thick ones by lower cells and absence of a brush border.

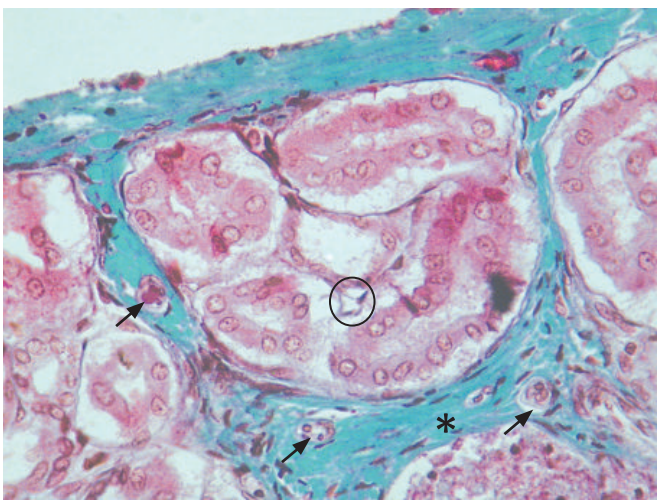


Fig. : 11.26 *Scyliorhinus canicula* (MT / HM)

Dogfish kidney bundle. The most amazing feature of the elasmobranch kidney is that more than 90% of the urea is resorbed from filtered primary urine : this involves a very high urea concentration in plasma and body fluids. These observations imply the existence of a specific mechanism enabling reabsorption of urea by the kidney. Indeed, histological studies have revealed that the renal tubules of marine elasmobranchs are highly elaborate and that passive transport of urea is correlated with countercurrent systems occurring in the bundles.

Each bundle consists of five canals which all belong to the same nephron. One canal (the neck segment NS) comes from the Bowman's capsule, two canals (the intermediate segment IS and the collecting duct CT) come from the *sinus* zone and two last canals (the first part of proximal tubule P1 and the early distal tubule EDT) leave the bundle and run into the *sinus* zone. A large collecting duct leaves the bundle at its distal end. NS and P1, as well as IS and EDT form the two hairpin loops and the whole forms a double countercurrent mechanism allowing passive reabsorption of urea. A small bundle vessel (circle) is normally visible between the tubules. Each bundle is surrounded by an important peritubular connective sheath (collagen in turquoise - ∗) containing some capillaries (arrows) and flattened connective tissue cells.

The identification of the different bundle tubules based only on Masson's trichrome is difficult : serial sections, specific histochemical reactions and electron microscopy are often necessary for a reliable diagnosis (see specialized literature).

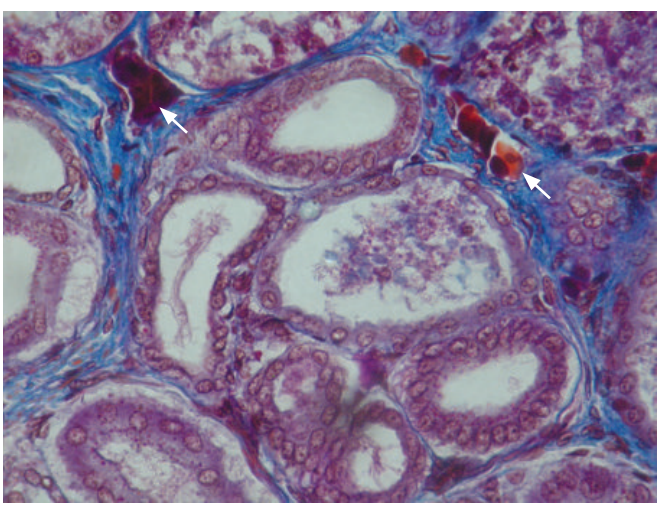


Fig. : 11.27 *Scyliorhinus canicula* (MT / HM)

As mentioned above each bundle actually contains five adjacent tubules and each of these groups corresponds to one nephron ! In a relatively important number of bundles, six or more tubular profiles can be seen : they result from cross sections through twisting and coiling parts of the second loop. This bundle is surrounded by a sheath of well-vascularized connective tissue : arrows point to small blood vessels and collagen is blue. *For the same reasons as in the previous image, the five tubular portions are difficult to differentiate.*

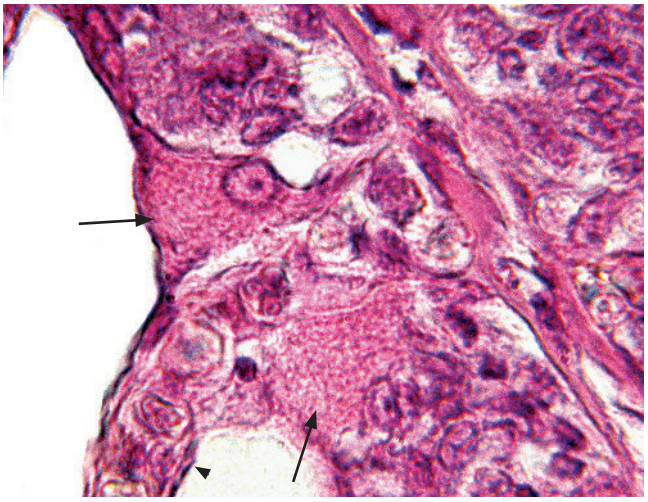


Fig. : 11.28 *Anguilla anguilla* (H-E / HM)

Chloride cells. Osmoregulation in teleosts is an integrated combination of transport activity by the kidney, the gut and the gills. The chloride cells of the gills are implicated in active Cl^- transport. This photomicrograph shows two chloride cells (arrows) located within the thick epithelium of a primary *lamella* (gill filament). The granular appearance and the eosinophilia of the cells are due to the abundance of mitochondria which are in close relation with the basolateral infoldings of the plasma membrane. Arrowhead shows the proximal part of a secondary *lamella*.

Chloride cells are sometimes present at the beginning of the digestive tract (see Fig. 7.22).

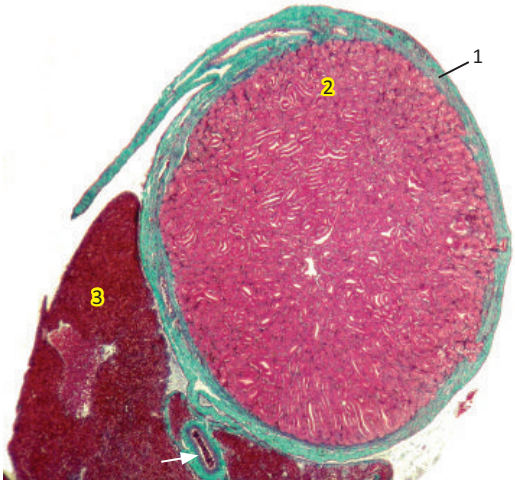


Fig. : 11.29 *Scyliorhinus canicula* (MT / LM)

Transverse section of the distal portion of the rectal gland. The rectal gland is a slender, blind-ended, finger-like appendix present in all elasmobranchs. This epithelial gland leads to the post-valvular intestine (rectum) via a large excretory duct. It is an organ of osmoregulation producing a fluid essentially consisting of a sea water-hyperosmotic NaCl solution. The rectal gland is suspended in the abdominal cavity by a mesentery. The arterial supply is the rectal gland artery (white arrow).

The rectal gland is a compound tubular gland composed of a peripheral capsular wall (1 – collagen in green), a glandular parenchyma (2) and a duct portion. The glandular portion is mainly composed of secretory tubules radially oriented to drain into the central region where the large collecting duct (not visible in this section) is lined by a stratified epithelium. The lower end of the epigonal organ (3) is attached to the rectal gland.

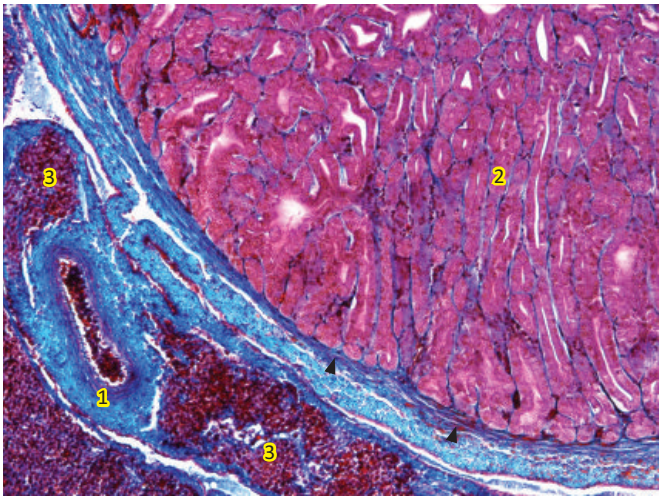


Fig. : 11.30 *Scyliorhinus canicula* (MT / MM)

Transverse section of the rectal gland wall. The capsule consists of an outer layer of *peritoneum* and a layer of smooth muscle fibers (elongated leiomyocytes in pink – arrowheads) bathing in fibro-elastic tissue (collagen in blue). It is richly supplied with arterioles of the rectal gland artery (1). Collagen as well as elastic fibers run into the glandular parenchyma (2) which consists of branched compactly arranged tubules lined by a simple cuboidal epithelium.

Lymphomyeloid tissue (3) of the epigonal organ is found close to the artery.

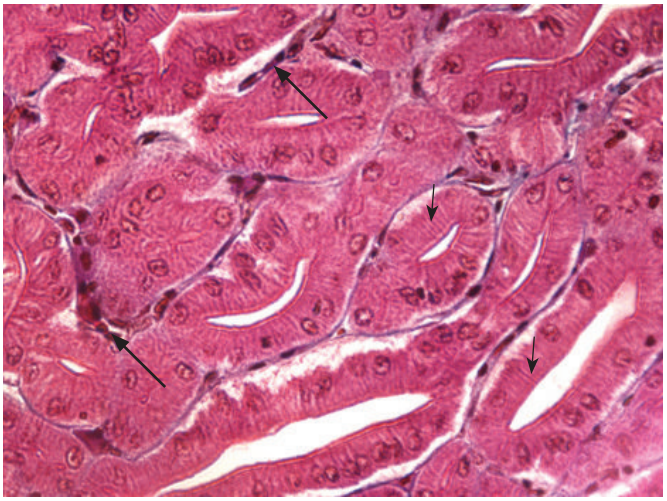


Fig. : 11.31 *Scyliorhinus canicula* (MT / HM)

Glandular parenchyma of rectal salt gland. The micrograph presents secretory tubules whose lumens are clearly lined with a simple cuboidal epithelium. Bouin-fixed sections usually give little information on cytological specializations associated with active electrolyte transport. However one can see striations (thin arrows - more obvious in Fig. 11.33) in the cytoplasm of the epithelial secretory cells. The appearance of striations noted with the light microscope, is found in electron micrographs to be due to the vertical alignment of long and numerous mitochondria closely related to deep invaginations of the basolateral plasma membranes. Numerous blood capillaries (arrows) run between the tubules.

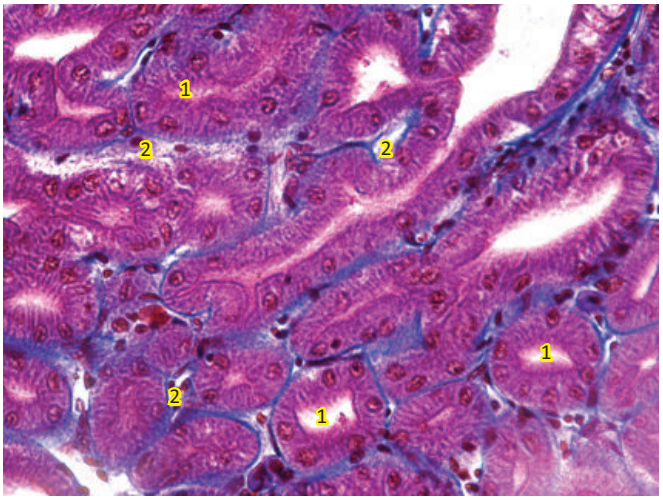


Fig. : 11.32 *Scyliorhinus canicula* (MT / HM)

Glandular parenchyma of rectal salt gland. Delicate strands of fibroelastic tissue (collagen in blue) coming from the capsule invade the glandular portion and surround the secretory tubules (1).

The rectal gland has a rich blood supply from the rectal gland artery which breaks up into numerous arterioles in the capsular wall. The arterioles then penetrate into the glandular parenchyma as a system of blood sinusoids (2) from which secretory cells can remove relatively large amounts of NaCl. Capillaries finally coalesce as the rectal vein that emerges from the gland, becoming the dorsal intestinal vein.

In the secretory tubules the fluid flows in the opposite direction to that of the blood in the capillaries thus constituting a countercurrent exchange.

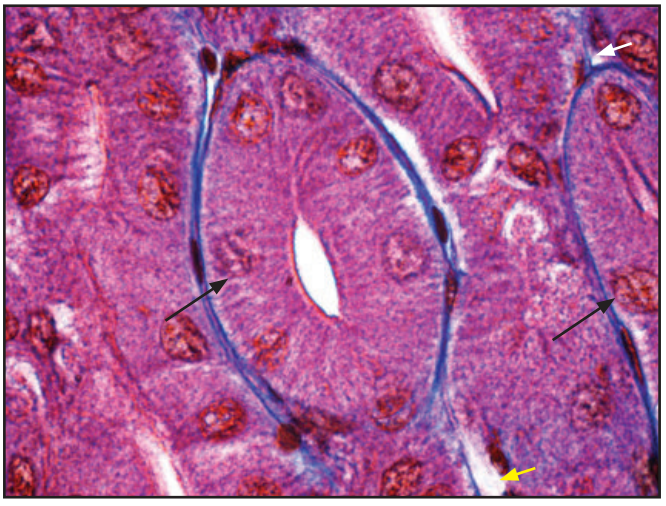


Fig. : 11.33 *Scyliorhinus canicula* (MT / IM)

Rectal salt gland. At the centre a cross-sectioned secretory tubule shows the typically striated appearance of the epithelial cells intensively involved in ion transport. This feature includes the presence of a very high mitochondrial content closely associated with deep infoldings of the plasma membrane. In secretory tubules two cell types occur : the predominating secretory cells (black arrows) and scattered goblet mucous cells mainly visible in the proximal part of the gland near the rectum.

The nucleus of secretory cells is rounded or oval and displays one or more nucleoli. The cells are provided with a short apical brush border which can be revealed among others by the AB method. These cells are a model for the transport of chloride : Cl^- enters the cell across the basolateral cell membrane via a cotransporter and leaves the cell across the luminal membrane via a CFTR-like channel. Note collagen in blue and some capillaries (short arrows).

12
Nervous system

The nervous system is defined as the structural and functional mechanism regulating the response of an animal to its internal and external environments or to changes in these environments.

In order for the nervous system to function in the mediation of responses to the environment, it is widely distributed throughout the body. The two major divisions of the nervous system are the central nervous system (CNS) and the peripheral nervous system (PNS).

The central nervous system

The CNS (Figs 12.1 to 12.28) is composed of the brain and spinal cord, and the PNS comprises all cranial and spinal nerves with their associated roots and ganglia. Although these two major divisions are clearly defined anatomically, it must be remembered that they are connected through the roots of cranial and spinal nerves.

In general outline, the brain, which is formed by the enlargement of the cephalic end of the spinal cord, is similar in all fishes, though they are considerable differences between the fish groups in the relative development of the different regions.

The five main brain regions are (Figs 12.1 & 12.2) :

telencephalon (olfactory lobes and cerebral hemispheres)

diencephalon ("between brain")

mesencephalon (optic lobes and *tegmentum*)

metencephalon (*cerebellum*)

myelencephalon (*medulla oblongata*)

The *medulla oblongata* tapers in the spinal cord, in which the narrow central canal is the posterior continuation of the brain ventricular system.

The fish telencephalon (Figs 12.3 to 12.5) is much reduced in comparison with mammals and is composed of the olfactory lobes and cerebral hemispheres (*cerebrum*). Ventricles are sometimes visible (Chondrichthyes - Fig. 12.4) but are usually not obvious. The *cerebrum* of teleosts is markedly different in histologic appearance from that of mammals in that it lacks a neocortex, the distinctive six-layered structure of mammalian cortex. The *cerebrum* of teleosts consists of fields of interconnected neurons supported by an extensive neuropil.

The diencephalon (Fig. 12.7) or the "between brain" is divided into the dorsal epithalamus comprising the pineal organ and the habenular ganglion, the thalamus itself and the hypothalamus ventrally. The hypothalamus, consisting of the *infundibulum* and the two inferior lobes, is the major anatomic structure of the diencephalon and functions to regulate the pituitary gland.

The mesencephalon is large in all fishes, roofed by the layered *optic tectum* (Fig. 12.7); its floor is the *tegmentum*. As in many anamniot vertebrates, the adult *torus semicircularis* (TS) of fish comes to lie on the top of the *tegmentum*. The TS is the principal target of the octavolateral hindbrain. The octavolateral system is a collective name for three following mechanosensory systems. The auditory, equilibrium and lateral line system have similar receptor cells called the hair cells. It is connected to many other areas of the brain and is involved in auditory and lateral line processing. The *optic tectum* is the main anatomical termination of the retinal ganglion cell axons. The development of this region reflects the degree of importance of the visual sense in different species. The *optic tectum* is divided grossly into two optic lobes

and has a characteristic laminar histological architecture that consists of five principal layers. These layers are distinguished by their relative content of nonmyelinated *versus* myelinated axons and presence or absence of neurons.

Behind the *infundibulum* of the pituitary gland lies the *saccus vasculosus* (Figs 12.20 & 12.21), a highly vascularized tissue resembling the choroid plexus of mammals. This structure produces cerebrospinal fluid for the third ventricle lumen with which it is continuous. The organ is composed of ribbons of cuboidal/columnar epithelium supported by a fibrovascular stroma with prominent capillaries.

The *cerebellum* is the major component of the dorsal metencephalon. It performs the same function of sensorimotor coordination in teleosts as it does in other vertebrates. The teleostean *cerebellum* is well developed and has three major divisions : the *valvula cerebelli*, the *corpus cerebelli*, and the vestibulolateral *cerebellum*. The *corpus cerebelli* and the vestibulolateral lobe are probably homologous to the *corpus cerebelli* and vestibular *cerebellum*, respectively, of mammals, but the valvula is present only in ray-finned fishes. Whereas the *corpus cerebelli* lies on top of the rostral rhombencephalon as in all vertebrates, the valvula projects forward under the *tectum* in the mesencephalic ventricular cavity. The cerebellar cortex contains the three characteristic layers (Figs 12.13 to 12.16): an outer molecular and inner granular with an intermediate ganglionic layer. The outer molecular layer contains parallel fibers, PURKINJE cell dendrites, eurydendroid cell (efferent neuron) dendrites and stellate cells (small neurons). The *somata* of these elements measure 6 by 8 μm. The parallel fibers course in the transverse plane, intersecting the sagitally oriented dendrites of PURKINJE and eurydendroid cells; the ganglionic layer is beneath the molecular layer. This layer corresponds to the PURKINJE cell layer in mammals but is referred to as the "ganglionic" layer in teleosts because it contains the cell bodies of both eurydendroid cells and PURKINJE cells (Figs 12.13 to 12.15). The *somata* of PURKINJE cells are rounded or pear-shaped. The cell bodies of the eurydendroid cells are rather triangular or rhomboid-shaped. Eurydendroid cells are slightly larger than the PURKINJE cells. The granular layer (Figs 12.12 to 12.16), beneath the ganglionic layer contains densely packed small multipolar neurons, the granule cells; the *somata* of these cells have a diameter of 3-4 μm.

In one group of teleosts, the mormyrids, the *cerebellum* (Figs 12.8 to 12.10) reaches amazing dimensions. In these forms the valvula (Figs 12.9 to 12.14) has grown out of the ventricle of the midbrain to become a superficial structure which covers almost all the brain. Different areas of this huge *cerebellum* are linked to the input of the different types of electroreceptors (see chapter 15).

The *medulla oblongata* represents the mass of the hindbrain and is continuous caudally with the spinal cord. It includes the nuclei of some cranial nerves (V to X) and is the site of origin of the Mauthnerian system (Figs 12.24 to 12.26), a neuromuscular specialization that is well developed in teleosts. It comprises a pair of very large neuronal bodies (Figs 12.24 & 12.25) located centrally in the *medulla* the axons of which (Fig. 12.26) run down the spinal cord in the ventral white matter as the largest fibers within it. Firing of the MAUTHNER cells causes a rapid and powerful tail flip that teleosts exhibit when frightened.

In the spinal cord (Figs 12.22, 12.23, 12.26 & 12.27), the grey substance shows a marked difference in arrangement from that of higher vertebrates in that the dorsal horns lie so close together that there is hardly any white substance between them. This gives the grey substance the shape of an inverted Y (Figs 12.26 & 12.27).

The neurons and neuroglia constitute the cellular elements of the brain and of the spinal cord. Neurons of fish are similar to those of mammals and contain abundant NISSL substance (Fig. 12.18), i.e. rough endoplasmic *reticulum*, in their cytoplasm, an extensive GOLGI apparatus and a large nucleus, possess dendrites and nonmyelinated or myelinated (Fig. 12.28) axons.

The central neuroglial cells identified in teleosts include astrocytes, oligodendroglia and ependymocytes; the two formers are difficult to distinguish in routine tissue sections. Immunohistochemical studies, using specific antibodies against proteins and peptides, are necessary to uncover any differences.

Teleost ependymal cells (Fig. 12.28) possess *cilia*, large quantities of glycogen and numerous huge mitochondria suggesting a specialized metabolic activity.

The peripheral nervous system

The PNS (Figs 12.29 to 12.42) is derived from the neural crest and is composed of ganglia (Figs 12.29 to 12.34 & 12.41, 12.42) and nerves (Figs 12.35 to 12.42).

As contrasted with the CNS, the PNS is simply constructed. It consists essentially of the nerves that penetrate to almost every region of the body : groups of fibers along which the sensory impulses are brought into the spinal cord and brain by afferent pathways, and through which resulting efferent impulses pass outwards to affect muscular or tegumental structures.

The ganglia contain the cell bodies of peripheral neurons.

In the head of vertebrates there is a special series of ten (cyclostomes, fish and amphibians) or twelve (reptiles, birds and mammals) pairs of cranial nerves (Figs 12.35, 12.36 & 12.40). The classification and name of these nerves are beyond the scope of this book (see anatomical works). It must be borne in mind that we have here a series of nerves which are amazing in variety but only seemingly haphazard in distribution.

Several pairs of spinal nerves (Fig. 12.42), all mixed, emerge from the spinal cord between the vertebrae. Each nerve has two roots (sensory neurons in the dorsan root and motor neurons in the ventral) which merge to form a mixed spinal nerve after leaving the spinal cord.

A large nerve (Figs 12.36 to 12.40) is composed of cylindrical bundles (*fasciculi*) of nerve fibers, each of which is internally supported by very delicate areolar tissue, called *endoneurium* (Fig. 12.40), and externally wrapped by *perineurium* (Fig. 12.36), a sheath of relatively dense connective tissue. *Fasciculi* are held together loosely by *epineurium*, a loose kind of connective tissue. A nerve is supplied by intra- and interfascicular blood vessels (Figs 12.36 & 12.39). Nerves do not harbor the perikarya of neurons. Except for certain highly specialized cranial nerves, nerves contain both sensory and motor fibers (mixed nerves).

In peripheral nerves, each individual axon is either enveloped by the myelin sheath (myelinated fibers) formed by SCHWANN cells, or surrounded by the cytoplasm of SCHWANN cells (unmyelinated fibers), not always easily detected at the light microscope level. The myelin is built up of successive layers of cell membrane of SCHWANN cells, which form a lipid-rich sheath around the axon. The fibers stain pale pink with hematoxylin/eosin. In longitudinal section, the most characteristic feature of peripheral nerves is the apparent wavy course of the axons (Figs 12.37 & 12.38).

The ganglion is enclosed by a dense connective tissue capsule, which divides into trabeculae to provide a framework for the neurons. Within the ganglion, myelinated axons in both cross and longitudinal sections can be observed (Fig. 12.30). In addition, blood vessels occur throughout the ganglion. Clusters of parasympathetic ganglion cells (myenteric or AUERBACH's plexus) are found between the two layers of the intestine *muscularis* (Fig. 12.41).

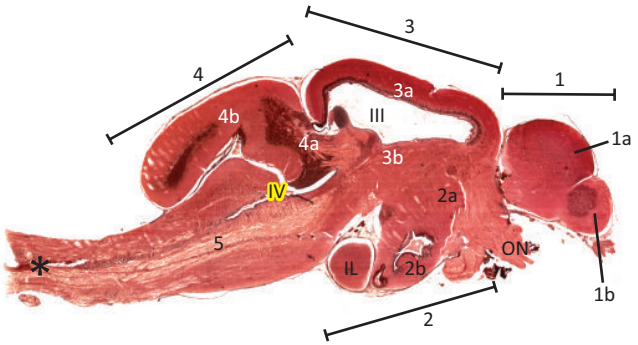


Fig. : 12.1 Young teleost specimen (MT / LM)

Parasagittal section of a teleost encephalon. This micrograph shows the anatomic relationship of structures comprising the five major parts of the brain.

From rostral (right) to caudal (left) one can see :

- telencephalon or *cerebrum* (1) : the most anterior part of the brain consisting of two cerebral hemispheres (1a) and a pair of solid olfactory lobes (1b)

- diencephalon (2) : divided into a dorsal epithalamus, a thalamus (2a) and a ventral hypothalamus (2b); it also consists of various appendages (pineal body, pituitary body…)

- mesencephalon (3) : consists of the dorsally placed optic lobes (3a - *optic tectum*) and the ventral *tegmentum* (3b)

- metencephalon (4) including the *cerebellum*, composed of the *valvula cerebelli* (4a) and of the *corpus cerebelli* (4b)

- *medulla oblongata* (5) is the elongated posterior region constituting the majority of the hindbrain and continuous with the spinal cord (∗)

III : third ventricle – IV : fourth ventricle – ON : optic nerve fibers – IL : inferior lobe

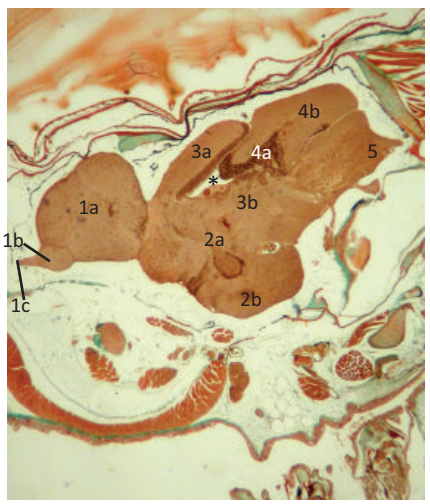


Fig. : 12.2 *Poecilia reticulata* (MT / LM)

Parasagittal section of the whole brain. From rostral (left) to caudal (right) :

- telencephalon with the cerebral hemispheres (1a), the olfactory lobes (1b) and the beginning of the olfactory tract (1c)

- diencephalon showing parts of the thalamus (2a) and of the hypothalamus (2b)

- mesencephalon with the well-developed optic lobes (3a) and the ventral *tegmentum* (3b)

- metencephalon dorsally composed of the *cerebellum* including the *valvula cerebelli* (under the optic lobes - 4a) and the *corpus cerebelli* (4b)

- myelencephalon (5)

∗ indicates the third ventricle

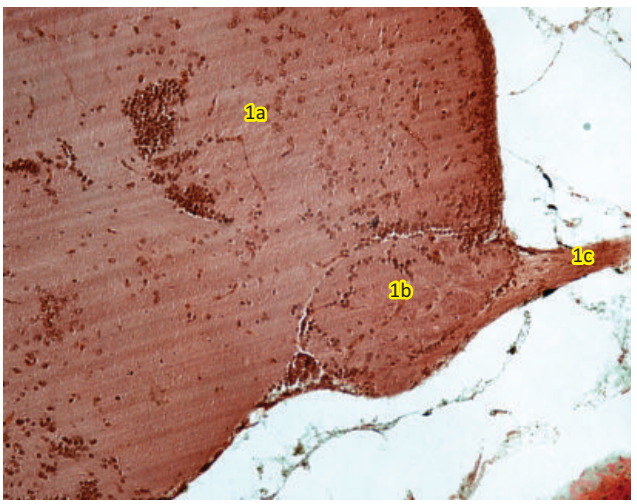


Fig. : 12.3 *Poecilia reticulata* (MT / MM)

Parasagittal section showing the anterior part of the telencephalon. One can recognize the cerebral hemisphere (1a), the olfactory lobe (1b) where sensitive nerve fibers of the olfactory nerve I end up. Fibers come from the smell receptors and join to form the olfactory tract (1c – partially visible).

The fish telencephalon lacks a neocortex, the distinctive six-layered structure found in mammals and instead consists of fields of neurons interconnected and supported by an extensive neuropil (nerve endings and dendrites).

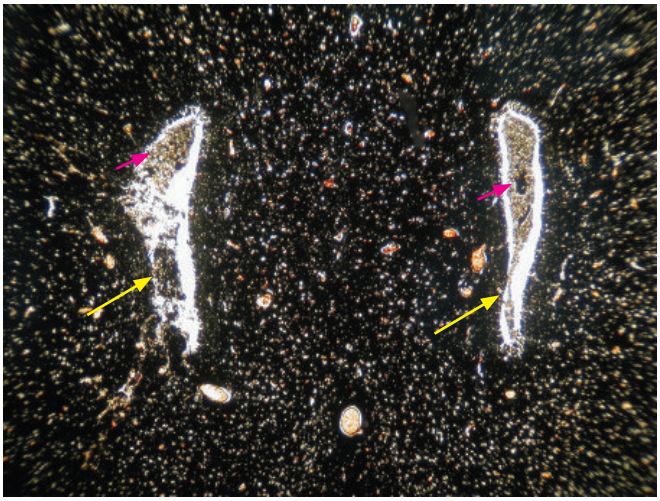


Fig. : 12.4 *Scyliorhinus canicula* (Ti-Ag / LM)

Dogfish telencephalon. Transverse section of the cerebral hemispheres showing the clearly separated first and second *cerebrum* ventricles (long arrows) inside which the paired anterior *tela choroidea* (short arrows) is found. In elasmobranchs, unlike Osteichthyes, the roof telencephalic (or cerebral) hemispheres are thick and constitute the *pallium* (complete cortex). The first and second lateral ventricles are in two separate ventricular lumens of the telencephalon and they open in the third ventricle via the *foramina* of MONRO. The nervous elements stained black and many capillaries display orange erythrocytes.

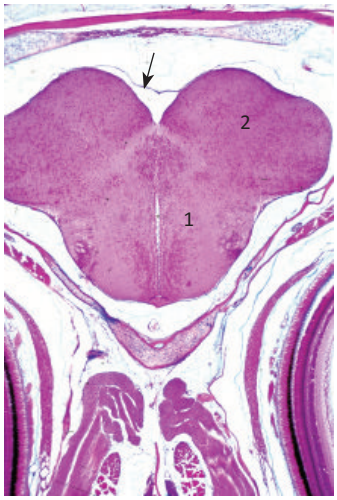


Fig. : 12.5 *Barbus caudovittatus* (H-E / LM)

Transverse section through the anterior part of the telencephalon. Teleost forebrain is quite aberrant and belongs to the everted type, absent in tetrapods : the lateral and dorsal parts of the hemispheres are rolled lateroventrally and thicken in two large masses containing the basal nuclei or *corpus striatum* (1) and the *epistriatum* (2). In Osteichthyes the roof of the telencephalon is a very thin (arrow) non nervous vascular sheath of ependymal cells which lines the central nervous system. The cerebral hemispheres are in communication one with the other and form only one (lateral) common ventricle.

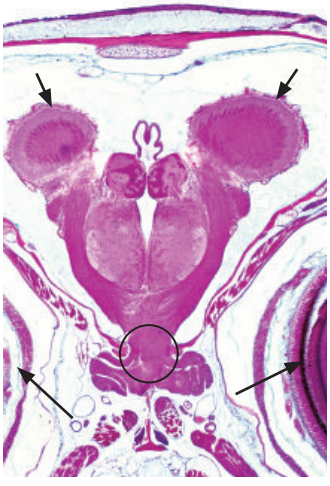


Fig. : 12.6 *Barbus caudovittatus* (H-E / LM)

Transverse section of the brain at the posterior part of the telencephalon. The cerebral hemispheres are in the form of solid masses (short arrows). The circle indicates the decussassing optic nerve and the long arrows point to the eyes. For more details see specialized literature.

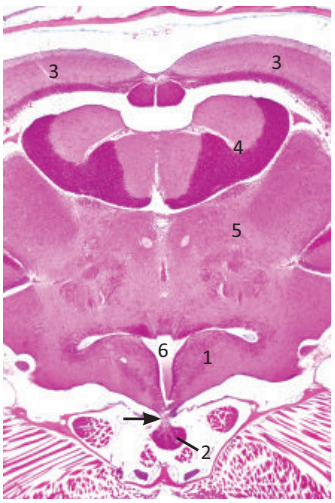


Fig. : 12.7 *Barbus caudovittatus* (H-E / LM)

Transverse section of brain at the level of the diencephalon. The picture also shows parts of the mesencephalon and of the metencephalon. The diencephalon is an area between the cerebral hemispheres and the optic lobes. Sometimes called «between brain» it attains its maximum development on the ventral side in the form of hypothalamus (1) and *infundibulum*. This latter is applied to the hypophysis (= pituitary gland - 2) in the mid-ventral line. The pituitary gland is the endocrine organ with the most widespread influence on bodily functions and is attached to the brain by means of a stalk (arrow – see also chapter 13).

3 : *tectum opticum* - 4 : *valvula cerebelli* - 5 : *tegmentum* (with *torus semicircularis*) - 6 : *aquaeductus of Sylvius* (ventral passage connecting the third and the fourth ventricles of the hindbrain).

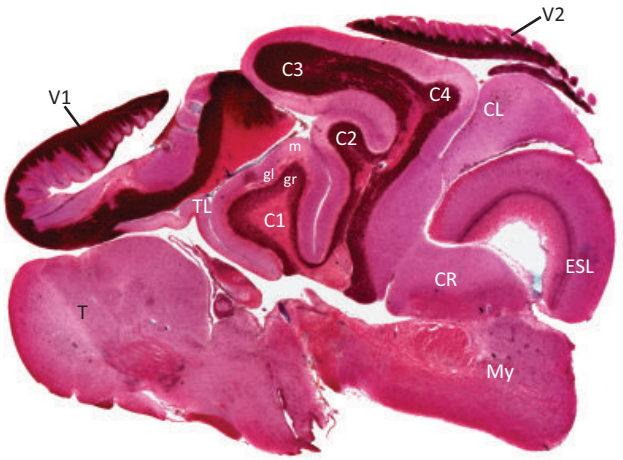


Fig. : 12.8 *Gnathonemus petersii* (MT / LM)

Parasagittal (nearly sagittal) section through the whole brain of the elephant nose fish. The *cerebellum* of the African fishes of the *Mormyridae* family is well developed and consists of the *valvula cerebelli*, the *corpus cerebelli* and the caudal lobes. Anterior (V1) and posterior (V2) lobes of the valvula are partly visible. This photomicrograph specially demonstrates the typical appearance of the *corpus cerebelli*. The latter is a large nervous mass dorsally located to the myelencephalon (My) and differentiated into four central lobes (C1 to C4).

In close relationship to the electrosensory system, the caudal lobe (CL - divided in anterior and posterior lobe) is located caudally to the central lobe C4 and dorsally to the electrosensory lobe (*linea lateralis lobus* - ESL) ; the posterior lobe (V2) of the valvula covers its dorsal surface.

The various central lobes show the three characteristic layers of the *cerebellum* : for instance C1 lobe possesses a molecular (m), a ganglionic (gl) and a granular (gr) layer.

Note also the telencephalon (T), the *cerebellum* crest (CR) and the transitory lobe (TL) of the *cerebellum*.

Fig. : 12.9 *Gnathonemus petersii* (Ti-Ag / LM)

Parasagittal section through the whole brain of the elephant nose fish. In Mormyriformes the greatly enlarged lateral lobes of the *valvula cerebelli* cover the brain dorsally and laterally : this arrangement is known as the *mormyrocerebellum*. In *Gnathonemus petersii* the valvula attains amazing dimensions and even extends in front (line) of the telencephalon (T) ! The posterior lobe (V2) of the valvula presents on its external surface numerous convolutions ; the anterior lobe (V1), which is a fold of the former, is smooth externally and the infoldings are oriented towards the inner side. ESL indicates the electrosensory lobe.

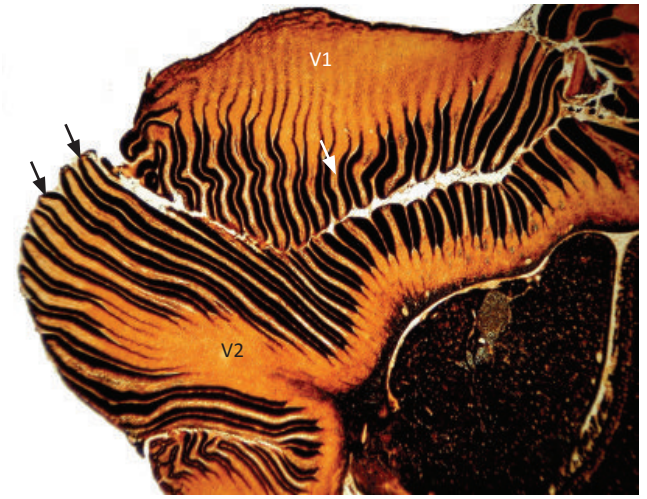


Fig. : 12.10 *Gnathonemus petersii* (Ti-Ag / LM)

Parasagittal section through the hypertrophied lateral lobes of the *valvula cerebelli*. As seen in this picture the valvula consists of a series of deeply convoluted folds (arrows) oriented towards the outer (posterior lobe – V2) or the inner (anterior lobe – V1) side. The axons are stained black with silver.

These lobes have grown out of the ventricle of the midbrain and this very remarkable hypertrophy of the mormyrid *cerebellum* involves an enormous capacity for processing electroreceptive information (see chapter 15). Fish with a large *valvula cerebelli* often have a better developed lateral line system.

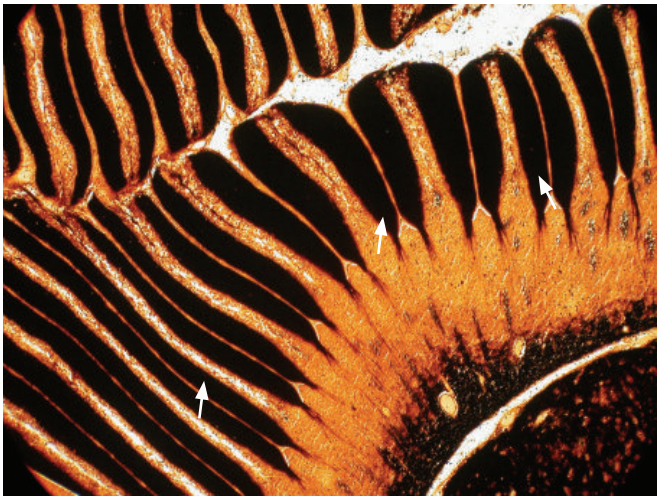


Fig. : 12.11 *Gnathonemus petersii* (Ti-Ag / MM)

Parasagittal section through the folds of the *valvula cerebelli*. Neuronal processes can be quite easily demonstrated in paraffin sections by using the silver impregnation according to Tinel. This rapid and fairly reliable method stains axons, fibrillary structures (neurofibrils) and dendrites of many neurons in black, with some differences depending on the procedure. The molecular layers (arrows) and neuronal processes are in black ; cell bodies (*somata*) of neurons are in shades of orange and brown.

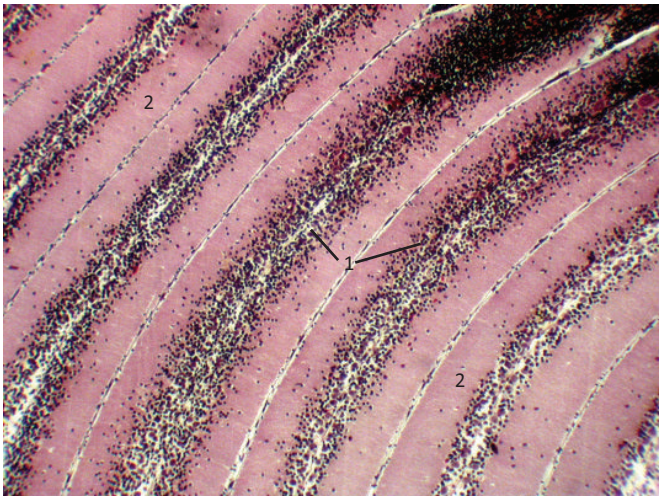


Fig. : 12.12 *Gnathonemus petersii* (MT / MM)

Section through the *valvula cerebelli*. The cytoarchitectonic properties of the teleostean (including mormyrids) *cerebellum* cortex are similar to other vertebrates. The valvula consists of the three characteristic layers : the molecular layer, the ganglionic layer and the granular layer. At this magnification only the granular (1) and the molecular (2) layer are distinguishable.

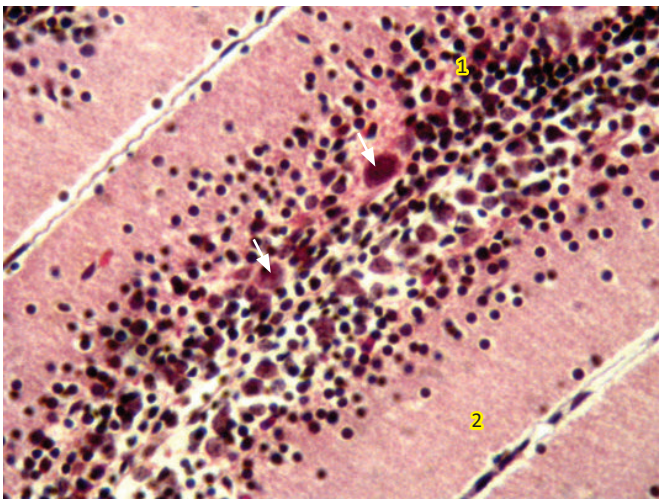


Fig. : 12.13 *Gnathonemus petersii* (H-E / HM)

Section through the *valvula cerebelli*. The valvula as the central lobes have a laminar organization. This image is a higher magnification of the previous and displays the granular layer (1) and the molecular layer (2). The granular layer consists of small, granular cells and numerous GOLGI cells. The molecular layer constitutes a continuous sheet of neuropil containing a huge amount of oriented dendrites (see Fig. 12.16). The intermediate or ganglionic layer contains PURKINJE cells (arrows) and numerous smaller cells. In contrast to the arrangement in most vertebrates, the PURKINJE cells are placed less regularly between the molecular and granular layers.

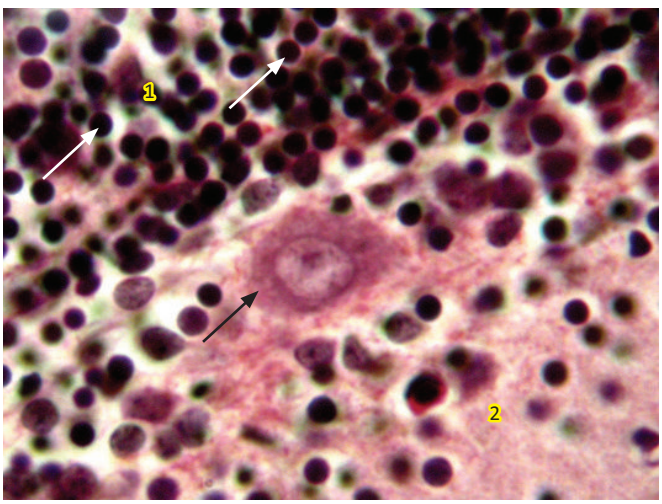


Fig. : 12.14 *Gnathonemus petersii* (MT / IM)

Section at the ganglionic layer level of the *valvula cerebelli*. A characteristic cell of this layer is the PURKINJE cell (black arrow). PURKINJE cells are randomly scaterred throughout the ganglionic layer and exibit large rounded or pear-shaped cell bodies (*somata*). Their dendrites ascend through the molecular layer (2) toward the external surface. The nuclei surrounding the PURKINJE cells are thought to be glial (satellite) cells. The small rounded nuclei (white arrows) are those of the neurons (granular cells and GOLGI cells) of the granular layer (1).

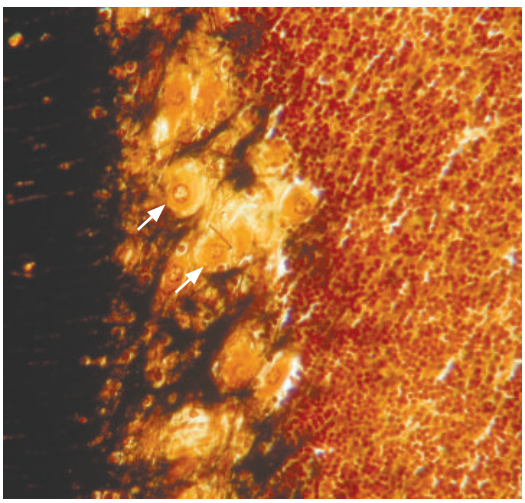


Fig. : 12.15 *Gnathonemus petersii* (Ti-Ag / HM)

Section through the elephant nose fish *cerebellum* showing the three characteristic layers. The ganglionic cell layer is illustrated in the centre of the photomicrograph. In addition to PURKINJE cells this layer also contains giant eurydendroid cells (arrows). These cells are less numerous and slightly larger than the PURKINJE cells. On the other hand the eurydendroid cell nucleus is smaller and the cell body/nucleus ratio is much higher. The granular layer is on the right and the molecular (black) is on the left.

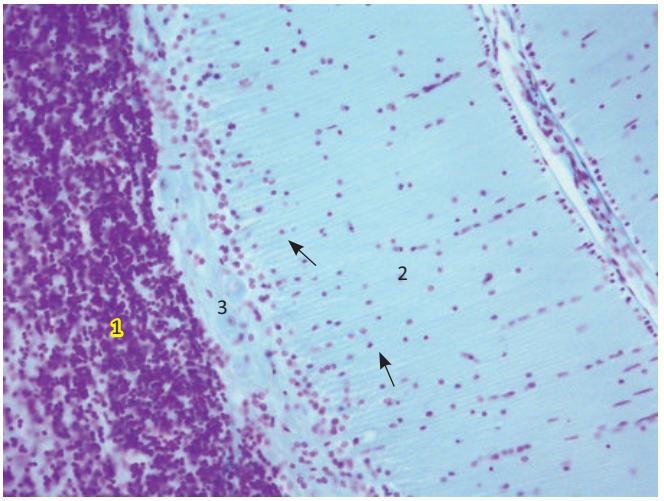


Fig. : 12.16 *Gnathonemus petersii* (FR-HB / HM)

Section through the elephant nose fish *cerebellum* showing the three characteristic layers. The granular (1), molecular (2) and ganglionic (3) layers of the grey matter are illustrated. Several main dendrites arise from the PURKINJE cells and give rise to branches which run strictly parallel to each other (arrows) through the molecular layer. This arrangement gives the latter its typical pattern.

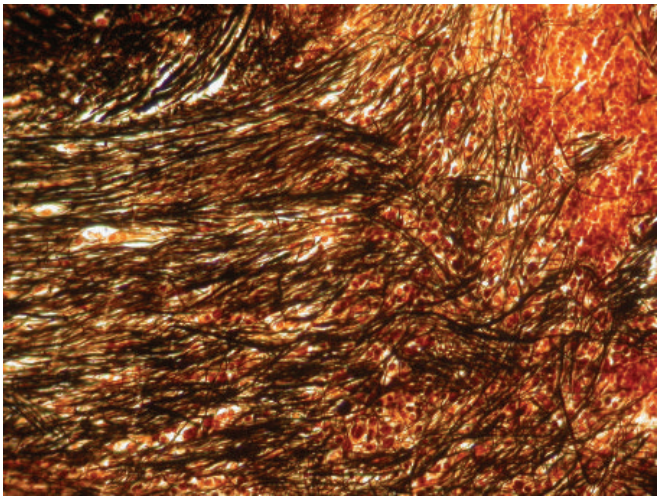


Fig. : 12.17 *Gnathonemus petersii* (Ti-Ag / HM)

Cerebellum. As one can see the silver impregnation according to Tinel stains axons, fibrillary structures (neurofibrils) and dendrites of many neurons in black.

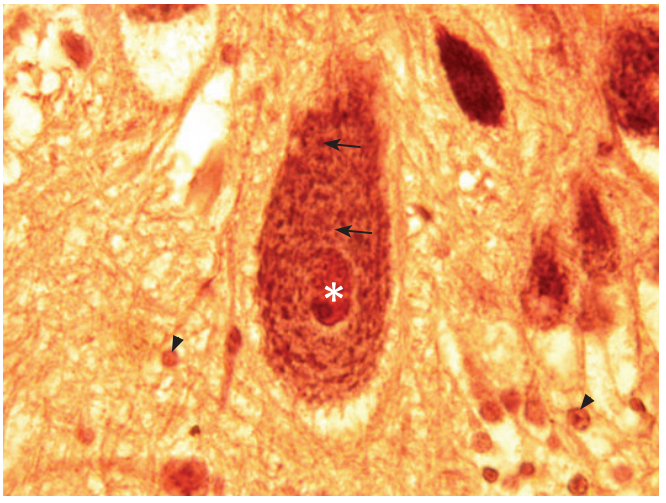


Fig. : 12.18 *Perca fluviatilis* (MT / HM)

Encephalon. Motor neurons containing NISSL bodies (arrows) give the neuronal cytoplasm a granular appearance. NISSL bodies are large granules which consist of rough endoplasmic *reticulum* (i.e. with ribosomes). The arrowheads point to glial cells (astrocytes, oligodendrocytes or microglial cells not distinguishable with trichrome method). The nucleus (∗) of the neuron is large, ovoid and centrally located in the cytoplasm.

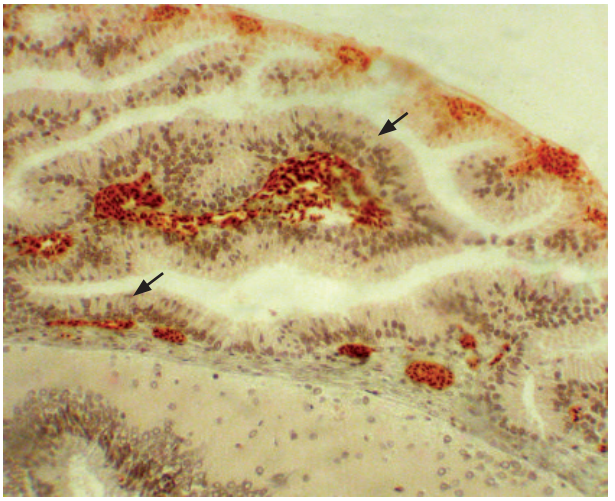
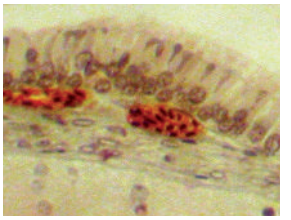


Fig. : 12.19 *Oncorynchus mykiss* (MT / MM)

Brain. The choroid plexi are vascular tufts located in the roof of the ventricles and extending from the *tela choroidea*. They consist of highly ramified vascular structures whose main functions are to secrete and absorb cerebrospinal fluid.

The choroid plexus is covered by a simple or pseudostratified columnar epithelium (arrows) composed of specialized cells. The underlying *lamina propria* is a loose connective tissue containing numerous thin-walled blood vessels (erythrocytes bright orange).



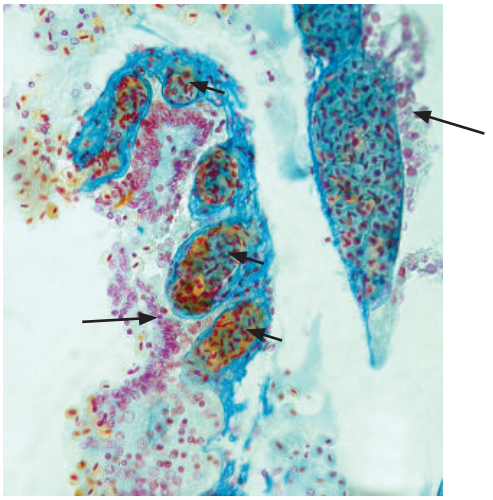


Fig. : 12.20 *Scyliorhinus canicula* (MT / MM)

Dogfish *saccus vasculosus*. The *saccus vasculosus* lies caudal to the inferior lobes of the *infundibulum* and dorsal to the pituitary gland. It is an oval thin-walled folded sac with ribbons of cuboidal/columnar neural epithelium (long arrows) supported by a highly and extensive vascular plexus (short arrows) bathing in connective tissue (blue).

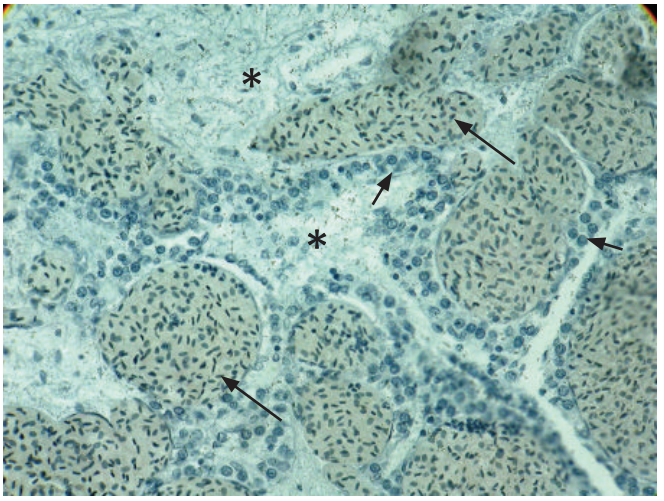


Fig. : 12.21 *Scyliorhinus canicula* (H-Pb / MM)

Dogfish *saccus vasculosus*. This section stained by the Mac Connail's lead hematoxylin clearly exhibits ribbons of cuboidal epithelium (short arrows) supported by a fibrovascular stroma (∗) with prominent capillaries (long arrows – greenish erythrocytes). The *saccus vasculosus* produces cerebrospinal fluid for the third ventricle and one hypothesis suggests that it can also detect water pressure.

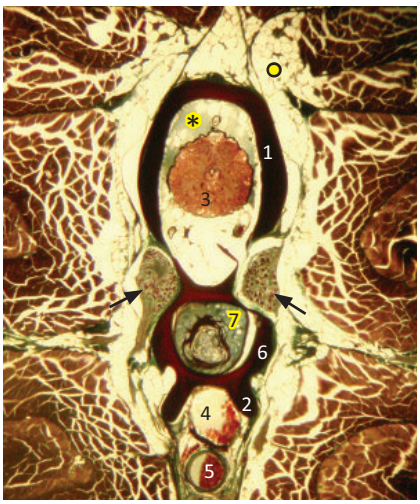


Fig. : 12.22 *Anguilla anguilla* (MT / LM)

Transverse section through the posterior region of a young specimen. The nervous components on this image are the two spinal ganglia (arrows) lying along the vertebral column. Numerous other structures are recognized by their respective locations : the bony vertebra bears processes above (neural arches - 1) and below (hemal arches – 2) which respectively protect the spinal cord (3) and the dorsal artery (4) and vein (5). The centre (centrum - 6) of the vertebra surrounds remains of the notochord (7).

The vertebral canal (space through which the spinal cord passes - ∗), some adipocytes (•) and skeletal muscular tissue (in red - all around) are also found.

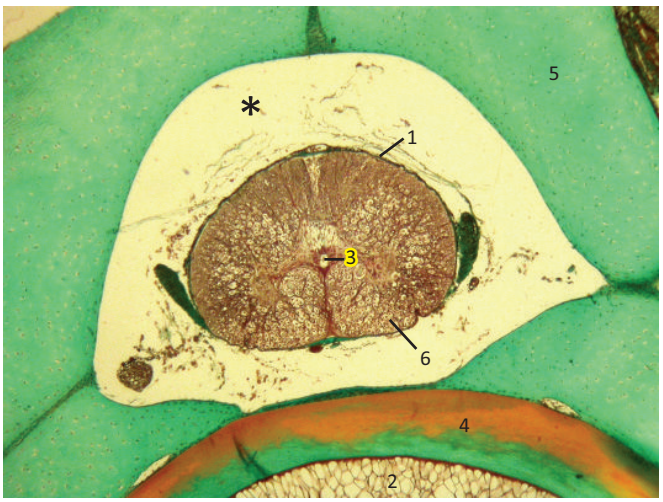


Fig. : 12.23 *Anguilla anguilla* (MT / MM)

Transverse section through the spinal cord of a young eel. The spinal cord is enclosed by the vertebral column and contained in the vertebral or spinal canal (∗). Spinal nerves and ganglia of the peripheral nervous system are metamerically arranged along the length of the cord. Most fish seem to have a single connective (primitive) meninx surrounded by fatty tissue. In some teleost fish the meninges can be divided in three layers.

1 : delicate meninx – 2 : vacuolated notochord cells - 3 : central canal (continuous throughout the length of the spinal cord) - 4 : vertebra – 5 : neural arch – 6 : cross sectioned myelinated axons.

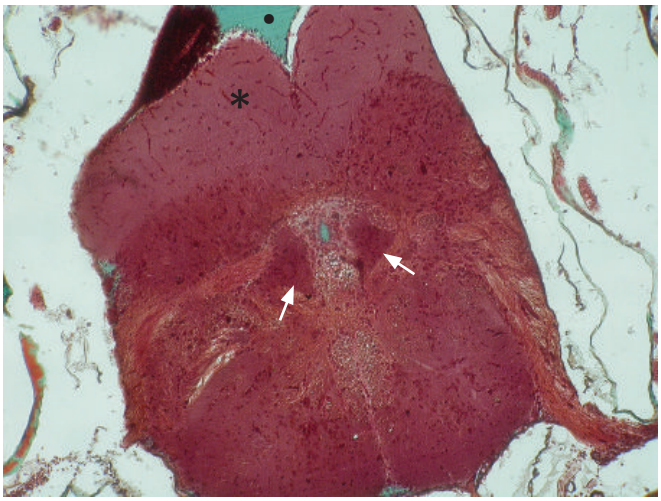


Fig. : 12.24 *Danio rerio* (MT / MM)

Transverse section through the *medulla oblongata* of the zebrafish brain. This area is the site of origin of the Mauthnerian system, a neuromuscular specialization which comprises a pair of very large triangular-shaped neuronal bodies (arrows) centrally located in the *medulla*. Firing of the MAUTHNER cells causes a rapid and powerful tail flip. The grey matter (∗) predominates at this level. • : cerebrospinal fluid.

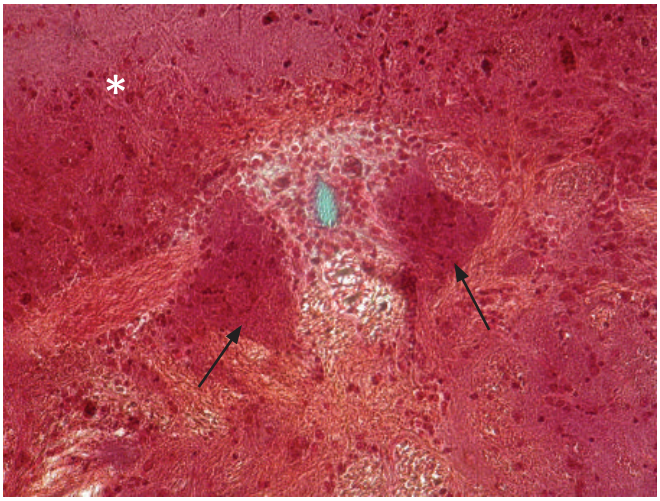


Fig. : 12.25 *Danio rerio* (MT / HM)

Higher magnification of the previous photomicrograph. The paired MAUTHNER neuronal bodies (*somata* - arrows) are obvious because of their huge size. The central canal containing cerebrospinal fluid (turquoise) is lined by ependymal cells (more visible on Fig. 12.28). ∗ : grey matter.

The Mauthnerian system is a neurolocomotory system well developed in teleost fish. Two (left and right) nerves possess giant axons which extend the length of the body, synapsing with motor neurons. Stimulations of the acoustic nerve VIII initiate rapid locomotion forward and away from the direction of stimuli.

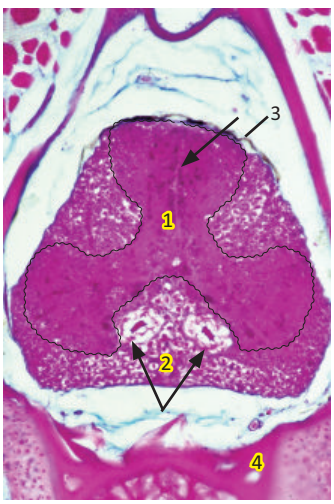


Fig. : 12.26 *Barbus caudovittatus* (H-E / MM)

Cross section through the spinal cord. In teleosts the spinal grey substance (1) shows a marked difference in arrangement from that of higher vertebrates in that the dorsal horns lie close together (arrow). This gives the grey substance the shape of an inverted Y (here demarcated by a curved line). Ventrally in the white substance (2), the bifurcated arrow points to the giant axons of MAUTHNER neurons. 3 : primitive meninx - 4 : vertebra

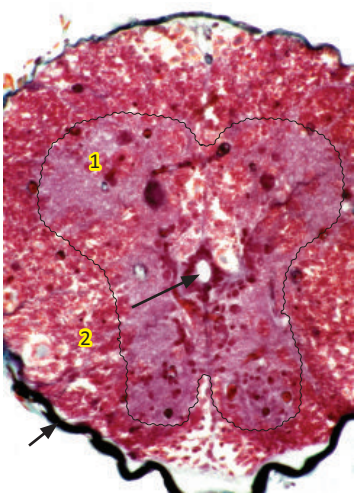


Fig. : 12.27 *Kryptopterus bicirrhis* (MT / MM)

Transverse section through the spinal cord of the glass catfish. The curved line indicates the limit between the grey (1) and white (2) matters. The grey matter is mainly composed of *somata* of neurons, dendrites and glial cells. The long arrow points to the central canal and the meninx appears like a black edge (short arrow).

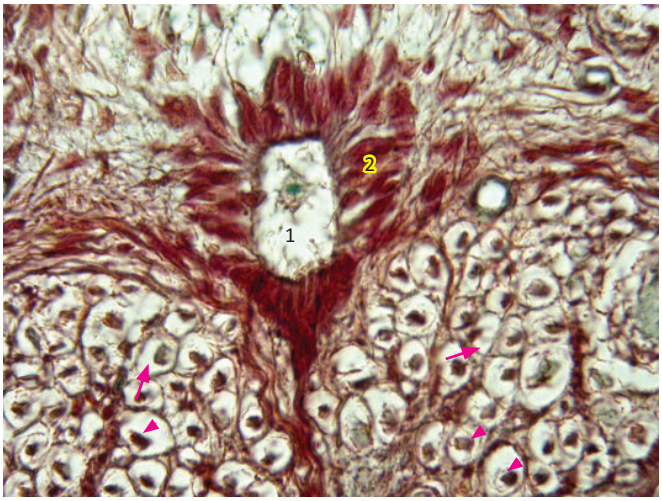


Fig. : 12.28 *Anguilla anguilla* (MT / HM)

Spinal cord in transverse section showing the white matter and the central canal (1). The white matter contains numerous nerve fibers like axons which appear as dark dots (arrowheads). The myelin sheath surrounding the majority of axons is composed of lipoprotein plasma membrane and is often partly dissolved (arrows) by the hydrophobic solvents used during the protocol. The central canal is lined by ciliated ependymal cells (2) responsible for helping to produce cerebrospinal fluid.

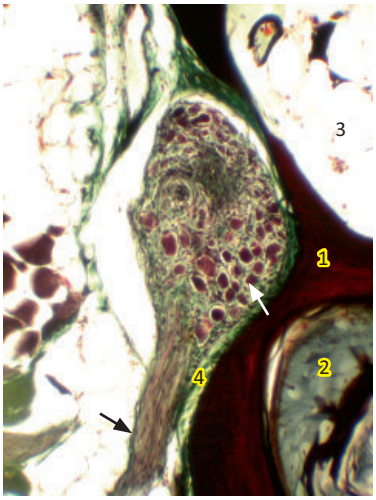


Fig. : 12.29 *Anguilla anguilla* (MT / MM)

Spinal ganglion. Spinal ganglia are swellings in the posterior nerve roots of the spinal nerves. They are located close to the spinal cord and consist of packed cell bodies (white arrow) of sensory neurons. The ganglion is enclosed by a dense connective tissue capsule (green) which is continuous with the associated nerve. Note the fascicle (black arrow) of nerve fibers. 1 : vertebra – 2 : notochord – 3 : fatty tissue surrounding the meninx - 4 : ganglion capsule

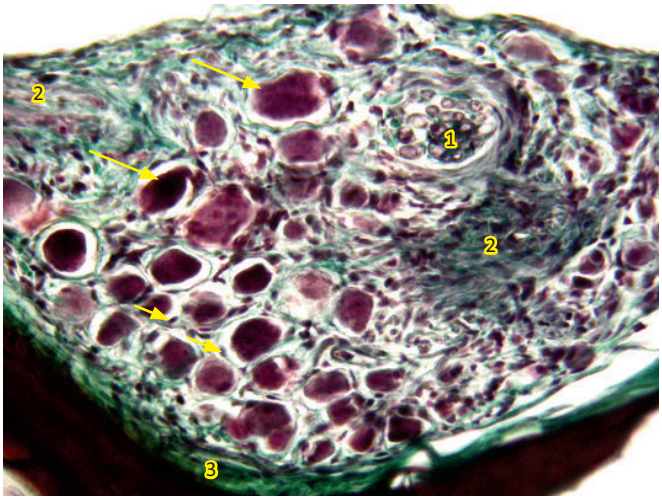


Fig. : 12.30 *Anguilla anguilla* (MT / HM)

Spinal ganglion. The neurons of spinal ganglia are large cells. The cell bodies (long arrows) are quite close together and surrounded by small nuclei (short arrows) of the flattened supporting satellite cells. Some nerve fibers are cut transversaly (1) or longitudinally (2). The ganglion dense connective capsule is stained green (3). Recall that ganglia belong to the peripheral nervous system.

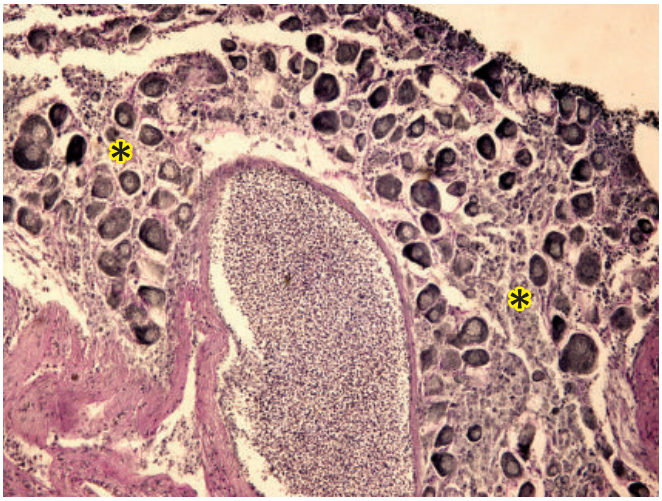


Fig. : 12.31 *Sparus aurata* (PAS-H / MM)

Parasympathetic ganglia are never far away from the effector organs and are located near or within them. One can see here a large cluster of neuronal cell bodies (black) in the terminal portion of the digestive tract. Ganglia of the autonomic nervous system exhibit cell bodies more widely spaced than in spinal ganglia because of the presence of numerous neurites (axons and dendrites - ∗) in between.

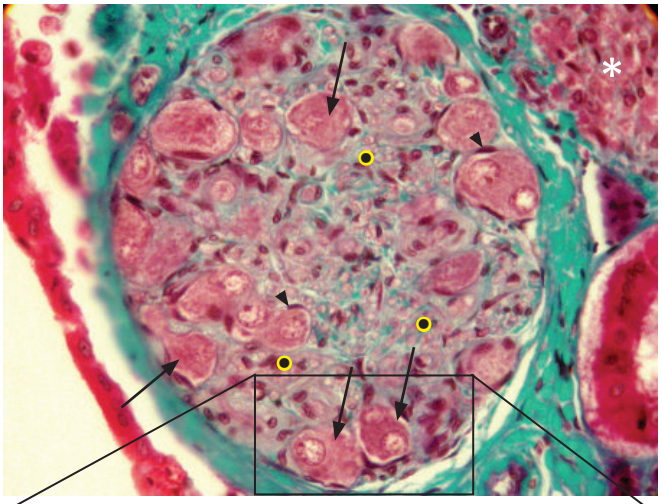


Fig. : 12.32 *Scyliorhinus canicula* (MT / HM)

Autonomic sympathetic ganglion associated with chromaffin tissue (∗) in the dogfish kidney. The micrograph shows a cluster of nerve cell bodies (arrows) with a large round nucleus. The ganglion cells are separated by numerous neurites (•). The arrowheads indicate satellite cell nuclei and the connective capsule is stained green.

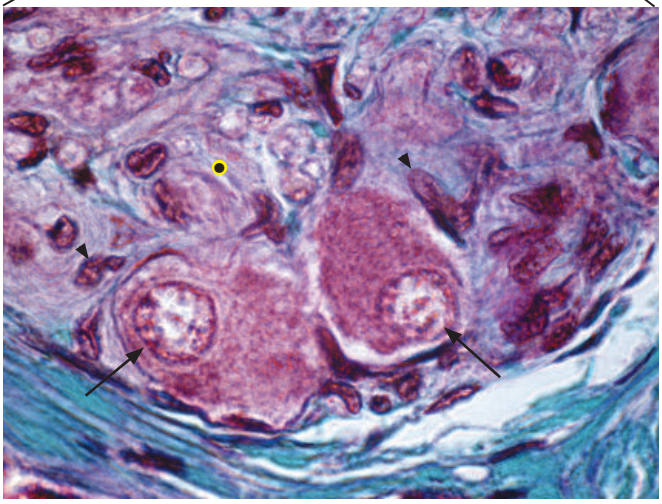


Fig. : 12.33 *Scyliorhinus canicula* (MT / IM)

Autonomic sympathetic ganglion associated with chromaffin tissue in the dogfish kidney. The ganglion cells are large neurons and possess a large round euchromatic nucleus tending to be eccentrically located in the cytoplasm (arrows). They are associated with flattened glial cells (satellite cells - arrowheads) quite irregularly placed due to the neuronal processes (•).

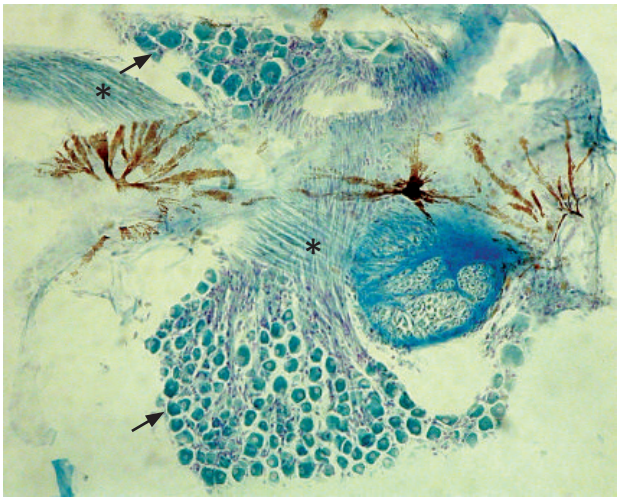


Fig. : 12.34 *Dicentrarchus labrax* (FR-HB / MM)

Parasagittal section of the medial trunk of the European seabass. This photomicrograph shows spinal ganglion cells (arrows), neurites (∗) and beautiful stellate chromatophores (brown).

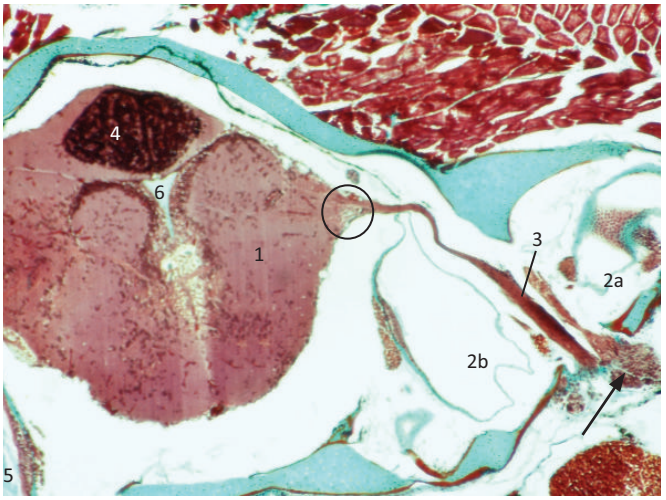


Fig. : 12.35 *Poecilia reticulata* (MT / MM)

Cross section at the *medulla oblongata* level. This micrograph illustrates the connection between the myelencephalon (1) and the inner ear (semicircular canal - 2a & otolithic chamber - 2b) via the VIIIth cranial nerve (vestibulocochlear or statoacoustic - 3). The vestibulocochlear nerve contains sensory fibers coming from various parts of the inner ear (*ampullae, sacculus, utriculus*). They are formed by true ganglion cells (arrow) situated close to the sensory structures and enter the *medulla oblongata* at a dorsal position (circle).

4 : hind *cerebellum* - 5 : otolith - 6 : fourth ventricle

Cartilage in turquoise and skeletal muscle (top) in red.

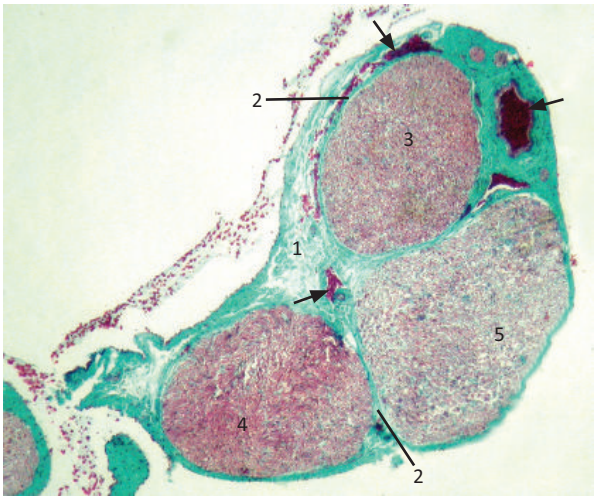


Fig. : 12.36 *Scyliorhinus canicula* (MT / MM)

Transverse section through three nerve fascicles which belong to two large peripheral cranial nerves. The three nerve sections are ensheathed in a common *epineurium* (loose collagenous layer binding the fascicles - 1) and each bundle or fascicle is surrounded by its own robust collagenous tissue called the *perineurium* (2).

The three thick fascicles are the maxillary (3 - innervates upper jaw) and the mandibular (4 - innervates lower jaw) branches of the trigeminal nerve V and also the buccal branch (5 - partly innervating the lateral line and rostrum) of the facial nerve VII. The buccal branch together with the maxillary branch form the large infraorbital nerve extending across the floor of the orbit.

Numerous vessels (arrows) running through the *epineurium* and the *perineurium* supply the peripheral nerves.

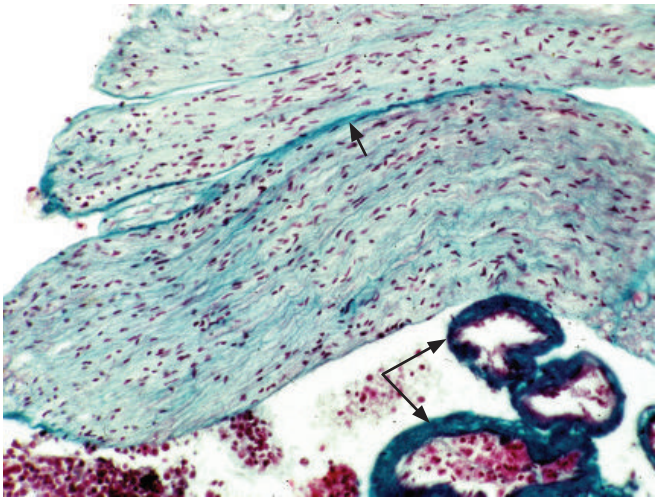


Fig. : 12.37 *Pangasius micronemus* (MT / MM)

Longitudinal section of peripheral nerve fascicles separated by the dense *perineurium* (short arrow). Most of the dark elongated nuclei seen within the fascicles are those of SCHWANN cells among which some fibroblasts (not distinguishable) are scattered. Note the wavy appearance of the fibers. Long arrows point to accompanying small arteries.

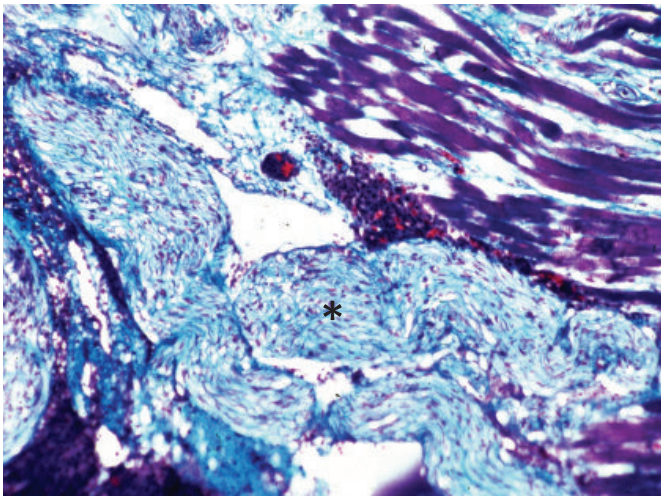


Fig. : 12.38 *Pangasius micronemus* (MT / MM)

Longitudinal section through a peripheral nerve (∗). This photomicrograph demonstrates the typical wavy appearance of the axons whose course is marked by the flattened nuclei of SCHWANN cells. The supporting SCHWANN cells lay down the myelin sheath around the axons in the peripheral nervous system. Rhabdomyocytes are in purple.

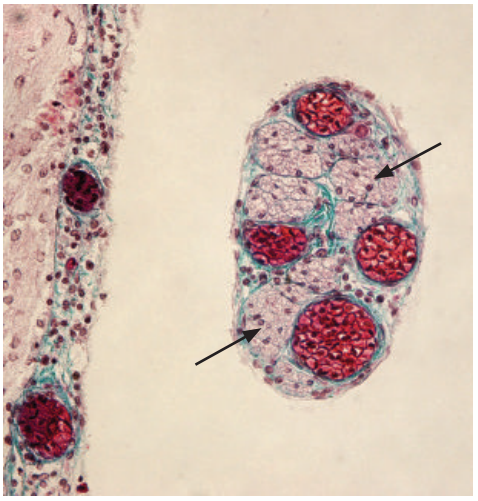


Fig. : 12.39 *Scyliorhinus canicula* (MT / MM)

Transverse section through a small olfactory nervous tract located between the cerebral hemispheres and an olfactive lobe of the telencephalon. The tract contains some nerve fascicles (arrows) and their rich blood supply (four large vessels in red). Each fascicle is encircled by connective tissue (collagen of the *perineurium* in green). On the left, part of the olfactive lobe.

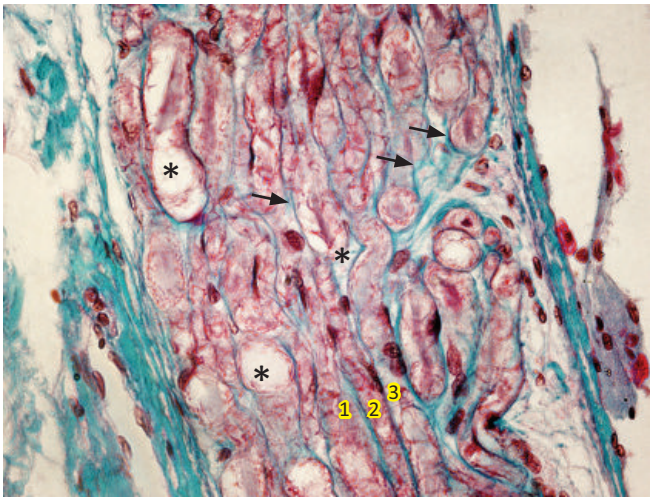


Fig. : 12.40 *Scyliorhinus canicula* (MT / HM)

Longitudinal section through the optic nerve II. In addition to the *epineurium* and the *perineurium* a loose vascular connective tissue, the *endoneurium*, surrounds each individual nerve fiber. The collagenous fibers (arrows) of the delicate *endoneurium* are stained turquoise by the Masson's trichrome. Axons (three adjacent ones are shown - 1-2-3) are here quite readily visible. The axis of each axon is pinkish and the light-coloured zones (∗) correspond to the partially dissolved myelin.

The optic nerve is a thick sensory nerve, but it more exactly represents a brain tract rather than a peripheral nerve. During the dissection it has been cut after removing the orbit. Nerve II is composed of the axons of ganglion cells whose *somata* (cell bodies) lie in the retina (see chapter 15). The ganglion cells receive impulses from the photoreceptors and the two optic nerves cross (decussate) each other (differently according to fish groups) at the optic chiasma before entering the brain.

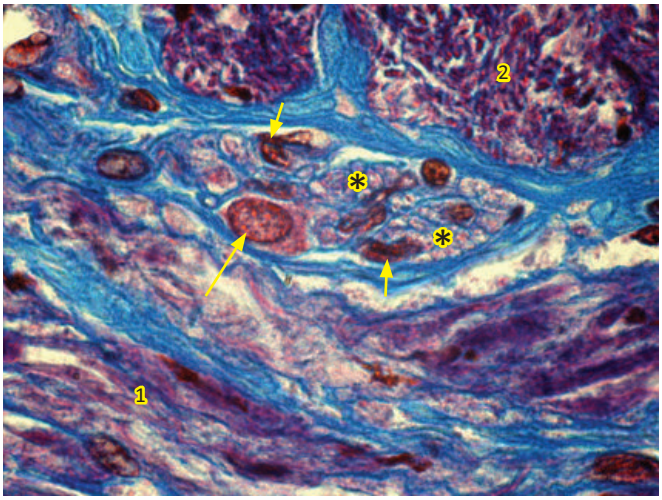


Fig. : 12.41 *Scyliorhinus canicula* (MT / MM)

Rectum wall. Nervous tissue forms interconnected plexuses within the gut wall. This tissue includes in particular parasympathetic fibers and the ganglia that innervate the smooth muscles of the digestive wall. This photomicrograph illustrates part of the myenteric plexus located between the two (inner circular – 1 and outer longitudinal – 2) smooth muscle layers in the rectal *tunica muscularis*. This small parasympathetic ganglion shows one ganglion cell (long arrow) with a large euchromatic nucleus. Afferent and efferent nerve fibers (∗) as well as small satellite cells (short arrows) are shown. In blue : connective tissue.

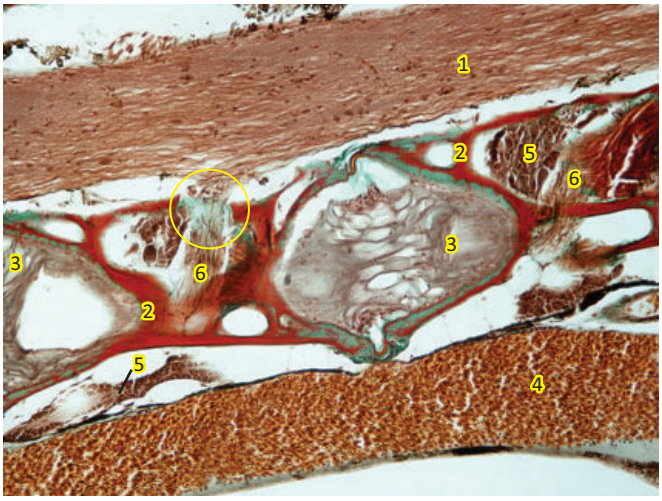


Fig. : 12.42 *Poecilia reticulata* (MT / MM)

Longitudinal section through the anterior trunk region displaying the spinal cord (1), vertebrae (2), remains of the notochord (3) and the dorsal aorta (4). Spinal ganglia (5) and spinal nerves (6) are also found. Spinal nerves are paired structures emerging from the grey matter of the spinal cord and passing out from the vertebral canal through spaces (the intervertebral *foramina*) between every pair of vertebrae. The circle indicates the intervertebral *foramen* area.

13
Endocrine glands

The endocrine system includes several glands which are distributed in various regions of the body. They control long-term activities of organs involved in digestion, development, metabolism, growth, reproduction, etc.

Animals have two types of glands, namely exocrine glands and endocrine glands. Exocrine glands have ducts to carry off their secretion. The endocrine glands have no ducts and their secretions, called hormones, are liberated into the bloodstream to be conveyed to other parts of the body where they produce a definite physiological effect. Hormones act specifically on target organs which possess specific receptors.

The endocrine glands, together with the nervous system, participate in the maintenance of a steady physiologic state, called homeostasis. Their functions are intimately linked, coordinated and sometimes even integrated as, for example, in the hypothalamo-hypophyseal complex.

Pituitary gland

The pituitary gland (Figs 13.1 to 13.7) occupies the same central part in the endocrine signalling system of fish that it does in higher vertebrates. It is located below the diencephalon (hypothalamus), behind the optic chiasma and anterior to the *saccus vasculosus*, and is attached to the diencephalon by a stalk (the pituitary stalk - Fig. 13.5). The pituitary gland is completely enveloped by a delicate connective tissue capsule. In fish the pituitary gland is a complex neuro-epithelial structure, which, although somewhat variable in structure between species, is remarkably similar within the major classes.

The part of epithelial origin (the *stomodaeum*) is known as the adenohypophysis (Figs 13.6 & 13.7). It is glandular in nature while the part having its origin in nervous tissue is known as the neurohypophysis, consisting of neurosecretory nerve endings of the hypothalamo-hypophyseal tract, blood sinuses and glial cells (pituicytes). There is considerable interdigitation of the two regions (Fig. 13.3).

The adenohypophysis of teleosts comprises a rostral and a proximal *pars distalis* and also a *pars intermedia*. This gland is composed of many different cell types which secrete a variety of hormones controlling basic physiological processes, including growth, gonad development, thyroid activity, regulation of steroidogenesis by the cells of the interrenal gland, water and electrolyte balance. Each pituitary hormone is generally associated with specific secretory cells, best identified by immunocytochemical methods, with a clear distribution within the three adenohypophysis regions. In most species the rostral *pars distalis* comprises corticotropic cells, which form a layer in the dorsal region adjacent to the the *pars nervosa*, and prolactin (PRL)-secreting cells. In some species thyrotropic hormone (TSH)-secreting cells may also be present in the rostral *pars distalis*. The proximal *pars distalis* contains growth hormone (STH)-secreting cells and gonadotropic and thyrotropic cells.

The majority of the pars intermedia cells are melanotropic cells that synthesize the pro-hormone proopiomelanocortin (POMC) peptide, which is converted intracellularly by peptidases into melanocyte-stimulating hormones (α-and β-MSH) and β-lipotropic hormone (β-LPH). α-MSH is mainly involved in the stress response in teleost fish, and may control dispersion of melanin in melanophores in some species. A second pars intermedia cell type is present in some teleosts. These cells synthetize and secrete the hormone somatolactin (SL), a protein molecule that belongs to the prolactin/growth hormone family.

As briefly mentioned above, the neurohypophysis of teleosts is composed of hypothalamic neurosecretory fibers, glial cells and blood ves-

sels. The nerve fibers are mainly non-myelinated and terminate in close relationship to blood capillaries. In teleosts as in most vertebrates, the glial cells or pituicytes are regarded as support cells but also as phagocytic cells being directly involved in the functioning of the neurohypophysis (*pars nervosa*).

Three neurohypophysial hormones are known in teleosts, isotocin (IT), arginine vasotocin (AVT) and melanin-concentrating hormone (MCH). IT and AVT are nonapeptides whereas MCH is a heptadecapeptide (= 17 amino acids). Although there has been great progress in our knowledge of the biochemistry and phylogeny of neurohypophysial hormones, their physiological functions in fishes remain unclear. There are many reports suggesting a role for AVT in fish osmoregulation although the precise role has not yet been established. Functional receptors have been identified in the gill and kidney of teleost fishes. Very little attention has been given to the actions of oxytocin-like peptides, although there is evidence for the presence of IT receptors in gill and liver of trout. Several studies have reported the involvement of IT and/or AVT in spawning (oviparous fish) and parturition (viviparous fish) of some teleosts. MCH is named for its role in concentration of melanin in melanophores.

Urophysis (caudal neurosecretory system)

In both elasmobranchs and teleosts a second neuroendocrine system and a neuro-hemal (in addition to the neurohypophysis) area is associated with the caudal end of the spinal cord; this is the urophysis (Figs 13.8 & 13.9). A well-defined neurohemal urophysis is seen only in teleosts. In elasmobranchs the neurosecretory cells, which are called DAHLGREN cells and have *perikarya* (cell bodies) some twenty times larger than ordinary motor neurons, extend along the terminal vertebrae region. The axons of these cells terminate at the blood capillaries lying on the latero-ventral surface of the spinal cord. In teleosts the unmyelinated axons of the neurosecretory cells congregate with blood vessels to form a neurohemal complex in a manner similar to that seen in the neurohypophysis. As with the mammalian neurohypophysis stalk the degree of constriction of the urophysis stalk varies. In the eel (*anguilla*) there is no stalk but only a slight ventro-lateral swelling of the spinal cord, while in other fish such as *Oryzias*, *Fundulus* and *Gillichthys* there is a well-defined stalk. The neurosecretory cell bodies lying in the spinal cord have polymorphic nuclei and a basophilic cytoplasm. Urotensin I and urotensin II are two types of peptide hormones isolated from teleost urophyses. These factors secreted by this complex have been linked to various vasoactive responses, notably causing an increase in blood pressure, and vasoconstriction in urinary bladder, intestinal tract, and reproductive tract. An ionoregulatory response in *Anguilla anguilla* has also been reported.

Interrenal and chromaffin tissues

The adrenal glands of mammals are located at the cephalic pole of each kidney. In gross section, the cortex, which constitutes the largest part of the gland, is clearly distinguishable from an inner thin *medulla*. The cortex and the *medulla* are both endocrine tissues, but they differ in their embryological origin and in their functions.

There is no «adrenal gland» in fish but interrenal (Figs 13.10 to 13.14) and chromaffin tissues (Figs 13.15 & 13.16).

In Chondrichthyes (Fig. 11.23), the steroidogenic cells (interrenal cells) and the catecholamine-producing cells (chromaffin tissue) form separate cell masses. The steroidogenic cells are situated along the medial edge of the kidneys; the catecholaminergic cells are located near the aorta and postcardinal veins. In teleosts, both types of cells assemble in groups on the ventral surface of the head kidneys. The catecholamine-producing cells occupy the more rostral position near the postcardinal vein. There is great variability in the interrenal morphology among teleost groups and even variability within families. The shape and size of the cells vary, sometimes being polygonal, columnar, cuboidal or even spindle-shaped. The form of these cells have been shown to change in response to hormones, drugs, stress or salinity changes. The interrenal cells secrete

corticosteroids. Chromaffin cells are generally more uniform and rounded in appearance than interrenal cells and their cytoplasm is slightly basophilic. They get their name from their staining reaction to chromic salts. They secrete sympathomimetic substances such as adrenaline, associated with immediate stress responses. In particular, raise in blood levels of catecholamines causes hyperglycemia and increases the gills functional area for gaseous and ionic exchanges.

Thyroid gland

In adult bony fishes, the thyroid gland (Figs 13.17 to 13.22) usually consists of numerous diffuse follicles (Figs 13.17 to 13.20) scattered around the ventral aorta and afferent branchial vessels. This pattern of scattered follicles can extend quite widely outside the pharyngeal region. This migration of so-called heterotopic follicles from the pharyngeal region is probably due to the fact that the gland is not encapsulated and surrounded by connective tissue. A few species of bony fishes (e.g. parrot fishes, *Scarus sp.* ; sword fish, *Xiphias*, and tunas, *Thunnus sp.*) and all cartilaginous fishes (Figs 13.21 & 13.22) have a thyroid contained within a connective tissue capsule.

The histological aspect of the gland in the teleosts is essentially similar to that of tetrapods; the thyroid tissue comprises epithelial cells, that vary in size depending on the degree of TSH stimulation from the hypophysis. The cells form a tight epithelium surrounding a colloid-filled lumen, and have *microvilli* projecting into the follicular lumen.

The distinctive feature of the gland is that the follicles are capable of trapping iodine and manufacturing the two thyroid hormones, 3,5,3'-triiodothyronine and thyroxine together with their precursors 3-monoiodotyrosine and 3,5-diiodotyrosine.

The thyroid plays a significant role in growth and its associated metabolism. Involvement in osmoregulation and migration movements has been shown. This wide-ranging spectrum of physiological changes suggests either that thyroid hormones in fish play a very basic role themselves in general metabolism or that they activate and modulate a number of different biochemical pathways.

The thyroid gland of chondrichthyean fish is a discrete organ. It is pear-shaped in sharks and somewhat flattened and disc-shaped in skates and rays. The thyroid follicle of elasmobranchs (Figs 13.21 & 13.22) is similar in structure to that found in bony fish and tetrapods. The follicle cells are normally cuboidal in shape. There is variation in follicle diameter, and larger follicles are found in older animals.

Endocrine pancreas

In addition to an exocrine component which was described in chapter 8, the pancreas comprises an endocrine component (Figs 13.23 to 13.27) of endodermal origin formed by the islets of LANGERHANS. These islets consist of clusters of lightly capsulated hormone-secreting cells (insulin, glucagon, somatostatin…) surrounded by a capillaries network. Because these cell types secrete peptide hormones, they exhibit the usual features of cells engaged in active protein synthesis. The size of islet cells may vary according to food and season. In many species, there is one major islet isolated from the rest of the pancreas, known as the BROCKMAN body.

Insulin causes hypoglycemia but fish do not exhibit the rapid blood glucose clearance response typical of mammals. Glucagon acts antagonistically to insulin in that it increases blood glucose by liver glycogenolysis. It also stimulates the incorporation of amino acids in the liver and stimulates gluconeogenesis.

Corpuscles of STANNIUS

These discrete encapsulated organs (Figs 13.28 to 13.30) first described in 1839, are probably unique to holostean and teleostean fish. They are located on the lateroventral or laterodorsal surface of the kidneys. Typically they are paired, but their number can reach ten or more. The glands are divided into cords or lobules by connective tissue *septa*, that are well-sup-

plied with blood vessels and nerves. The glandular cells contain large secretory granules at the basal side. The granules can be coloured by the PAS staining method. These cells are known to synthesize a glycoprotein hormone called stanniocalcin (or hypocalcin) which is involved in calcium homeostasis. It is now well established that the corpuscles of STANNIUS are a primary force in calcium regulation in teleosts. Removal of the glands (stanniectomy) causes a prompt rise in plasma calcium levels (hypercalcemia). Treatment of intact teleosts, such as eels, with extracts of corpuscles causes a marked fall in calcium levels. Sea water is high in calcium compared to fresh water and histological observations on the corpuscles of STANNIUS indicate that the glands are more active in sea water than in fresh water fish.

Ultimobranchial gland

Just as the thyroid gland develops as a ventral outgrowth of the pharynx at the level of the two first branchial pouches, so another structure, the ultimobranchial gland (Figs 13.31 to 13.34), is derived from the last branchial pouch area in the embryonic fish. This tissue migrates backwards, during the development of the fish, to a position lying near the *pericardium*.

The gland is difficult to identify, even under the dissecting microscope. In teleosts the gland is unpaired lying in the midline and is generally located in the transverse *septum* between the abdominal cavity and the *sinus venosus* just ventral to the esophagus. In some cyprinids (carp, goldfish, zebrafish – Figs 13.31 & 13.32) the gland is composed of small follicles, whereas in the eel and the trout, it consists of two glandular units lining a central lumen. In small-sized fishes (Figs 13.33 & 13.34), guppy, medaka…the gland shows a sheet-like structure and its observation is very limited. Calcitonin (CT) is produced by the endocrine cells. Manipulation of the ultimobranchial gland helped establish roles for CT in the regulation of fluids, electrolytes, and mineral metabolism. The ultimobranchial gland also controls, apparently with more precision than the corpuscles of STANNIUS, the serum calcium concentration, particularly in the female during the reproduction cycle.

Pseudobranch and choroid body

The pseudobranch (Figs 13.35 to 13.36) is a red, gill-like structure derived from the first gill arch and attached to the internal surface of the *operculum*. It is composed of gill *lamellae*, connective tissue and blood vessels. The *lamellae* consist of pseudobranchial cells on an underlying basement membrane. This latter is applied to a network of parallel blood capillaries which can be supported by thin cartilaginous rods. The pseudobranch has a direct vascular connection with the choroid of the eye (Fig. 13.37), which is composed of similar arrays of capillaries (*rete mirabile*) alternating with rows of fibroblast-like cells. The pseudobranch is not present in all teleosts. Those fish which do not possess such structure (some *Siluridae, Ictaluridae, Notopteridae, Cobitidae, Anguillidae...*) invariably also lack a *choroid rete*. Although it is considered to have an endocrine and regulatory function as well as a hyperoxygenation function for the retinal blood supply, these are still to be defined in full.

Pineal gland

For many years it has been thought that the pineal complex as a dorsal evagination of the brain was a sensory organ in lower vertebrates and a glandular organ in higher vertebrates. It is now well accepted that the pineal (Figs 13.38 & 13.39) in fish has a well-defined glandular activity and that indeed throughout the vertebrates, it is a «photoneuroendocrine organ».

In bony fish the general histological appearance of the saccular pineal closely resembles a sensory organ with photoreceptors, supporting cells and larger cells with nerve cell characteristics. The abundant sensory cells of the organ are large and club-shaped with well-defined nuclei and mitochondria; outgrowths from these cells penetrate the lumen of the pineal. This gland, connected to the third ventricle is completely enclosed in a connective layer capsule and possesses a epiphyeal stalk lined by epen-

dymocytes. Electron-microscopic studies have been presented which indicate morphological evidence of endocrine activity in the pineal organ of several teleost fish.

The gland contains melatonin, the enzyme HIOMT (hydroxyindole O-methyltransferase) necessary for the formation of melatonin, serotonin and a number of free amino acids which may all act as chemical transmitters and have some physiological role.

Endocrine cells of the gut

Like in higher vertebrates small «regulatory peptides» have been identified in endocrine cells of the fish gut. Their identification was performed with immunohistochemical procedures using appropriate (specific) antibodies.

The endocrine cells of the gastrointestinal tract are located in the *mucosa* and deliver their content to the bloodstream, the target organ can thus be at any distance from the endocrine cells. Ten or more subtypes of endocrine cells have been reported in teleost fishes (specific for bombesin, enkephalin, gastrin/cholecystokinin, neurotensin, substance P, etc.). The physiological role of these peptides, often common to the gut and the brain, is beyond the scope of this atlas.

Gonads

In the testis interstitium are found small numbers of endocrine cells, called LEYDIG cells (see following chapter). A lot of steroids (androstenedione, testosterone, progesterone,...) have been isolated from the testes of several teleosts. The details of the interaction between the SERTOLI and LEYDIG cells in the steroidogenic pathway in fishes are far from clear.

The thecal and granulosal cells surrounding the oocytes comprise the steroidogenic tissue of the ovary. The primary ovarian oestrogen, 17β-estradiol, is one of the primary factors involved in the stimulation of synthesis of vitellogenin, the main constituent of the yolk of the oocytes. 17β-estradiol also stimulates hepatic synthesis of proteins that form the chorion of the oocyte. Progestagens in fish are also thought to promote ovulation. In brief, the steroids exert both local and peripheral effects.

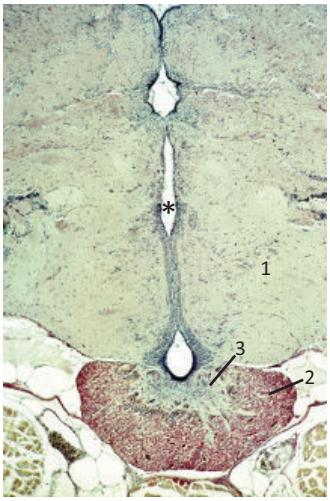


Fig. : 13.1 *Astatotilapia burtoni* (MT / MM)

Transverse section of the hypothalamus-hypophyseal system. This system is a neuroendocrine complex in which blood vessels (portal veins) and axons link the hypothalamus (1) and the pituitary gland allowing endocrine communication between these two structures. The pituitary gland or hypophysis is divided into anterior epithelial (adenohypophysis - 2) and posterior nervous (neurohypophysis - 3) parts.

∗ indicates the third ventricle of the diencephalon.

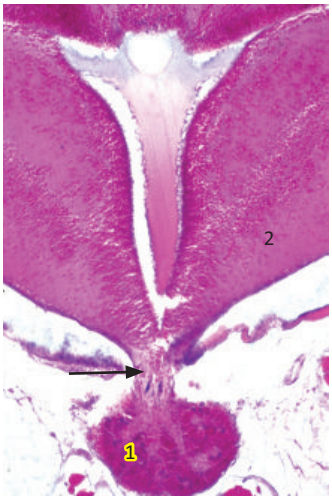


Fig. : 13.2 *Barbus caudovittatus* (AB-H-E / MM)

Transverse section of the hypothalamus-hypophyseal system. This survey photomicrograph of the pituitary gland demonstrates the relationship of the gland (1) to the hypothalamus (floor of the diencephalon - 2) from which it is suspended by the pituitary stalk (arrow). The cavity within the diencephalon is the third ventricle, filled with cerebrospinal fluid.

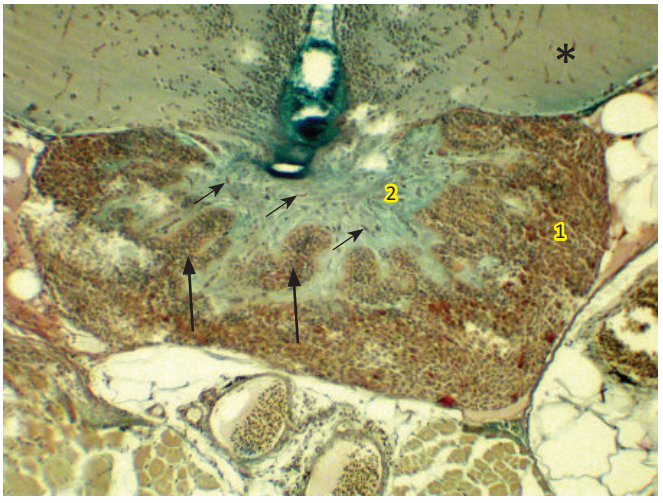


Fig. : 13.3 *Haplochromis burtoni* (AB-PAS-H / HM)

Transverse section through the hypophysis. On the basis of histology, two well-outlined areas, the adenohypophysis (1) and the neurohypophysis (2) can be distinguished.

The adenohypophysis is of epithelial origin and glandular in nature ; the neurohypophysis has its origin in nervous tissue and consists of neurosecretory nerve endings of the hypothalamo-hypophyseal tract, blood capillaries and supporting glial cells (pituicytes – thin arrows) bathing among the axons. As one can see there is considerable interdigitation of the two regions, the neurohypophysis intermingling with cords of the adenohypophysis (arrows).

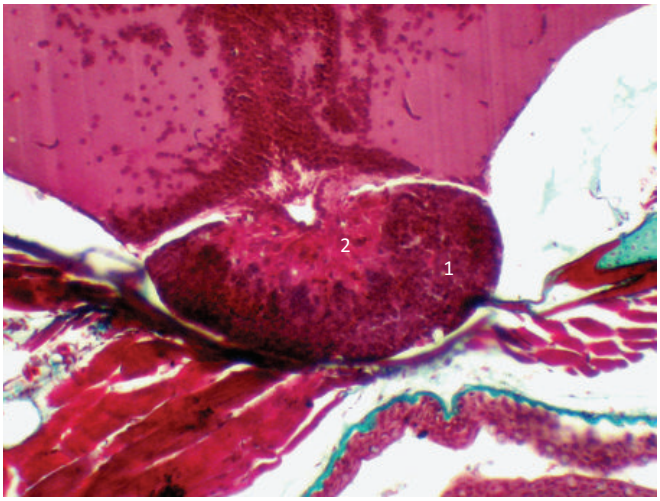


Fig. : 13.4 *Poecilia reticulata* (MT / HM)

Transverse section through the hypophysis. The glandular nature of the adenohypophysis (1) can be easily distinguished from the nervous (without cell bodies) «uninspiring» aspect of the neurohypophysis (2).

The neurohypophysis stores and releases hormones (isotocin, arginine vasotocin...) which seem to be involved in osmoregulatory mechanisms.

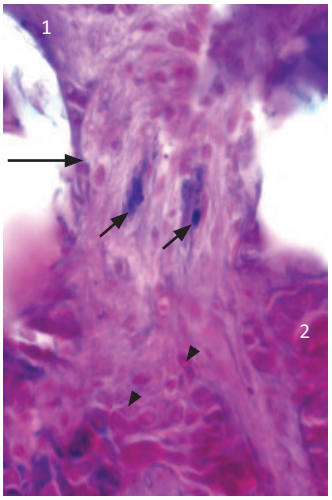


Fig. : 13.5 *Barbus caudovittatus* (AB-H-E / HM)

Pituitary stalk. The posterior pituitary hormones are synthesized by neurone cell bodies of the hypothalamus and pass down the axons through the pituitary stalk (arrow). These nerve fibers arising from the hypothalamus (1) and forming the *pars nervosa* intermingle with cellular cords of the adenohypophysis (2). Note neurosecretory material (short arrows) along the nerve fibers. The *pars nervosa* of the pituitary gland is composed of cells (pituicytes) which are thought to be neuroglial in nature. These cells, which possess more or less oval nuclei (arrowheads) appear to support numerous unmyelinated axons travelling from the hypothalamus.

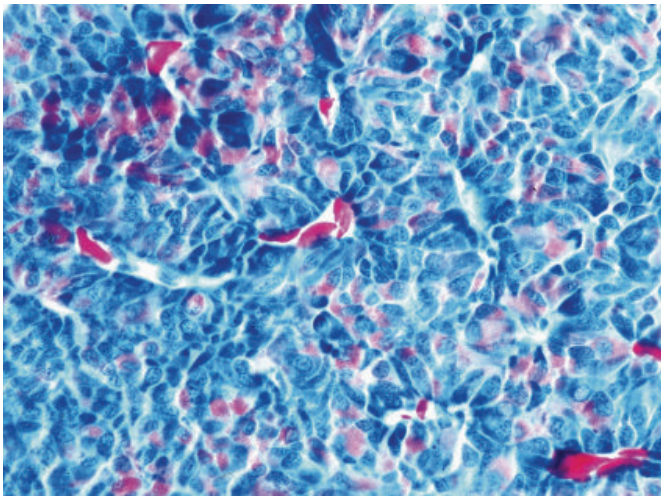


Fig. : 13.6 *Garra congoensis* (MT / MM)

Secretory cells of the adenohypophysis. The anterior pituitary is composed of large cords of cells that branch and anastomose with each another. These epithelial cells secrete a variety of hormones controlling essential physiological processes and can be differentiated by immunocytochemical methods. These cellular cords are surrounded by an extensive network of capillary sinusoids (bright red).

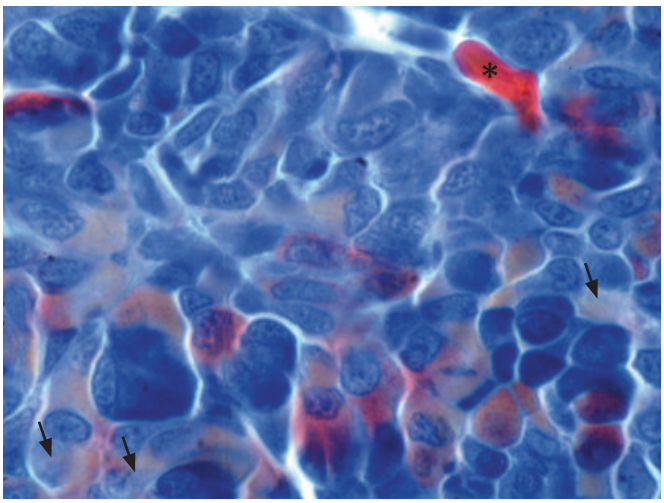


Fig. : 13.7 *Garra congoensis* (MT / HM)

Secretory cells of the adenohypophysis. This photomicrograph is a higher magnification of the previous document. By means of trichrome staining the parenchymal cells of the adenohypophysis can only be divided into two groups : chromophiles and chromophobes. The former stain blue, pink or orange, while the latter stain poorly (arrows). ∗ : erythrocyte in a capillary.

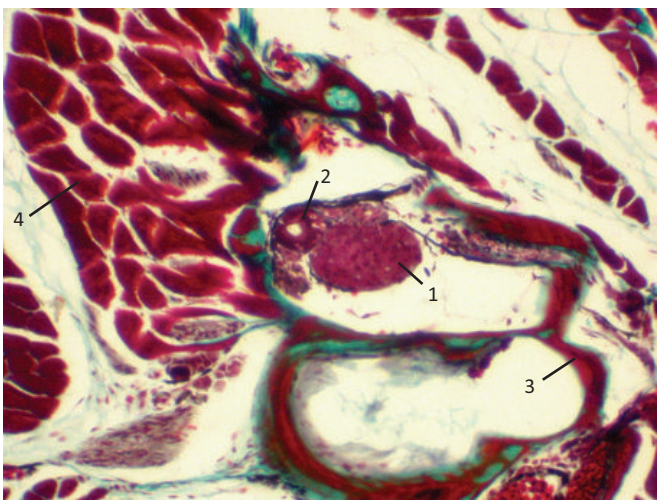


Fig. : 13.8 *Poecilia reticulata* (MT / MM)

Urophysis. Also known as the caudal neurosecretory system the urophysis (1) is an endocrine gland associated with the caudal end of the spinal cord (2). Toward the end of the tail large neurosecretory cells are found in the spinal cord and their unmyelinated axons extend posteriorly to a network of capillaries. At this medium magnification one of the last vertebrae (3) and caudal epaxial muscles (4) are also present.

Both Chondrichthyes and Osteichthyes (but not all) have an urophysis, however a well-defined one is only seen in teleosts.

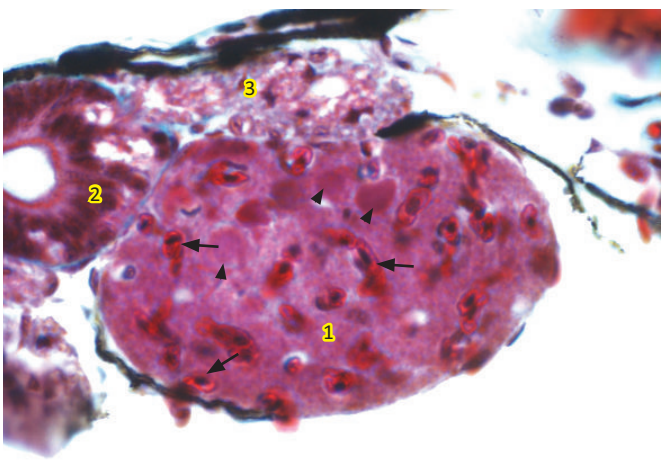


Fig. : 13.9 *Poecilia reticulata* (MT / IM)

Urophysis. This photomicrograph is a higher magnification of the previous figure. The urophysis (1) occupies the center of the previous image and the end of the spinal cord (2) is on the left. Hormones secreted by the neurosecretory cells pass down their axons through the neurosecretory tract (urophysis stalk - 3) to the urophysis. They are stored in the terminal parts of the axons («bulbous tips» - arrowheads) which are in intimate contact with a well-developed vascular bed containing erythrocytes (arrows). The neurosecretory cell bodies (not visible) lying in the spinal cord have polymorphic nuclei and a basophilic cytoplasm.

The hormones (urotensins) secreted by this complex appear to play important roles in blood pressure, vasoconstriction and osmoregulation.

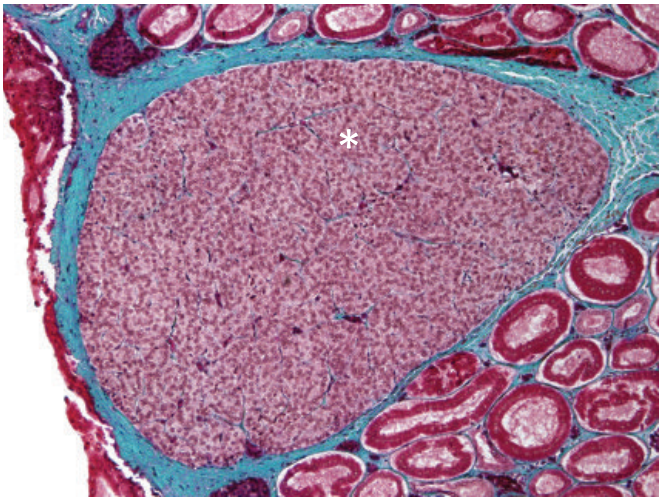


Fig. : 13.10 *Scyliorhinus canicula* (MT / MM)

Interrenal tissue. In the Chondrichthyans the interrenal gland is completely separated from the chromaffin tissue (see Fig. 11.23). The interrenal itself lies along the medial edge of the posterior kidneys. This photomicrograph shows interrenal cell masses (∗) surrounded by connective tissue (in turquoise) and nephron tubules.

There is no "adrenal gland" in fish but interrenal and chromaffin tissues.

In elasmobranch fishes the adrenocortical tissue organizes into a single compact gland which is located on and between the kidneys.

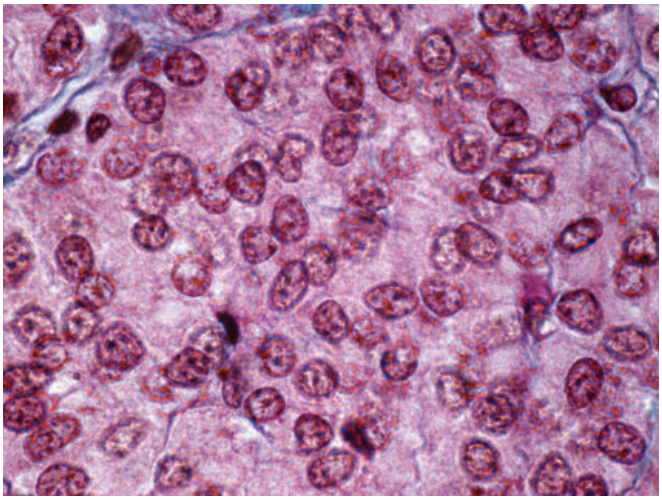


Fig. : 13.11 *Scyliorhinus canicula* (MT / HM)

Interrenal tissue. It is generally found that interrenal cells are quite homogeneous. The secretory cells are round or polygonal with a large prominent nucleus and one or more nucleoli clearly visible. The interrenal cells secrete steroids (1a-hydroxycorticosterone is the major corticosteroid in elasmobranchs) and the form of these cells has been shown to change in response to various substances like hormones or drugs. Delicate collagenous *trabeculae* (in blue) separate cells into irregular masses but there are no true lobules.

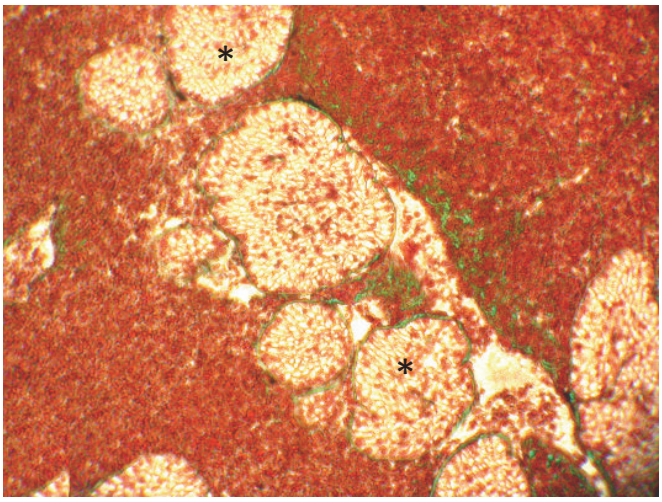


Fig. : 13.12 *Acipenser gueldenstaedtii* (MT / LM)

Interrenal tissue of the Russian sturgeon. In the teleosts the interrenal tissue exhibits considerable morphological variation among taxonomic groups and even variability within families. In Chondrosteans it is dispersed throughout the kidney. The image shows masses of interrenal tissue (∗) embedded in the anterior kidney (hematopoietic cells in deep orange). The whitish secretory cell aspect is due to the lipid droplets poorly stained by routine histological methods. The few collagen fibers are in green.

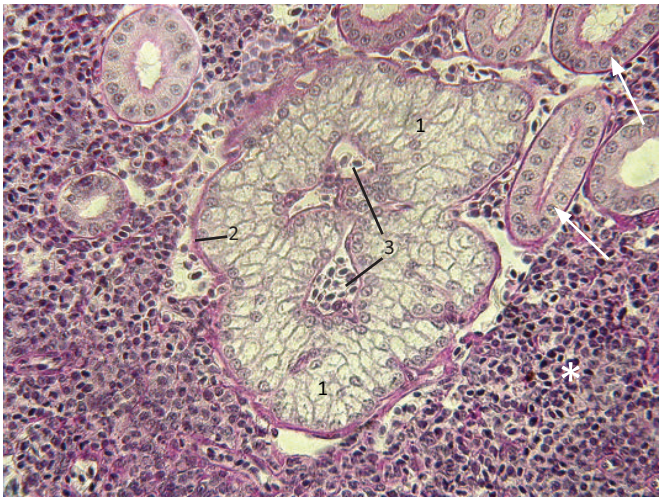


Fig. : 13.13 *Acipenser gueldenstaedtii* (PAS-H / MM)

The interrenal tissue in sturgeons usually forms compact bodies which are scattered along the length of kidneys and along the postcardinal veins. It is formed by cords of cells (1) lined by connective tissue (2) and permeated by blood sinuses (3). The steroidogenic cells (cortisol is the principal corticosteroid produced in Actinopterygians) possess a large, round nucleus and are characterized by a strong vacuolization. Like the trichrome (previous image) the PAS method does not stain the cytoplasms whose lipid droplets have been dissolved by hydrophobic solvents used during the protocol. Note renal tubules (arrows) and hematopoietic tissue (∗).

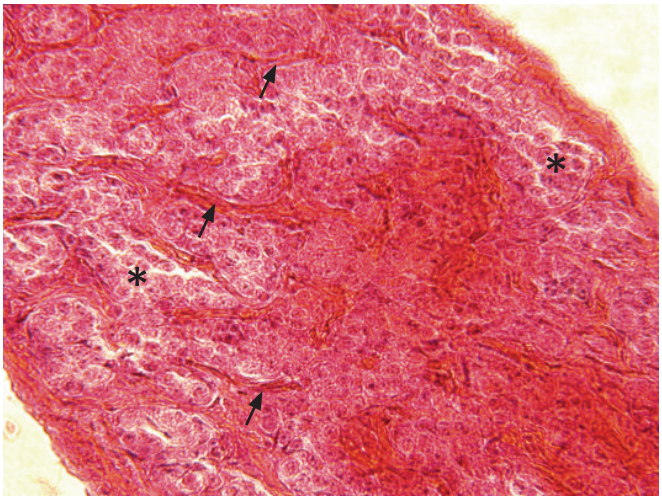


Fig. : 13.14 *Anguilla anguilla* (MT / HM)

Interrenal tissue. In eels the corticoid tissue is widely spread around the head kidney and forms islets surrounding and investing the walls of the cardinal veins and their branches. Interrenal cells are often arranged in cords (∗) separated by fine strands of collagenous fibers (arrows) containing numerous large capillaries.

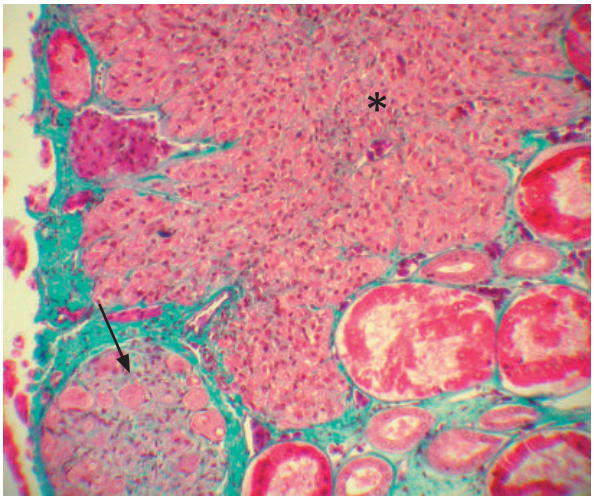


Fig. : 13.15 *Scyliorhinus canicula* (MT / MM)

Chromaffin tissue. In Chondrichthyes the chromaffin tissue (∗) is clearly separated from the interrenal tissue and is organized in groups of chromaffin cells which are closely associated with the paravertebral sympathetic ganglia (arrow – see also Fig. 12.32). In the dogfish islets of chromaffin tissue lie along the inner borders of the kidneys and are distributed near the aorta and the postcardinal veins. The chromaffin mass is surrounded by green connective tissue and renal tubules.

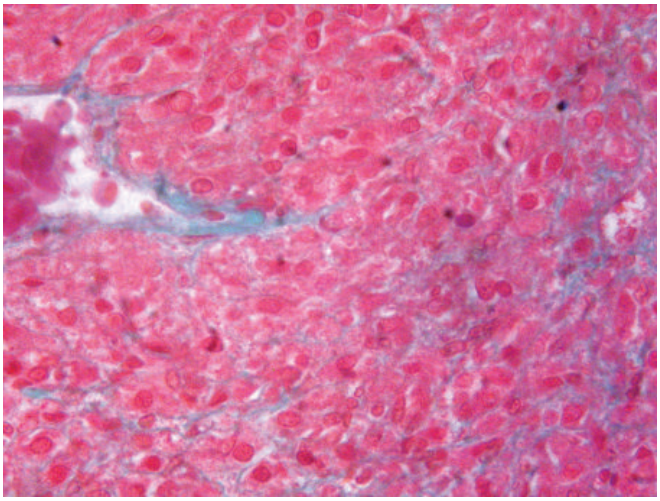


Fig. : 13.16 *Scyliorhinus canicula* (MT / HM)

Chromaffin tissue. Chromaffin secretory cells are generally more uniform and rounded in appearance than the interrenal ones. They secrete catecholamine hormones (adrenaline, noradrenaline) and are capable of rapidly releasing large quantities of these substances in case of stress. The secretory cells usually have basophilic cytoplasm filled with the stored catecholamine granules : they do not present the vacuolated cytoplasm which characterize steroid-secreting cells in routine sections.

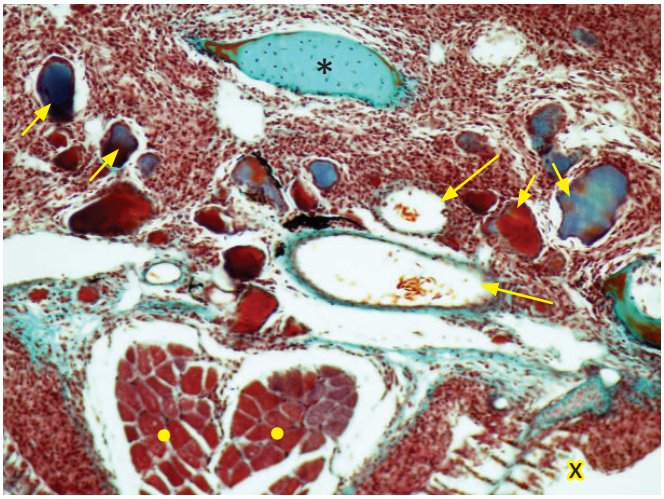


Fig. : 13.17 *Poecilia reticulata* (MT / MM)

Thyroid gland. In most bony fish the thyroid gland is found in the branchial region along the ventral aorta and afferent branchial arteries (long arrows). Thyroid is usually not a compact gland and consists most of the time of numerous scattered units called follicles (short arrows).

The thyroid gland secrete two iodine-containing hormones : thyroxine (T4) and tri-iodothyronine (T3) which play significant roles in maintaining general tissue metabolism.

∗ : piece of cartilage - • : cross-sectioned rhabdomyocytes - x : gill cavity

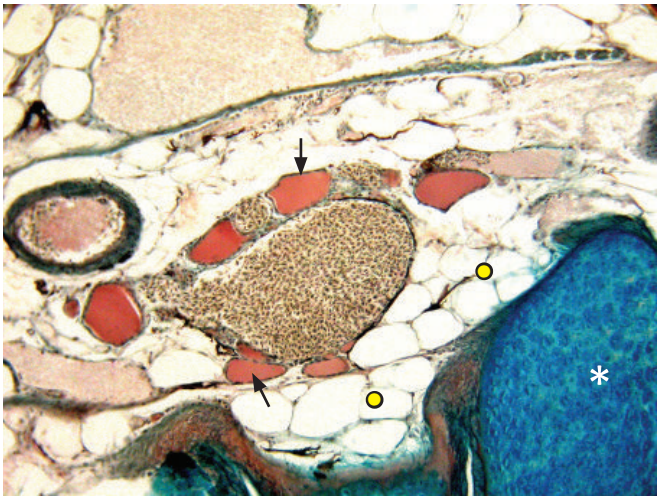


Fig. : 13.18 *Haplochromis multicolor* (AB-PAS-H / MM)

The thyroid follicles (arrows) congregate into several clusters near to the zones where the afferent branchial arteries leave the ventral aorta. The cavities of the follicles are filled with thyroglobulin (brick red), a glycoprotein which stores T3 and T4.

∗ : piece of hyaline cartilage / • : adipocytes

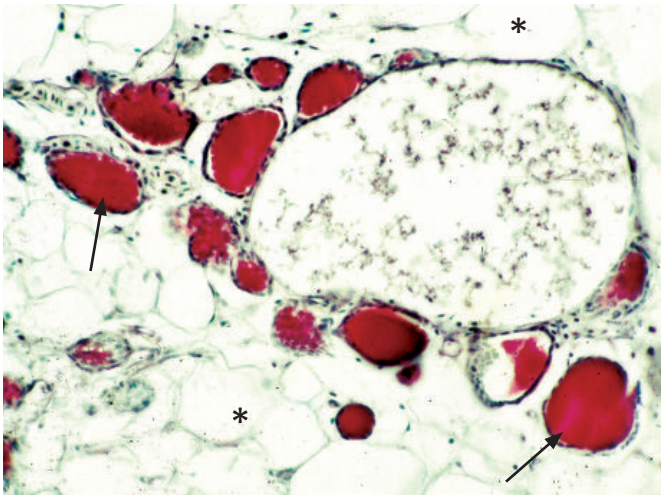


Fig. : 13.19 *Garra congoensis* (MT / MM)

As usual, thyroid follicles (arrows) lie around branchial blood vessels ; however this pattern of scattered follicles can extend quite widely outside the pharyngeal region. In some teleosts bits of thyroid tissue are found in unexpected areas like heart, kidney, eyes... This migration of so-called heterotopic follicles is probably due to the fact that the gland is not encapsulated. Note the abundance of adipocytes (∗).

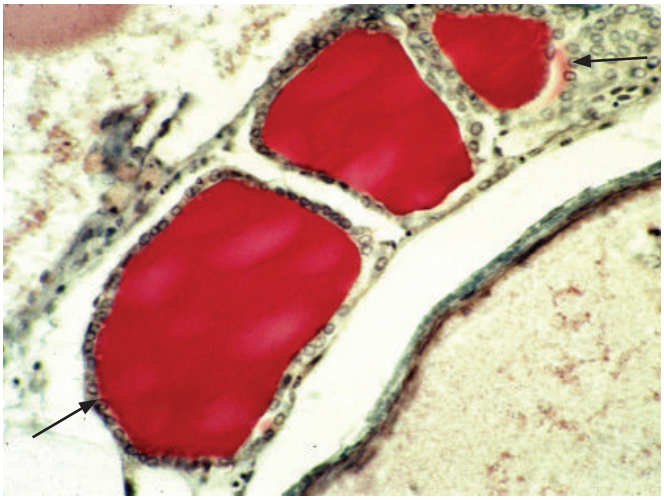


Fig. : 13.20 *Garra congoensis* (MT / HM)

This high magnification shows that thyroid follicles are units lined by a simple cuboidal epithelium. The follicular epithelial cells (arrows) surround a lumen containing a mass of homogeneous and coagulable fluid called colloid (= thyroglobulin - in red).

In this low-active thyroid gland picture the follicular cells appear flattened and the follicles are distended by a large amount of colloid.

In active glands the follicle epithelium is much taller and colloid is less abundant. In addition reabsorption vacuoles on follicular colloid can be seen and thyroglobuline displays variously stained areas.

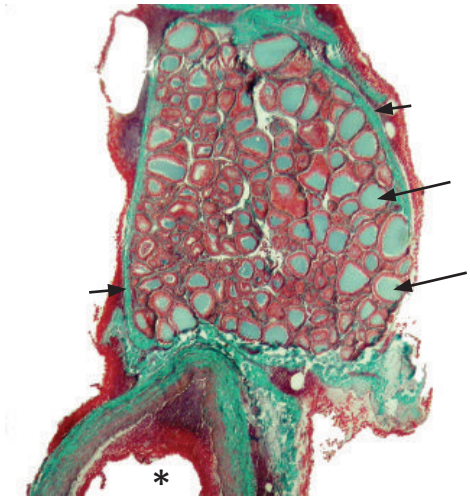


Fig. : 13.21 *Scyliorhinus canicula* (MT / LM)

General view of a lesser spotted dogfish thyroid gland. In elasmobranchs and a few species of bony fishes the thyroid is a single compact gland covered by a connective tissue capsule (green - short arrows). It lies ventrally on the anterior bifurcation («T») of the ventral aorta (∗) coming from the *conus arteriosus*. Numerous green-stained follicles (long arrows) are found. Their size largely depends on the section angle.

The follicular structure of this thyroid gland is remarkably similar to that found in mammals.

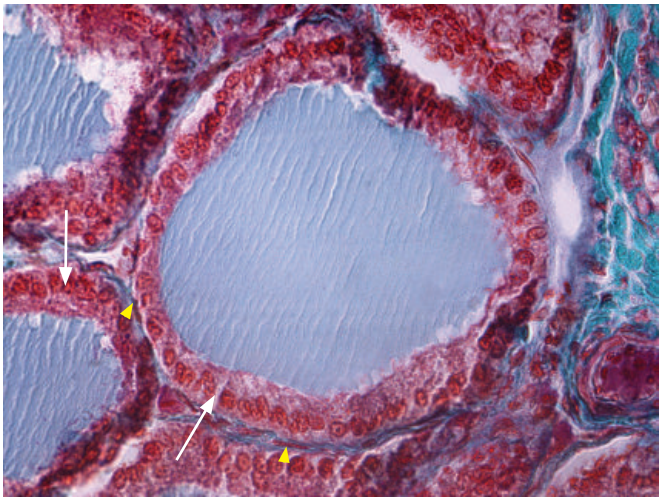


Fig. : 13.22 *Scyliorhinus canicula* (MT / HM)

This magnification exhibits a thyroid follicle that represents the functional unit of this endocrine gland. The follicles are spherical or ovoidal structures which present cavities filled with colloid (ice blue). The thyroid follicles are surrounded and bound together by connective tissue (arrowheads). All cartilaginous fishes have a thyroid contained within a connective tissue capsule.

The follicular cells form a tight epithelium (arrows) and have *microvilli* (slightly discernible here) projecting into the follicular lumen. As compared to that of Fig. 13.20 the height of the epithelium is taller indicating a more active thyroid gland.

Striations in the colloid are artefacts.

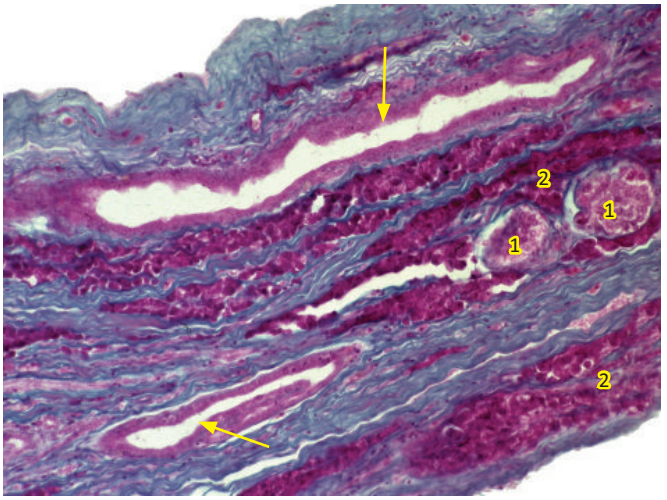


Fig. : 13.23 *Parachanna obscura* (MT / MM)

Endocrine pancreas of a snakehead fish. In addition to an exocrine component lying mostly in fat of the intestinal mesentery (see chapter 8), a pancreas endocrine component constitutes the islands or islets of LANGERHANS.

The size of islet cells may vary with season and some teleosts show an aggregation of these cells into one or two pea-shaped glands called BROCKMAN bodies. Others, such as the present snakehead fish, have islets of tissue (1) scattered among cords of diffuse exocrine tissue (2). Arrows point to exocrine ducts and collagen is stained blue.

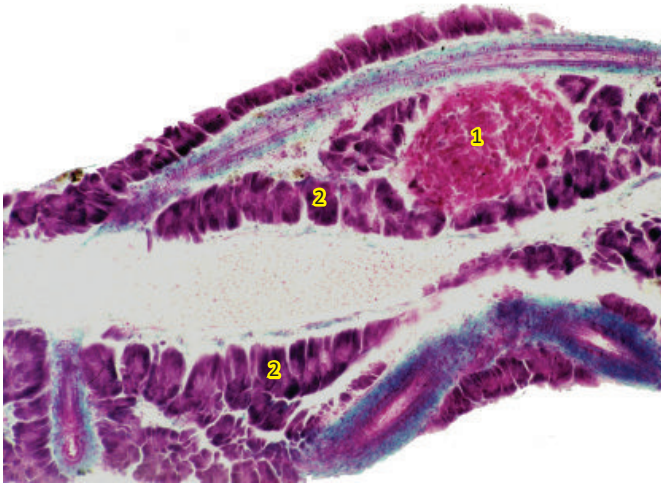


Fig. : 13.24 *Gnathonemus petersii* (MT / MM)

Endocrine pancreas. In most fish the endocrine pancreas consists of areas scattered through the exocrine part. This endocrine tissue forms islets of LANGERHANS (1) composed of small cells with a pale stained granular cytoplasm ; in contrast, the large protein-secreting cells of the exocrine gland stain strongly (2). With H-E and trichrome stained preparations, the - at least - three cell types of the islets are undistinguishable from one another and immunostaining methods are required to differentiate between them.

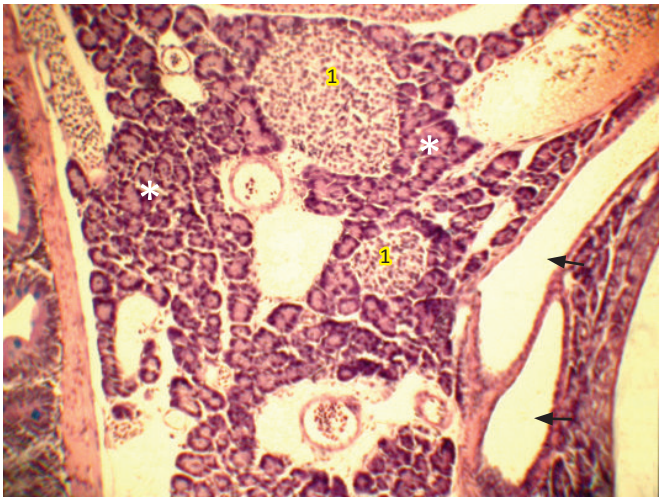


Fig. : 13.25 *Corydoras paleatus* (MT / MM)

Endocrine pancreas of an armored catfish. This microphotograph shows two encapsulated endocrine pancreatic tissues (islet of LANGERHANS - 1) and cords of diffuse exocrine pancreas (∗) located within connective tissue of intestinal mesenteries.

Islets of LANGERHANS, as any endocrine gland, are not furnished with ducts : the large pancreatic ducts here present (arrows) belong to the exocrine pancreatic part and drain proenzymes toward the duodenum.

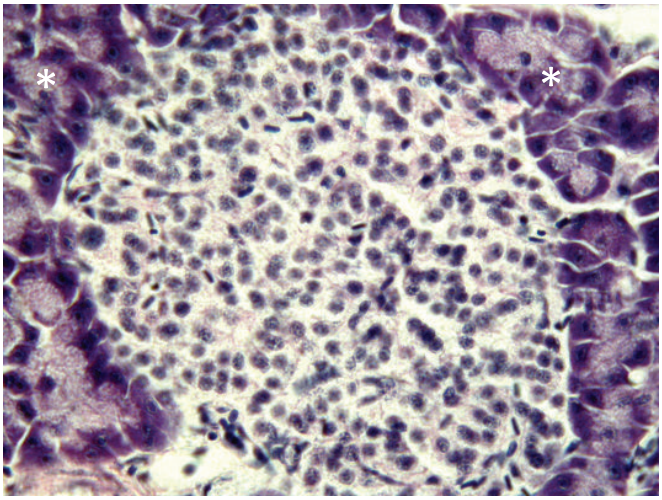


Fig. : 13.26 *Corydoras paleatus* (MT / HM)

Endocrine pancreas. This large islet of LANGERHANS lies among exocrine pancreatic component. It consists of clusters of hormone (insulin, glucagon...)-secreting cells. The several endocrine cell types are generally small and possess a poorly stained cytoplasm in comparison with that of the exocrine acinar cells (∗).

Insulin causes hypoglycemia and glucagon acts antagonistically.

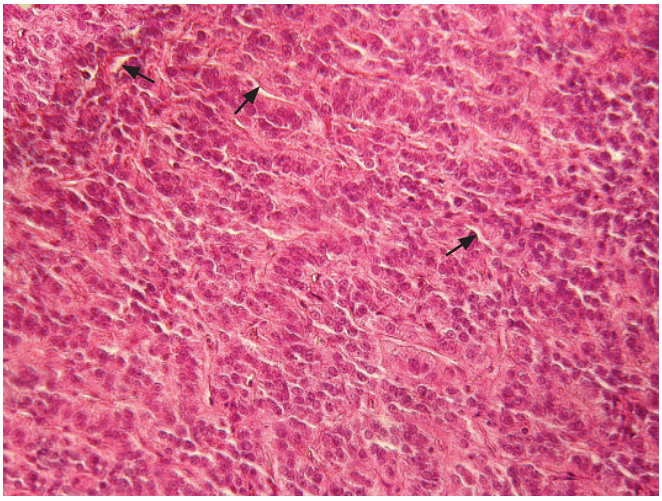


Fig. : 13.27 *Anguilla anguilla* (MT / HM)

Endocrine pancreas. This image, taken within an island of LANGERHANS, demonstrates the appearance of this endocrine tissue. The clumps of secretory cells are supported by delicate collagenous fibers (not distinguishable here) with a rich network of fenestrated capillaries (unstained spaces - arrows) in between.

There is considerable diversity in the arrangement of the hormone-secreting cells of the fish endocrine pancreas.

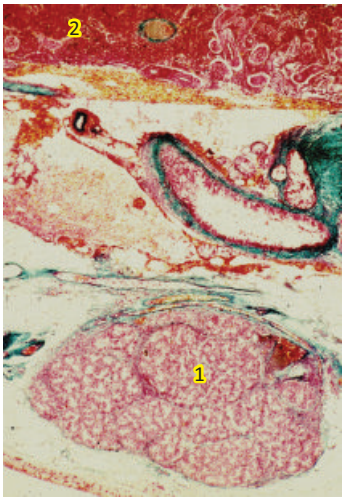


Fig. : 13.28 *Cyprinus carpio* (MT / LM)

Corpuscle of STANNIUS (1). Holosteans and teleosts possess small bodies usually located on the latero-ventral surface of the kidneys (2) and called corpuscles of STANNIUS. Typically they are paired, but their number can reach ten or more. These are endocrine bud-like evaginations arising from the embryonic kidney and involved in calcium homeostasis.

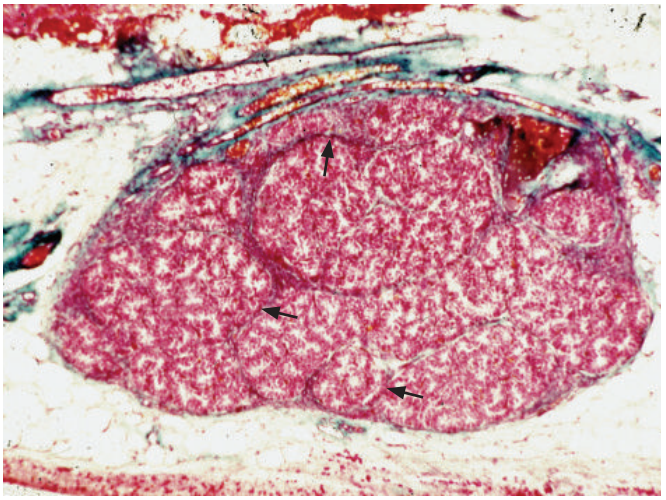


Fig. : 13.29 *Cyprinus carpio* (MT / MM)

Corpuscle of STANNIUS. This photomicrograph illustrates a large corpuscle of STANNIUS. Connective tissue (arrows) from the peripheral capsule penetrates the cellular mass dividing it into lobes consisting of cords or lobules.

It is generally admitted that the corpuscles are less numerous and larger in teleosts in comparison with those of gar pikes and bowfins (Holosteans).

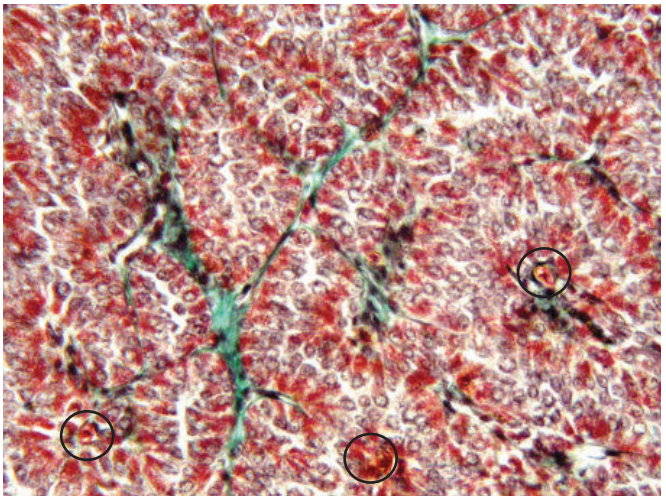
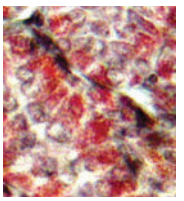


Fig. : 13.30 *Cyclopterus lumpus* (MT / MM)

Corpuscle of STANNIUS parenchymal cells. The penetrating connective tissue divides the corpuscle into several incompletely delimited lobes, within which the connective tissue (green) and capillaries (circles) ramify and establish contact with most secretory cells. At the basal side the glandular cells contain large secretory granules (with stanniocalcin – in red - see insert below) which will directly diffuse into the bloodstream.



Calcium concentrations are higher in seawater as compared to freshwater and histological observations on the corpuscles of STANNIUS seem to indicate that the glands are more active in marine fish.

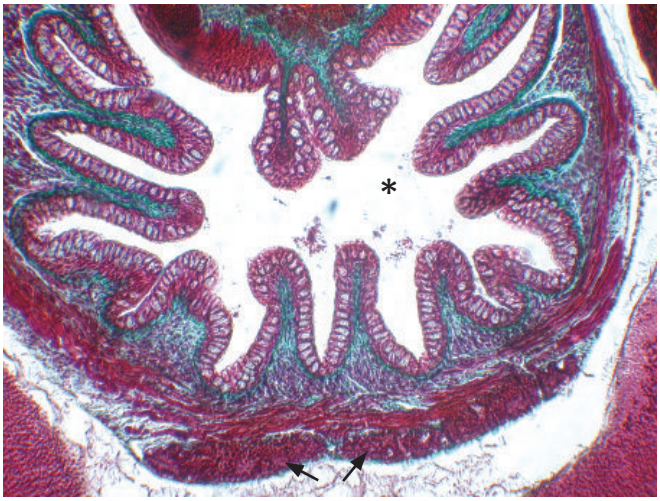
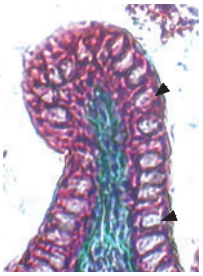


Fig. : 13.31 *Danio rerio* (MT / MM)

Ultimobranchial gland. This endocrine gland is derived from the last branchial pouch area in the embryonic fish. In most fish the ultimobranchial gland consists of one or two glandular units (arrows) generally located in the transverse *septum* beneath the esophagus (∗). On this image collagen is stained in green and the numerous esophageal mucus-secreting cells are unstained (arrowheads).



Ultimobranchial glands produce the hormone calcitonin, which reduces the amount of calcium in blood and are partly equivalent to the parafollicular cells of the mammalian thyroid gland.

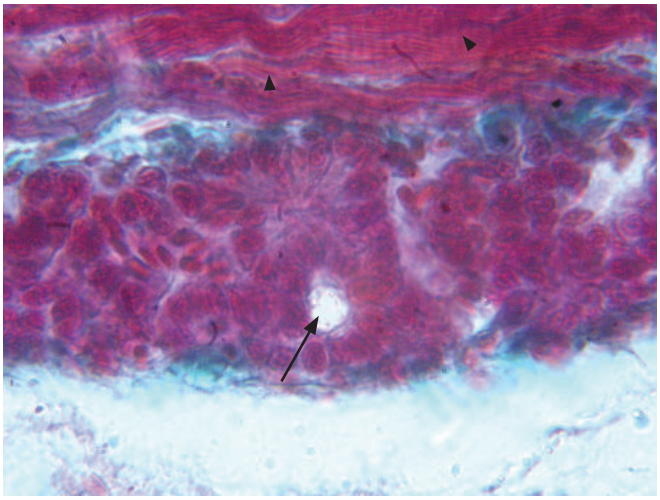


Fig. : 13.32 *Danio rerio* (MT / HM)

Ultimobranchial gland. The parenchyma of the ultimobranchial gland is composed of a non-structural cell layer and in some cyprinids like the zebrafish the gland comprises small follicles surrounding a narrow lumen (arrow). The arrowheads point to the striated muscle fibers of the esophagus.

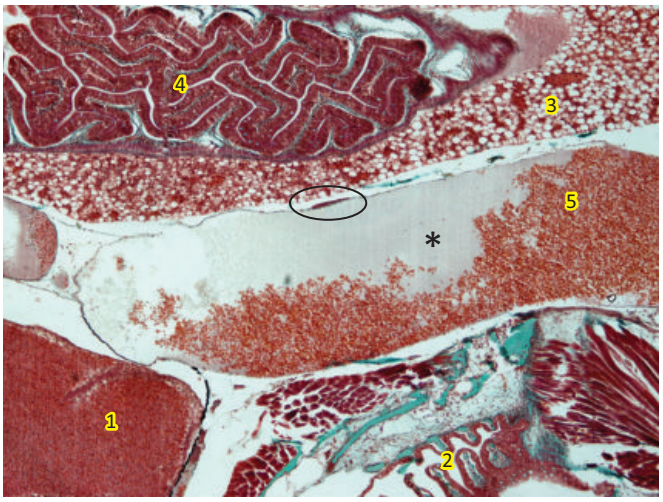


Fig. : 13.33 *Poecilia reticulata* (MT / MM)

Ultimobranchial gland. In small-sized fishes the gland is difficult to identify : the gland shows a sheet-like structure and its observation can be very limited. This general view shows the location of the gland (ellipse) in close association with the *sinus venosus* (∗).

1 : *atrium* / 2 : pharynx / 3 : liver / 4 : small intestine / 5 : erythrocytes in the *sinus venosus*.

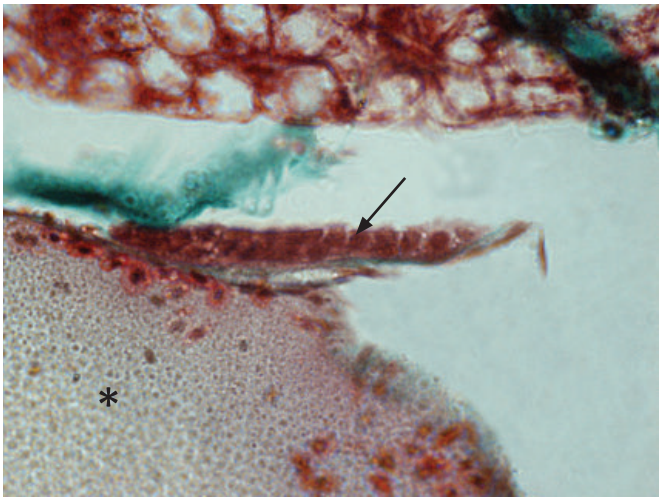


Fig. : 13.34 *Poecilia reticulata* (MT / HM)

Ultimobranchial gland. The arrow indicates the elongated sheet-like tissue of the ultimobranchial gland attached to the cardiac *sinus venosus* (∗). The gland is very thin and composed of one or two layers of secretory cells. Liver is visible at the top of the picture.

The peptide calcitonin is produced in ultimobranchial glands and monitors blood calcium levels apparently more precisely than the corpuscles of STANNIUS.

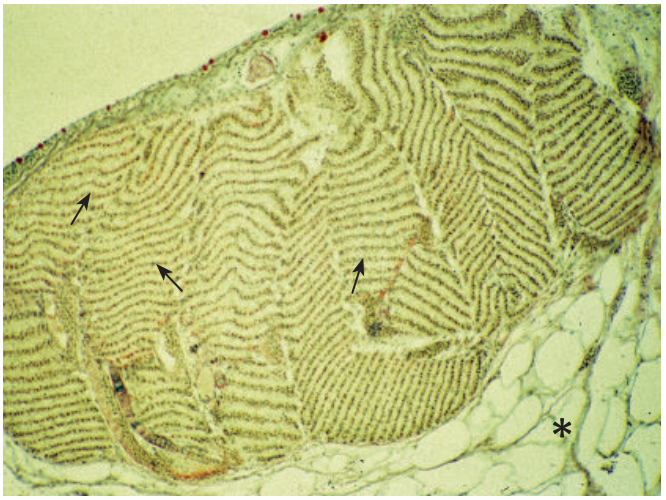


Fig. : 13.35 *Pelvicachromis pulcher* (AB-PAS-H / LM)

Pseudobranch. The pseudobranch is a gill-like structure derived from the first gill arch and located on the dorsal part of the *operculum*. This endocrine gland is composed of gill *lamellae*, connective tissue and parallel rows of blood vessels (capillaries). On this survey photomicrograph only the latter are clearly visible (arrows). ∗ adipocytes.

The pseudobranch is not present in all teleosts and its functions are still highly conjectural.

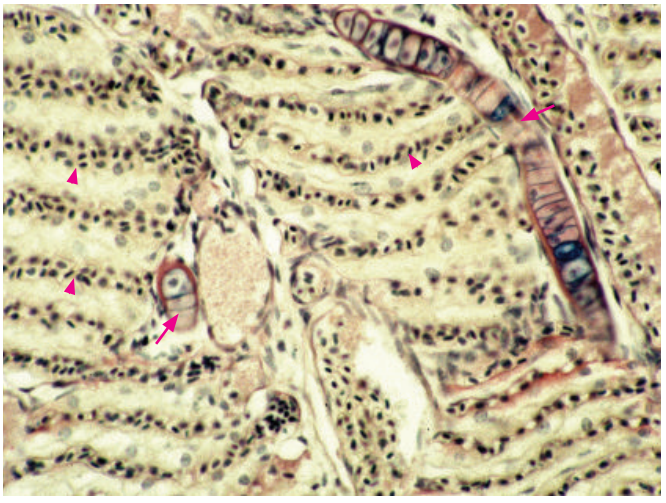
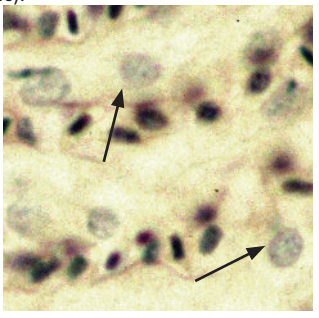


Fig. : 13.36 *Pelvicachromis pulcher* (AB-PAS-H / MM)

Pseudobranch. At this medium magnification the epithelial (pseudobranchial) cells of the *lamellae* (long arrows - below) and the network of parallel blood capillaries (arrowheads) containing nucleated erythrocytes are clearly visible. Parts of the pseudobranch can be supported by thin cartilaginous rods (short arrows).



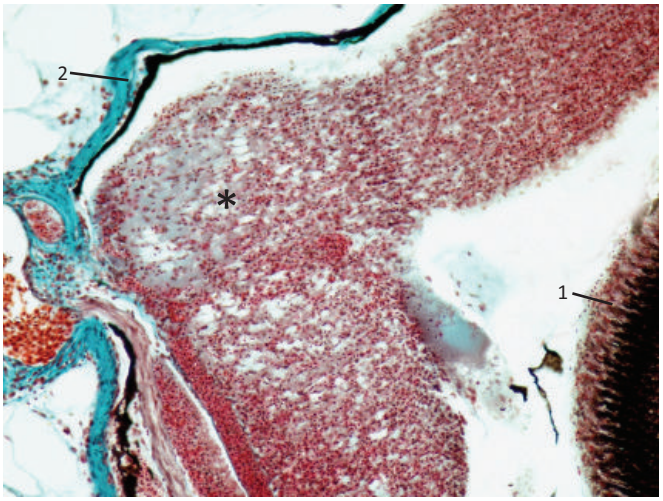


Fig. : 13.37 *Poecilia reticulata* (MT / MM)

Choroid body of the eye. This photomicrograph demonstrates the choroid *rete* (*rete mirabile* - ∗) composed of arrays of capillaries alternating with rows of slender fibroblast-like cells.

It is believed that the pseudobranchs are linked to the ocular choroid gland for the purpose of providing a well-oxygenated blood supply to the retina. Fish who do not possess pseudobranchs invariably also lack a choroid *rete*.

1 : retina / 2 : sclera (see chapter 15)

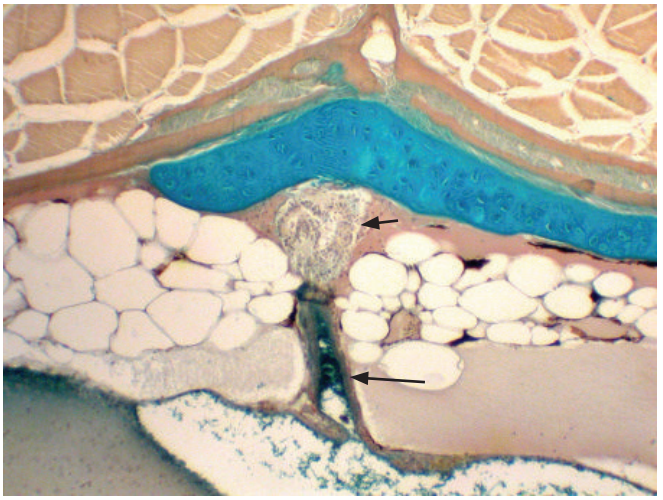


Fig. : 13.38 *Haplochromis burtoni* (AB-PAS-H / MM)

Epiphysis or pineal gland. This small endocrine gland is a non-image forming photodetector connected to the dorsal part (epithalamus) of the «between brain». In fish the pineal organ is basically a tubular or hollow sac-like structure. The photomicrograph shows the thin proximal stalk (long arrow) filled with cerebrospinal fluid and the distal thickening (short arrow). One can also see polyhedral rhabdomyocytes (on the top), cranial cartilage (in blue) and large unstained adipocytes.

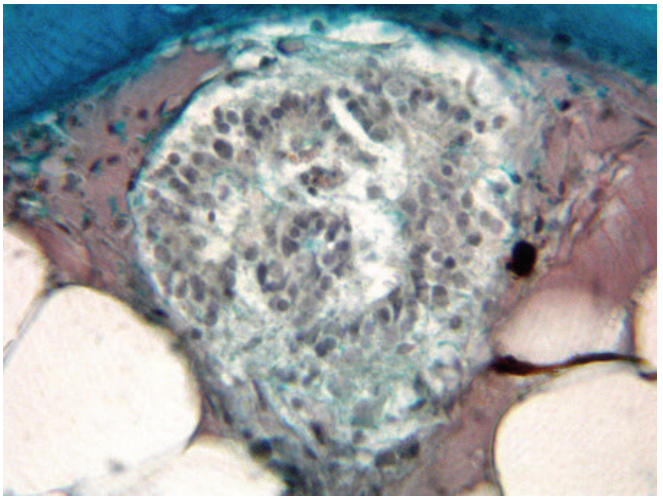


Fig. : 13.39 *Haplochromis burtoni* (AB-PAS-H / HM)

Photomicrograph of the pineal gland end-vesicle. The pineal neuroepithelium is composed of several cell types, among which photoreceptors, neurons and glial cells separated by delicate collagenous fibers (pale blue).

Hyaline cartilage is in blue and as usual the adipocytes are unstained.

The epiphysis appears to receive data on the presence/absence of light. It produces melatonin, a hormone that seems to communicate information about environmental lighting and to have actions on the pigment dispersion within the melanophores. In fish one can consider the pineal gland as a true «photoneuroendocrine organ».

14
Reproductive system

MALE

Testes (Figs 14.1 to 14.17) are either lateral paired organs, or a single fused medial organ located within the dorsal abdomen below the swimbladder. They may vary greatly in size depending on the age of the animal and the season. In Actinopterygii, mature spermatozoa leave the testis by *ductuli efferentes* and reach the genital pore by two *ducti deferentes* or spermiducts (Fig. 14.9) that usually merge caudally. In elasmobranchs *ductuli efferentes* (Figs 14.18 to 14.20), lined by a pseudostratified columnar epithelium course through the epigonal organ (see chapter 5) and enter the epididymis (Figs 14.18 to 14.21). Pairs of *ductuli efferentes* join to form the *ductus epididymidis* which receives secretions from the branched tubular LEYDIG's gland. Secretions from this gland appear to be similar to those of the mammalian prostate and seem to be involved in the formation of spermatozoa into bundles. The testis itself is organized into lobules oriented towards the central lumen.

Germ cells progress through several distinct cytological stages during spermatogenesis. Germ cells located within the stroma of the tubule wall give rise to primary spermatogonia. These are large cells with eosinophilic cytoplasm and a distinct nucleus containing dense chromatin. Each primary spermatogonia undergoes a series of mitotic divisions to produce a cluster of secondary spermatogonia encapsulated within a cyst. Each cyst contains cells at the same stage of differentiation, forming a clone. The cyst wall arises from a SERTOLI cell associated with the original germ cell. Clusters of cells resulting from divisions of the original germ cell maintain a consistent stage of development within the cyst. Secondary spermatogonia are smaller than primary spermatogonia with large lightly basophilic nuclei and little cytoplasm. Primary spermatocytes, the result of another round of mitotic divisions, are smaller still with increasingly basophilic nuclei. Primary spermatocytes undergo the first meiotic division to produce secondary spermatocytes (haploid). Still contained within the cyst, these cells are again smaller and have increasingly dense basophilic nuclei. Secondary spermatocytes undergo a second meiotic division. The resulting spermatids have condensed, intensely basophilic, nuclei and very little cytoplasm. At this stage the cyst ruptures releasing the spermatids into the testis lumen where final maturation takes place. Each spermatid develops into a spermatozoon. In the male guppy all the sperm of a number of cysts ripen at the same time and are discharged as *spermatozeugmata* (or packets of spermatozoa - Figs 14.7 & 14.8) into the lumen of the sperm ducts containing secretions of seminal fluid. In the testis interstitium are found small numbers of LEYDIG cells (Figs 14.22 & 14.23). These interstitial cells are polymorphous with spherical nuclei and show some characteristics of steroid hormone production. Furthermore, interstitial cells undergo maximal development (thus seeming to signal secretory activity) just before and during the breeding period; their presence may be partly obscured, before spawning, by the crowding and distention of the testis with sperm.

The spermatogenetic process is seasonal in some fish and more or less continuous in others. For example, the testis of the perch is smallest from late June to late August (Northern hemisphere). Spermatogenesis then proceeds rapidly enough so that by early November the gonad has reached its greatest size. Testicular volume, an indicator of spermatogenetic rate, in the top-minnow, *Gambusia affinis*, is eight times as great in summer as in winter.

FEMALE

The morphological variety seen in the female reproductive tract and ovaries is immense. This is mirrored by the fact that oviparity, with eggs being shed into the water either for a brief spawning period each year, or at short inter-

vals throughout the year, ovoviviparity and also viviparity are all common phenomena in this fantastic group.

Teleosts are unique among vertebrates in having hollow ovaries resulting from the embryonic development of longitudinal ovarian folds that eventually fuse to enclose a coelomic cavity. A short oviduct (Fig. 14.42) conducts eggs to the outside via an exit between the anus and urinary pore. In Salmonids the solid ovary has no excurrent duct, eggs being discharged into the coelomic space and escaping by way of the abdominal pore.

Histological examination of ovarian tissue commonly reveals eggs at all stages of development (Figs 14.28 to 14.39) and degeneration (atresia - Fig. 14.32). For some species, such as cyprinids, this is due to the fact that selected groups of oocytes undergo maturation, with spawning activity being rhythmic through the reproductive period. For salmonid species that have synchronized spawning of all the viable oocytes at a single time, the appearance of the oocytes may be more uniform.

The luminal surface of the *tunica albuginea* folds into ovigerous *lamellae* oriented perpendicular to the long axes of each ovarian lobe. Lamellar walls are composed of germinal and follicular epithelia supported by a vascular connective tissue stroma. Previtellogenic and vitellogenic follicles can be observed (Figs 14.28 to 14.37).

During the ovarian cycle, the oocytes can be classically divided into five to six stages, according to major morphological characteristics of oocyte growth. In this text, three main phases will be emphasized : the previtellogenic, the vitellogenic and the mature oocyte phases.

During the previtellogenic phase, the oocytes (sometimes of very large diameter) show homogenous strong basophilia of the ooplasm. The nuclei contain many (up to 20) prominent nucleoli, often next to the nuclear membrane. The oocytes are surrounded by a single layer of squamous follicle cells (Fig. 14.38). Together with oocyte growth, the size of the nucleus also increases and cortical alveoli are detected in the ooplasm; these spherical structures appear empty. The cortical alveoli progressively increase in number to form peripheral rows.

The apparition of yolk granules (proteins) and fat vacuoles in the ooplasm defines the vitellogenic oocytes which reach their maximum size at the end of this stage. The cytoplasm begins to fill with yolk spheres, granules or globules (Figs 14.39 to 14.41). These structures maintain their integrity throughout the oocyte growth, without merging into a continuous mass of fluid yolk. The ooplasm seems paler. The nuclear membrane shows an irregular aspect.

The start of the ripe (mature) stage is indicated by the peripheral migration of the nucleus (germinal vesicle) toward the animal pole and the dissolution of the nucleus membrane.

Owing to the large size of the ova resulting from the accumulation of yolk reserves, the granulosa and thecal cells become tightly stretched and flattened as the follicle matures, making these cell layers difficult to identify in sections prepared for light microscopy.

Following the final stage of oogenesis, ripe oocytes are released into the ovarian lumen. This final stage is difficult to follow because of the shrinkage and distortion of these cells during normal processing.

The spent ovaries are composed of atretic (degenerate) follicles, immature oocytes and mature eggs left unspawned and are often bloodshot in appearance. This image is a good indicator of recent spawning and also that the fish have reached maturity. As you have read the organization of the developing ovarian follicle differs between the mammalian and non mammalian vertebrates. *Corpora lutea* have been described in some fish ovaries. In species of oviparous teleosts in which fertilization is external, the *corpora lutea* are poorly developed transient structures and, when studied, lack histochemically detectable steroidogenic enzymes.

A predominant feature of all vertebrate ovaries is that follicles can degenerate (Fig. 14.32) at any stage of development leading to the production of *corpora atretica*. Vitellogenic follicles are more prone to atresia than previtellogenic follicles.

Ovoviviparous and viviparous fish are live-bearing species that require internal fertilization. (claspers or *myxopterygia* in Chondrichthyes - Fig. 14.24 / *gonopodium* in *Poeciliidae* – Figs 14.25 to 14.27). In ovoviviparous forms the eggs are retained and fertilized within the body, but the young receive no nutrients from the mother – they must rely solely on what is provided in the yolk (lecitotrophy). In the genus *Heterandria (Poeciliidae)*, a simple kind of pseudoplacenta is found. In this case, the walls of the ovarian follicles acquire an elaborate network of capillaries that extend out as *villi* and make intimate association with the external surface of the developing embryos.

Many sharks (blue, hammerhead, lemon, spottail…) are viviparous. In the same way, inside the order Cyprinodontiforms, the *Goodeidae* (indigenous to the central plateau of Mexico) are considered true viviparous species. They developed interesting particularities in their reproductive biology, such as a gestation period of several weeks within the hollow ovary. Morphological and functional adaptations of both the female fish and embryo make this possible. Structures that facilitate the transfer of nutrients and oxygen from the maternal organism to the embryo, and mechanisms for the disposal of metabolic waste products, play an essential role. This has necessitated the development of specific placental exchange surfaces and/or the transitory use of other organs for the support of the developing embryo. In *Goodeidae*, the most conspicuous structural adaptations are the so-called "*trophotaeniae*", which are food absorbing processes that grow out of the embryo hindgut (*proctodaeum*). These temporary outgrowths form a placental exchange site, which is in intimate contact with the ovarian liquor. *Trophotaeniae* are covered by a modified intestinal epithelium.

In cyprinodonts with follicular gestation, matrotrophy (the mother provides the majority of resources to the developing offspring after fertilization) is accompanied by an enormous expansion of trophoderm that covers the pericardial sac. The development of a trophotaenial placenta represents the evolutionary end in the formation of structural adaptations for viviparity in the cyprinodonts.

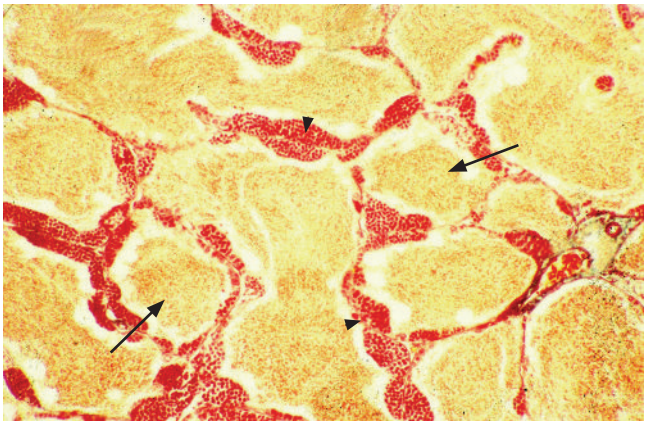


Fig. : 14.1 *Cyprinus carpio* (PAS-H-AUR / LM)

Seminiferous lobules. Sperm may be available at all times during the reproductive season or mature in a single or several succeeding periods. The cystic type of spermatogenesis, where large numbers of sperm develop in unison inside envelopes (= cysts) that eject their contents into the seminiferous lobules, is most usual in fish. That is especially suited to species where a great number of gametes are suddenly required for external fertilization.

This picture shows various sections through seminiferous lobules. At this stage the cysts rupture releasing the spermatids into the lumen where final maturation will take place (long arrows). Cysts with clones of germ cells at an identical stage of spermatogenesis are also seen (arrowheads).

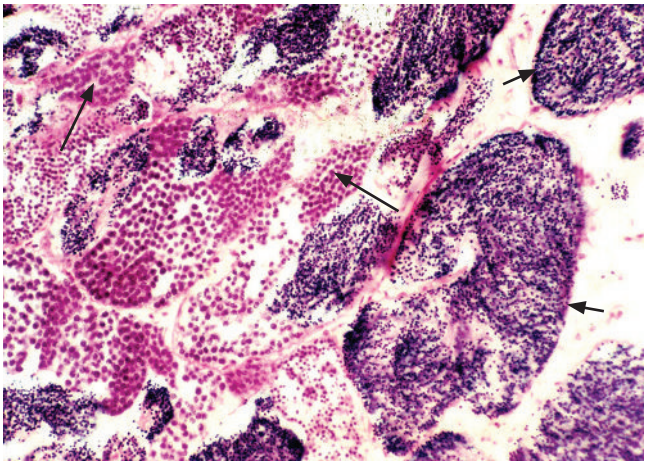


Fig. : 14.2 *Ctenopoma ansorgei* (MT / MM)

Testis. Spermatogenesis is said to be unrestricted meaning that maturation of sperm occurs along the entire length of the seminiferous lobules. Testes are covered by an *albuginea* connective capsule containing smooth muscle and blood vessels. The capsule sends *septa* into the organ forming seminal lobules composed of various cysts.

This photomicrograph illustrates seminiferous lobules containing numerous cysts at more (short arrows) or less (long arrows) advanced stages.

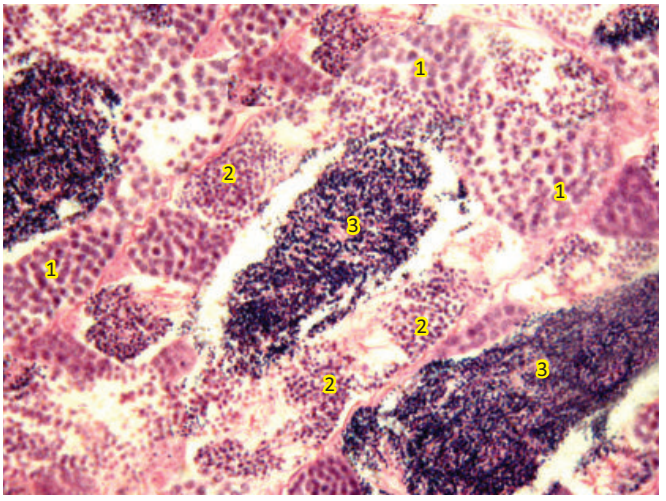


Fig. : 14.3 *Ctenopoma ansorgei* (MT / MM)

Testis. Several cysts at different stages of maturation are illustrated. Germ cells progress through several distinct cytological stages during spermatogenesis.

1 : cysts with secondary spermatogonia / 2 : cysts with spermatocytes / 3 : cysts with spermatids or spermatozoa.

The different maturity stages are in sequence : germ cells –> primary spermatogonia (diploid 2n – the largest cells with a clear cytoplasm and showing no cysts) –> secondary spermatogonia (2n - smaller and encapsulated within a cyst comprising a few cells) –> primary spermatocytes (2n – with a prominent nucleus and filamentous chromatin) –> secondary spermatocytes (haploid n - smaller and with slightly condensed chromatin) –> spermatids (n – scarce cytoplasm and condensed basophilic nucleus) –> spermatozoa (n - the smallest cells of the spermatogenic lineage with a very dense nuclei).

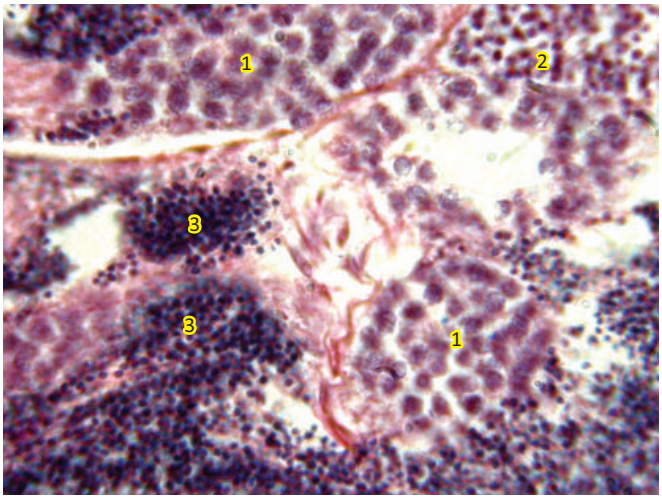


Fig. : 14.4 *Ctenopoma ansorgei* (MT / HM)

Testis. Several cysts, each at the same development stage, are shown. Some of them contain spermatogonia (1) or spermatocytes (2) others display spermatids (3). Cyst wall arises from a SERTOLI cell associated with the original germ cell.

Each cyst containing cells at the same stage of differentiation forms a clone.

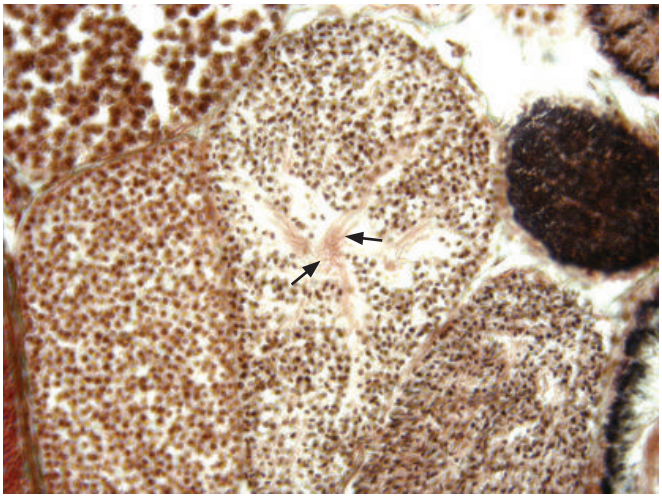


Fig. : 14.5 *Ctenopoma ansorgei* (MT / MM)

A spawning testis showing three lobules. In the centre of the picture the lumen is filled with immature spermatozoa (arrows). Spermatozoa concentrate in the seminal lobules after breaking through the cyst wall. Cysts with germ cells at different stages of development are also displayed.

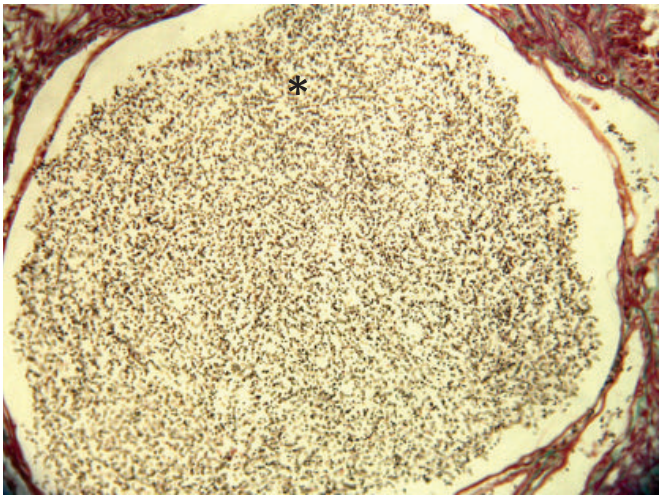


Fig. : 14.6 *Cyclopterus lumpus* (MT / HM)

Late maturing testis. Lobules are swollen with sperm (∗) that are typical of fish in breeding condition. The cyst wall has broken up whereby the spermatozoa are released into the lobule lumen.

The maturing testis is fairly voluminous and may occupy up to 25% of the abdominal cavity. Active spermatogenesis occurs and spermatids as well as spermatozoa are more abundant in the lumen of the seminal lobules.

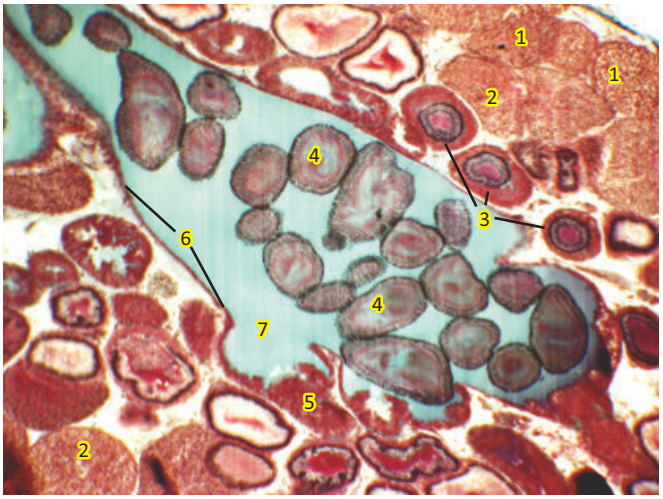


Fig. : 14.7 *Poecilia reticulata* (MT / MM)

Sagittal section through the testis of an adult guppy. In this species the testis is bilobed and is composed of cysts-containing tubules which radiate from the two main sperm ducts toward the periphery where spermatogonia I are associated with SERTOLI cells. As spermatogenesis proceeds, cysts with the different spermatogenetic stages (spermatogonia II - spermatocytes I - spermatocytes II - spermatids - spermatozoa) migrate from the periphery toward the sperm ducts. The spermatozoa of the guppy are produced in the form of unencapsulated sperm bundles called *spermatozeugmata*. In the latter the heads of spermatozoa point outward (attached to the SERTOLI cells lining the cysts) while their tails point to the centre. Then the SERTOLI cells fuse with the wall of efferent duct, the cysts open and *spermatozeugmata* are released in the main sperm duct. Finally *spermatozeugmata* enter the *gonopodium* before mating.

1 : spermatocytes - 2 : spermatids - 3 : *spermatozeugmata* surrounded by Sertoli cells - 4 : *spermatozeugmata* released into the lumen of the main efferent duct - 5 : hypertrophied SERTOLI cells transforming into efferent duct cells - 6 : cuboidal or columnar epithelium lining the efferent duct - 7 : protective secretion produced by the duct epithelium

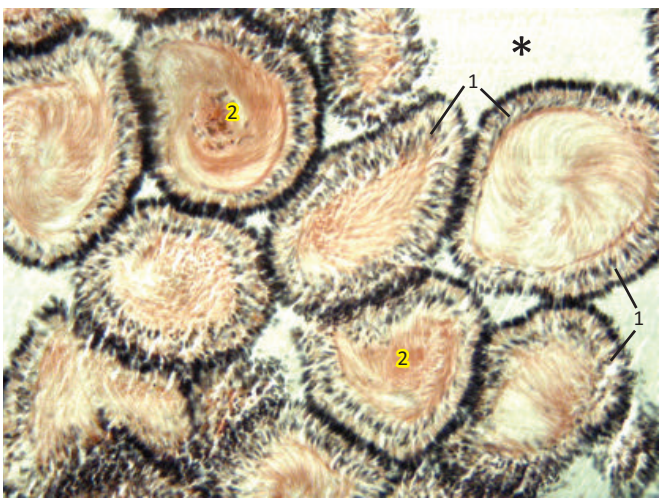


Fig. : 14.8 *Poecilia reticulata* (MT / HM)

Mature testis of a guppy. This micrograph shows free *spermatozeugmata* in the lumen (∗) of the efferent duct. The *spermatozeugmata* are aggregations of spermatozoa in which the heads point to the periphery (1) simulating a columnar epithelium ; the tails are spirally oriented to the centre (2).

Into the female reproductive tract, the *spermatozeugmata* rapidly dissociate into free-swimming spermatozoa before their migration to the ovarian cavity.

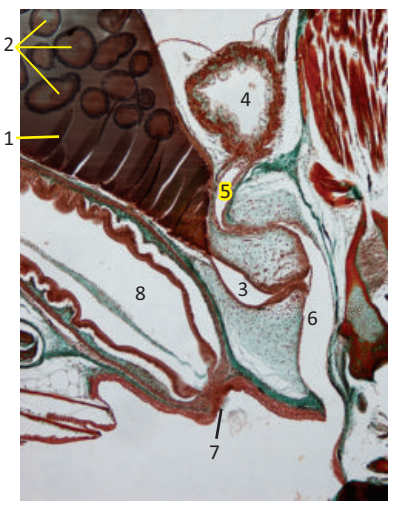


Fig. : 14.9 *Poecilia reticulata* (MT / MM)

Section close to the sagittal plane at the level of the urogenital and anal regions. In the testis (1) the lumen of the end of the main efferent duct is filled with numerous *spermatozeugmata* (2) which will pass through the spermiduct (3). Urine exits the bladder (4) via the urethra (5). The spermiduct and the urethra join into the urogenital *sinus* (6) which opens just behind the anus (7). Remember that *spermatozeugmata* enter in the *gonopodium* before mating. The epithelium of the rectum (8) is slightly folded and does not form *villi*.

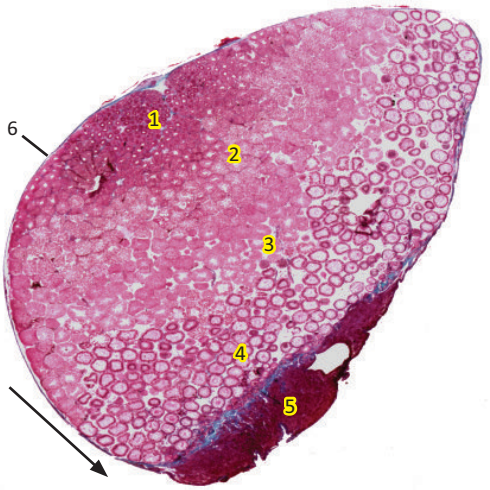


Fig. : 14.10 *Scyliorhinus canicula* (MT / LM)

General view (transverse section) of a mature dogfish testis. The testis contains spheroidal lobules arranged in zones corresponding to the distinct stages of spermatogenesis. Each lobule is made of (spermato)cysts surrounding a central cavity. Lobules originate in the ventrolateral germinative zone and progress linearly (follow the arrow) to the opposite dorsal zone where spermiation occurs.

Four zones are usually visible by light microscopy. 1 : germinative zone with immature lobules and spermatogonia - 2 : lobules with primary or secondary spermatocytes - 3 : area composed of lobules with early spermatids - 4 : late spermatids or immature spermatozoa.

The epigonal organ (5 – see Figs 5.13 & 5.14) partially envelops the elasmobranch gonads and the testis is enclosed by a thin capsule of collagenous tissue (*albuginea* - 6).

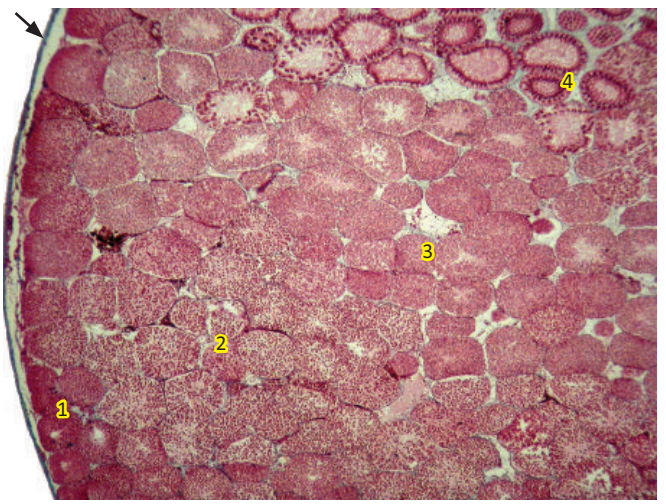


Fig. : 14.11 *Scyliorhinus canicula* (MT / LM)

Low magnification of dogfish testis showing zonated arrangement of lobules. The four zones of lobules with spermatogonia (1), spermatocytes (2), early spermatids (3) and late spermatids or immature spermatozoa (4) are distinguishable.

Lobules radiate from the germinal zone (1) and progress linearly across the testis. They are made up of cysts which are comprised of one SERTOLI cell associated with germ cells all at the same stage of development.

Testes are covered by a connective capsule (*tunica albuginea* - arrow) containing blood vessels and a few smooth muscle fibers.

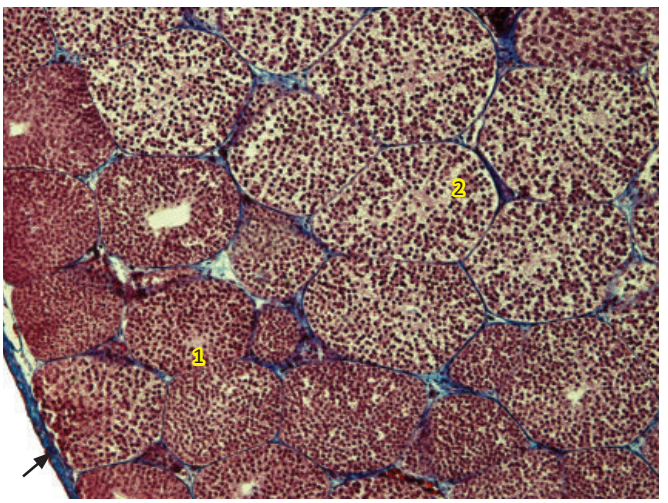


Fig. : 14.12 *Scyliorhinus canicula* (MT / MM)

Dogfish testis. This micrograph shows the two peripheral zones of the testis : the zone with lobules containing spermatogonia (1) and the zone (2) where the primary spermatocytes (secondary spermatocytes are relatively rare) represent the main cellular type. The thin capsule is arrowed.

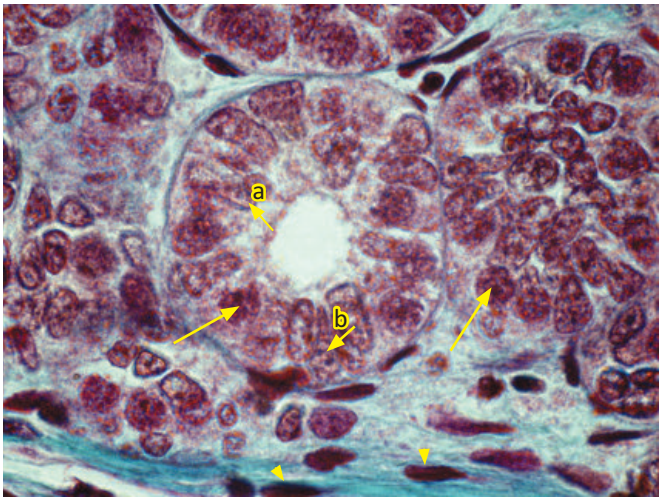


Fig. : 14.13 *Scyliorhinus canicula* (MT / HM)

Lobules of the germinative zone. At the centre of the picture a small spermatogonial lobule displaying a central cavity is clearly seen. Histological observation shows spermatogonia (long arrows) and SERTOLI cell nuclei (short arrow) which can be located both in an adluminal (a) or a peripheral (b) position. Lobules are surrounded by connective tissue (turquoise) with dark elongated fibroblast nuclei (arrowheads).

Through the course of spermatogenesis, SERTOLI cells increase in size and in the abundance of steroidogenic organelles.

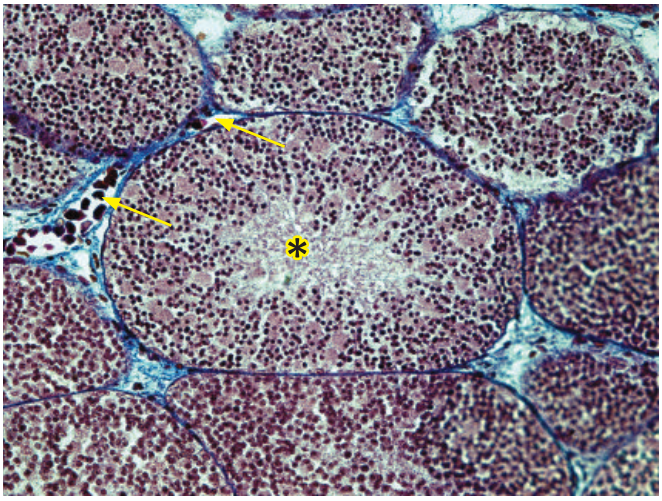


Fig. : 14.14 *Scyliorhinus canicula* (MT /MM)

Lobules with early spermatids. This stage is characterized by the radially arrangement of the early spermatids and by the condensation of their nuclei. Sections through flagella can be seen at the centre (∗).

Lobules are separated by little connective tissue (blue) containing blood vessels (arrows).

In all stages of development, interstitial tissue was sparse and only some relatively undifferentiated LEYDIG-like cells are present.

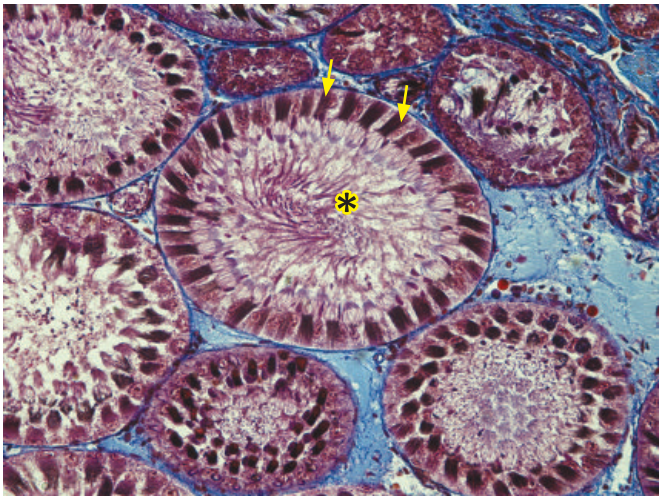


Fig. : 14.15 *Scyliorhinus canicula* (MT / MM)

Lobules with spermatids during the last stages of spermatogenesis. On the side opposite to the germinative zone, near the epigonal organ, lobules contains cysts with well-differentiated spermatids. One can recognize these stages by the peripheral arrangement of spermatids in tight compacted bundles (arrows) and by the massive presence of curved flagella (∗).

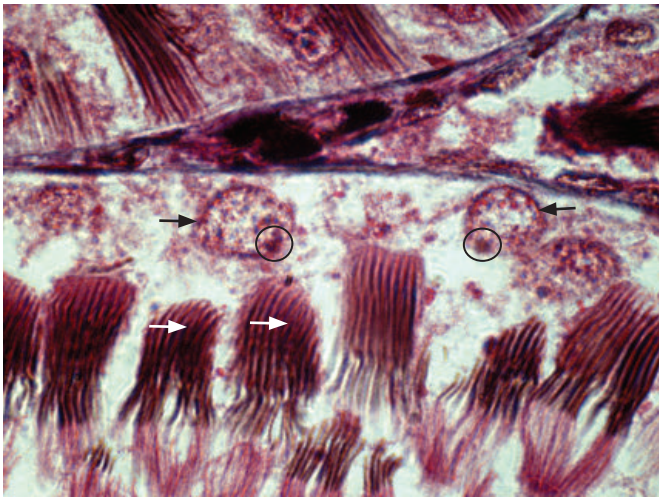


Fig. : 14.16 *Scyliorhinus canicula* (MT / HM)

Lobules with spermatids during the last stages of spermatogenesis. At this stage the SERTOLI cell nuclei (arrows) are still located in periphery of the lobule. The SERTOLI cell nucleus is easily recognizable by the presence of the nucleolus (circles). White arrows point to the tightly compacted parallel arrangement of the elongated spermatid nuclei.

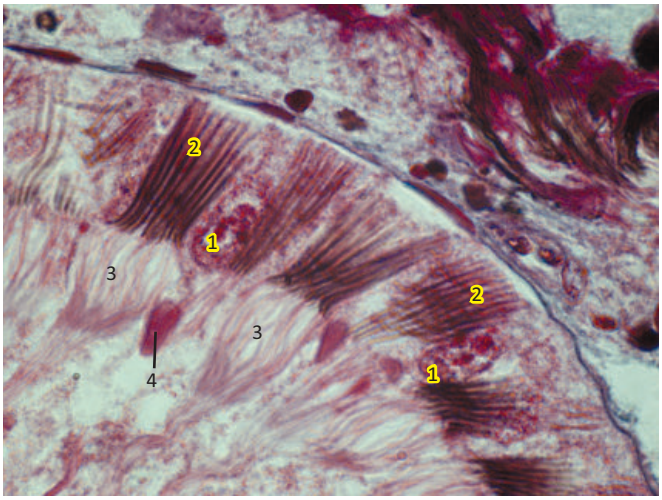


Fig. : 14.17 *Scyliorhinus canicula* (MT /HM)

This high magnification shows one of the latest stages of spermatogenesis. In the present stage the SERTOLI cell nuclei (1) move and fix between the tight bundles of spermatids (2).

Numerous flagella (3) and a body of proteinaceous nature (4) secreted in the cytoplasm of each SERTOLI cell are also found.

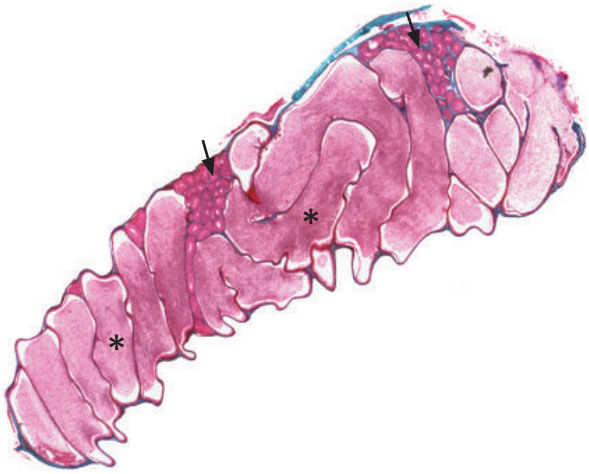


Fig. : 14.18 *Scyliorhinus canicula* (MT / LM)

Efferent ductules and epididymis. The male genital ducts of the lesser spotted dogfish are arranged in this sequential order : *ductuli efferentes* (*vas efferens*) –> *epididymis* –> *ductus deferens* and seminal vesicles. Sperm passes from the testes to the kidneys within the efferent ductules, and after passing through the anterior kidney it enters the *ductus deferens* toward the cloaca.

This micrograph illustrates the highly convoluted epididymis filled with spermatozoa (∗) cut in various planes of section. Efferent *ductuli* are visible (arrows).

The LEYDIG's gland is the portion of the anterior mesonephros which has lost its urinary function. It is made up of secretory tubules whose secretions are discharged into the epididymis.

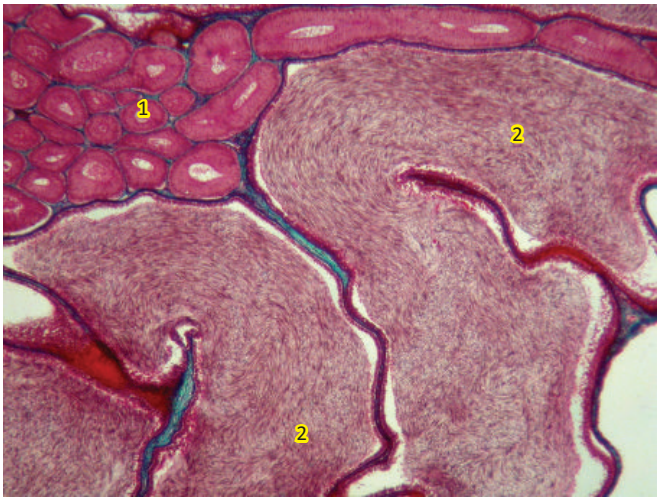


Fig. : 14.19 *Scyliorhinus canicula* (MT / MM)

Ductuli efferentes and epididymis. In elasmobranchs *ductuli efferentes* (1) course through the epigonal organ (see chapter 5) and enter the epididymis (2) filled with numerous spermatozoa. Pairs of efferent ducts join together to form the *ductus epididymidis* which receives secretions from the branched tubular LEYDIG's gland. Cross sections of the *vas efferens* usually present a regular and circular profile.

In the epididymis spermatozoa develop the capacity for motility and form bundles in the distal portion.

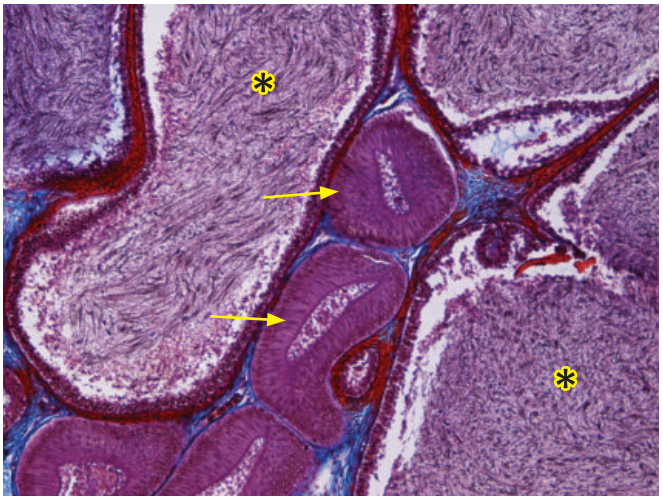


Fig. : 14.20 *Scyliorhinus canicula* (MT / MM)

The centre of this photomicrograph displays sections through the efferent ductules. The latter are surrounded by the convoluted epididymis mainly filled with spermatozoa (∗).

Ductules are lined by a tall pseudostratified ciliated columnar epithelium (arrows). The underlying connective tissue is rich in collagen fibers (in blue). The highly coiled *ductus epididymidis* is lined by a high (initial segment) or low (terminal segment) pseudostratified epithelium.

The epithelia lining the segments of the epididymis and the secretory tubules of the LEYDIG's gland are specialized for protein secretion.

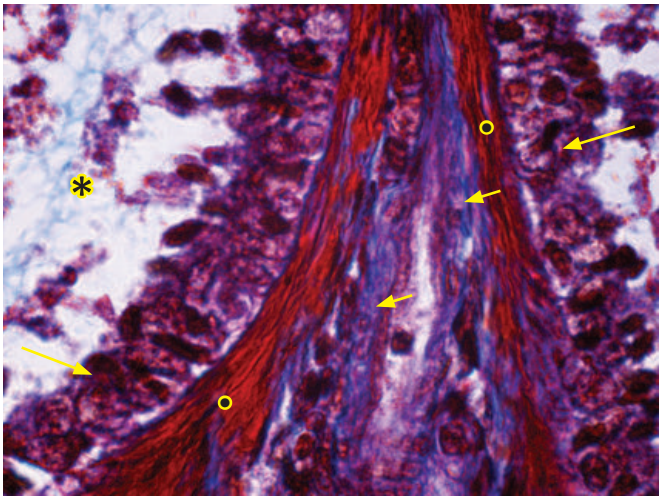


Fig. : 14.21 *Scyliorhinus canicula* (MT / HM)

Wall of the epididimys. The lumen (∗) of the *ductus epididymidis* is mainly filled with spermatozoa but also with SERTOLI cells and fragments of spermatocysts. The pseudostratified columnar epithelium (long arrows) is supported by connective tissue (indigo blue – short arrows) and some smooth muscular cells (•) may be present in the distal wall.

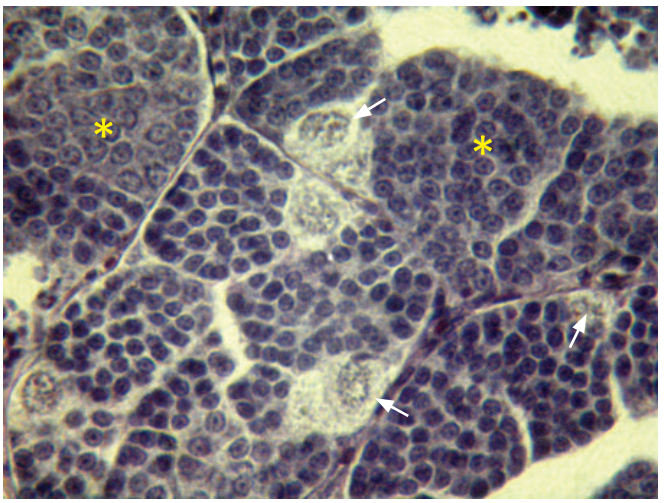


Fig. : 14.22 *Rutilus rutilus* (H / MM)

Interstitial cells in a low active testis. In the interstitium small numbers of LEYDIG cells (arrows) which synthesize and secrete the male sex (steroid) hormones are found. These interstitial cells are polymorphous with spherical nuclei. The cytoplasm contains numbers of lipid droplets dissolved by hydrophobic solvents used during the routine histological protocol. The other cells (∗) of the micrograph are SERTOLI cells and spermatogonia : this testis has been dissected after the breeding period.

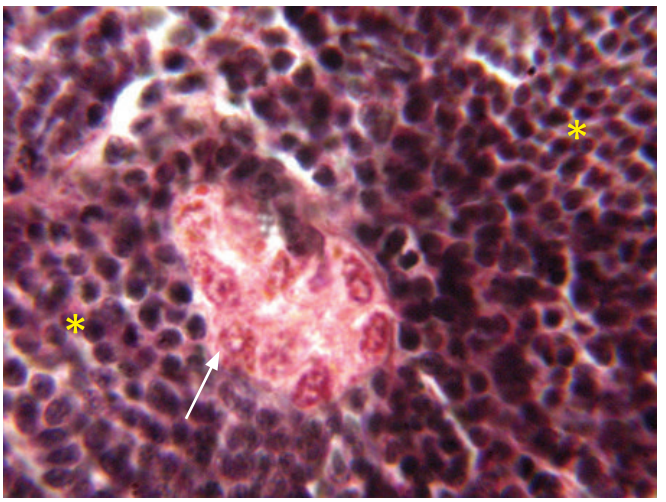


Fig. : 14.23 *Rutilus rutilus* (MT / HM)

This image shows a cluster of some interstitial cells (arrow) surrounded by closely-packed SERTOLI cells and spermatogonia (∗). The MASSON's trichrome poorly stains the steroid-containing vacuoles of the cytoplasm.

Like in the previous picture the testis has been removed after the breeding period. LEYDIG cells mainly secrete testosterone.

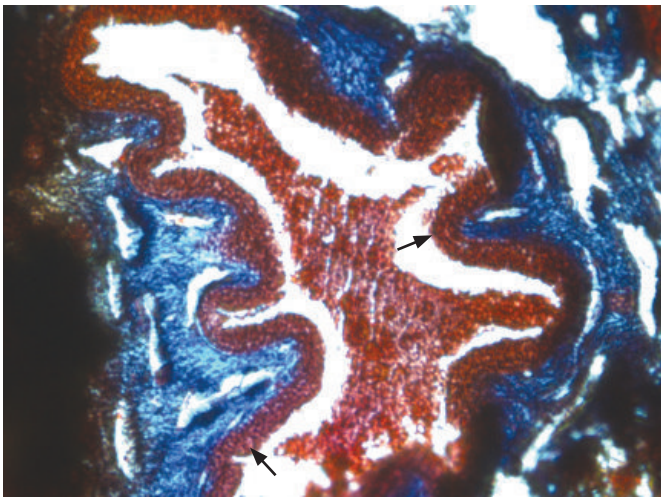


Fig. : 14.24 *Scyliorhinus canicula* (MT / MM)

Transverse section of a dogfish clasper organ. In a appreciable number of fish (some teleost families, Chondrichthyes) the fertilization is internal. Males thus have intromittent organs like the claspers (present figure) in elasmobranchs or *gonopodia* (see the three next images) in *Poecillidae* for instance.

In male Chondrichthyes the medial portion of the pelvic fin is modified into the clasper organ. Claspers also known as *myxopterygia* are paired organs supported by fin cartilages and that contain a groove along which sperm packets are conveyed into the female cloaca. Spermatozoa are ejected by muscular organs (siphon sacs) which use seawater to propel the sperm along the groove. This photomicrograph shows a cross section through the groove which is lined by a stratified cuboidal epithelium (arrows) supported by dense connective tissue (blue). Sharks (like *Scyliorhinus canicula*) with a primitive mode of reproduction are oviparous. Eggs are laid in the form of egg cases (made up keratin) which attach to algae or rocks.

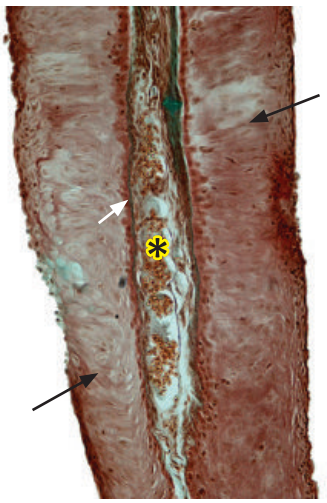


Fig. : 14.25 *Poecilia reticulata* (MT / MM)

Longitudinal section of the *gonopodium*. In the *Poeciliidae* for instance, the males have a *gonopodium* which is an intromittent organ developed from modified anal fin rays. It is a tube-like structure through which the sperm is ejected into the female's genital tract.

The epithelium (long arrows) covering the *gonopodium* is composed of several layers (stratified squamous epithelium). ∗ shows erythrocytes bathing in large central blood sinuses. The thick epithelium is supported by a basement membrane (short arrow).

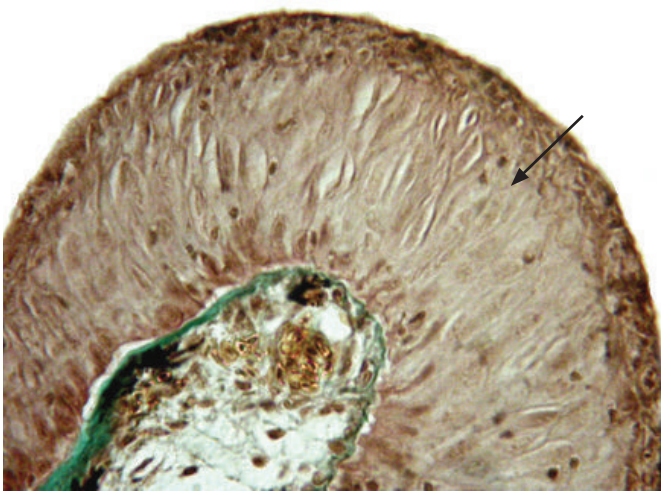


Fig. : 14.26 *Poecilia reticulata* (MT / HM)

Parasagittal section of the *gonopodium* showing the distal end. This micrograph shows the thick stratified squamous epithelium (arrow) which is similar to that covering the fins except for mucus-secreting cells. The *gonopodium* is formed of specialized rays at the front of the anal fin. At the centre of the image some collagenous fibers (in green) and erythrocytes (in orange) are seen.

A sagittal section at this level would have shown the genital pore through which *spermatozeugmata* (see Figs 14.7 & 14.8) are transferred into the female.

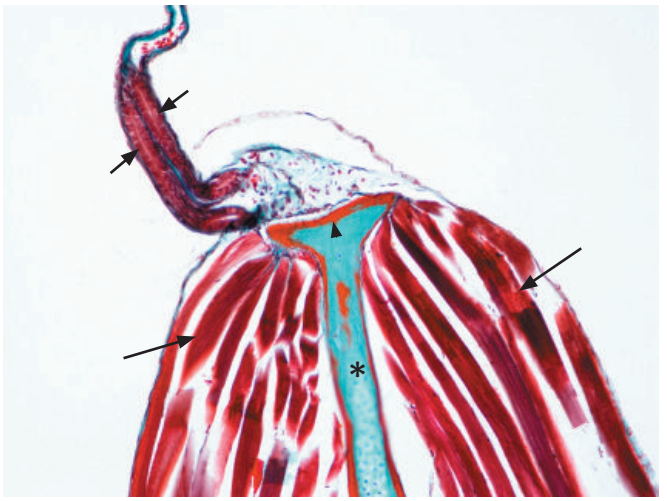


Fig. : 14.27 *Poecilia reticulata* (MT / MM)

Gonopodium musculature. *Gonopodia* of male teleosts are often elongated modifications of fin rays and are directed posteriorly. For copulation, the *gonopodium* swings forward and sperm bundles (*spermatozeugmata*) are directly transported into the female cloaca. *Gonopodium* is raised, or erected, by skeletal muscle at its base (long arrows). These muscular fibers (rhabdomyocytes) are often supported by skeletal elements (cartilage in turquoise – ∗ / bone in orange - arrowhead). On top the ureters (short arrows) are found.

In very few teleost species, female have a copulatory organ they insert into the male genital pore for receiving sperm.

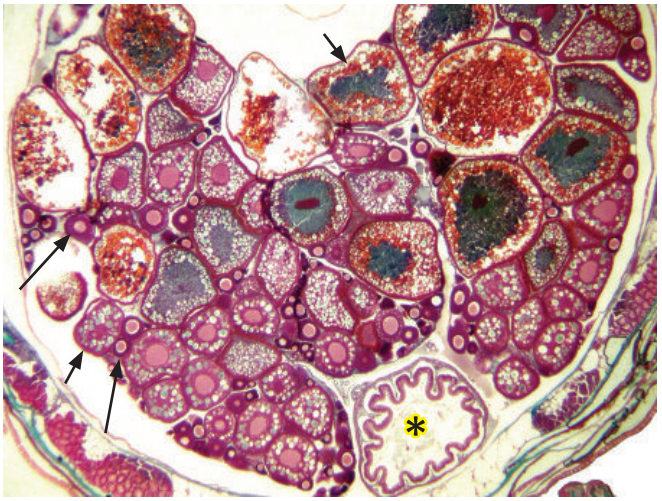


Fig. : 14.28 *Danio rerio* (MT / LM)

Ovary. In most teleost fishes the ovary is a hollow organ into which extend numerous longitudinal ovigerous folds lined by germinal epithelium. The germ cells multiply and transform into primary oocytes (previtellogenic follicles) which undergo vitellogenesis (vitellogenic follicles) when yolk is deposited in the cytoplasm. In addition to the previtellogenic and the vitellogenic phases, a mature oocyte phase can also be distinguished.

This low power view shows various oocyte developmental stages. The long arrows and short arrows point, respectively, to previtellogenic and vitellogenic oocytes.
On the bottom of the image one can see a transverse section through the posterior intestine (∗).

The next micrographs show nine views of beautiful oocytes at different stages of development. We do not resist the pleasure of showing you these fabulous brightly-coloured images !

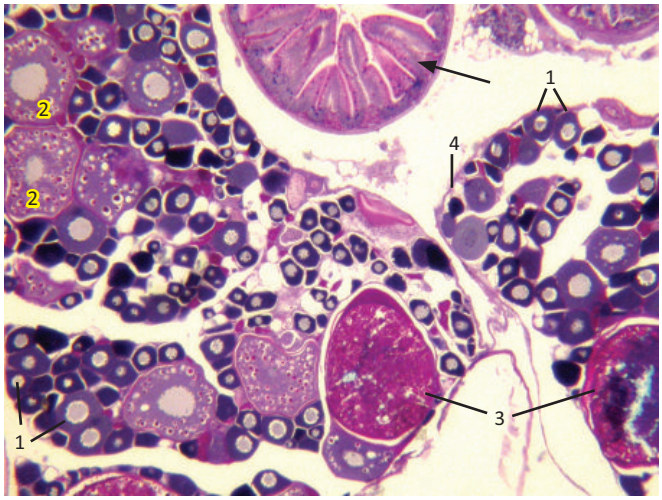


Fig. : 14.29 *Danio rerio* (AB-PAS-H / LM)

General view of the ovary. Fish ovaries are elongate structures oriented longitudinally within the abdominal cavity. This part of ovary contains dark previtellogenic oocytes (1), early vitellogenic oocytes (2) and two nearly mature oocytes (3). The arrow indicates a cross section through the small intestine.

Like the testes the ovaries are enclosed in a fibrous connective tissue called *tunica albuginea* (4).

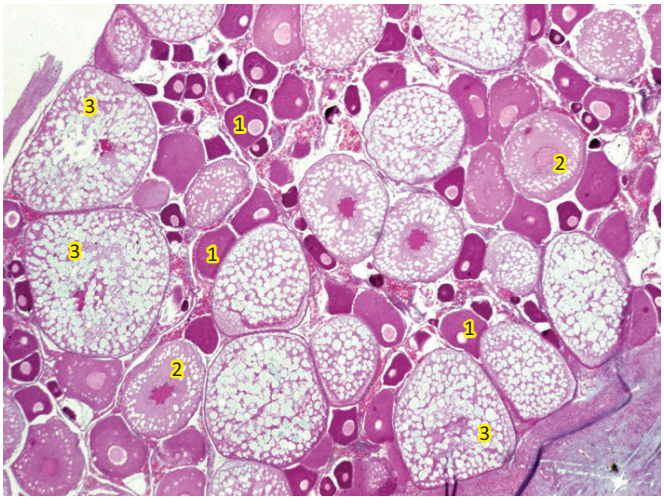


Fig. : 14.30 *Parachanna obscura* (H-E / LM)

Ovary of snakehead fish captured from the wild. This species is an asynchronous spawner and the gonads contain individual oocytes in three or more development stages. For a more detailed description of the ova development several stages can be defined according to major morphological characteristics of oocyte growth.

Stages I & II : oocytes located within the germinal epithelium ; respectively with lightly eosinophilic to basophilic cytoplasm.
Stage III : oocytes break from the germinal epithelium ; they are enveloped by a simple squamous follicular epithelium ; (provitelline) nucleoli appear at the periphery of the nucleus.
Stage IV (beginning of vitellogenesis) : appearance of yolk granules and fat vacuoles in the ooplasm ; a distinct chorion (= vitelline envelope) seats beneath the follicular epithelium.
Stage V : increasing of yolk vesicles which fill the entire ooplasm except beneath the chorion ; the nuclear envelope begins to degenerate and the nucleus migrates peripherally before the final maturation stage.

The previtellogenic oocytes display strongly basophilic ooplasms. Stage III oocytes (1) - stage IV oocytes (2) - stage V oocytes (3).

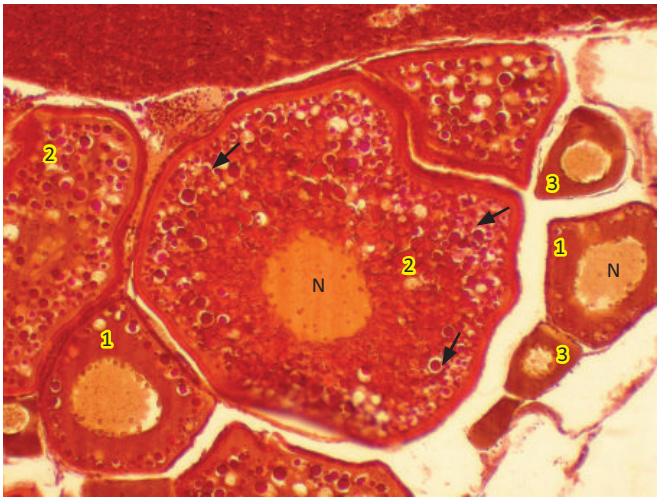


Fig. : 14.31 *Danio rerio* (PAS-H-OR / MM)

Ovary. Early (1) and late (2) stage IV vitellogenic oocytes with cortical alveoli and yolk granules (arrows). The apparition of yolk granules (proteins) and fat vacuoles in the ooplasm defines the vitellogenic oocytes which reach their maximum size at the end of this stage (stage V). The ooplasm begins to fill with yolk spheres, granules or globules. These structures maintain their integrity throughout the oocyte growth, without merging into a continuous mass of fluid yolk.

N: nucleus / 3 : stage III oocytes (previtellogenic).

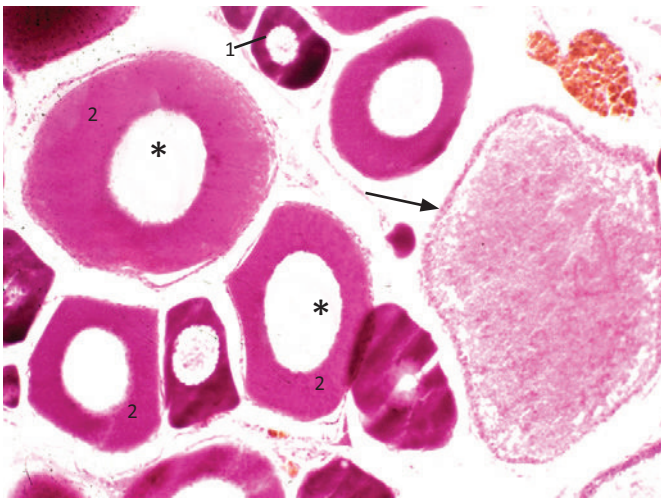


Fig. : 14.32 *Schilbe mystus* (MT / HM)

Part of ovigerous *lamellae* showing stages II (1) and III (2) oocytes of various sizes. The nuclei have not been preserved during the protocol (artefacts - ∗). The arrow indicates an atretic follicle and the liquefaction of yolk granules. Histological examination of ovarian tissue commonly reveals eggs at all stages of development and degeneration (atresia).

Follicular atresia is a common phenomenon in vertebrate ovaries and involves both the oocyte and the follicular wall degeneration.

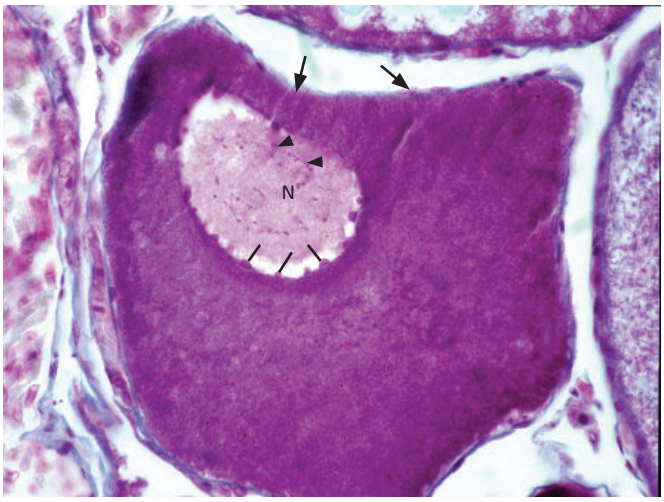


Fig. : 14.33 *Parachanna obscura* (PAS-H / HM)

Photomicrograph of a primary growth oocyte (stage III). Note the heavily basophilic cytoplasm (purple) around the germinal vesicle (nucleus - N). The latter contains numerous perinuclear nucleoli (thin lines).

Several lampbrush chromosomes (arrowheads) are apparent within the germinal vesicle. Lampbrush chromosomes are chromosomes which have begun to spread out and which form numerous symmetrical loops. The oocyte is surrounded by a single layer of squamous follicle cells (*zona granulosa* - arrows).

During the previtellogenic phase, the oocytes show homogeneous strong basophilia of the cytoplasm. The nuclei may contain many (up to 20) prominent nucleoli, often next to the nuclear membrane.

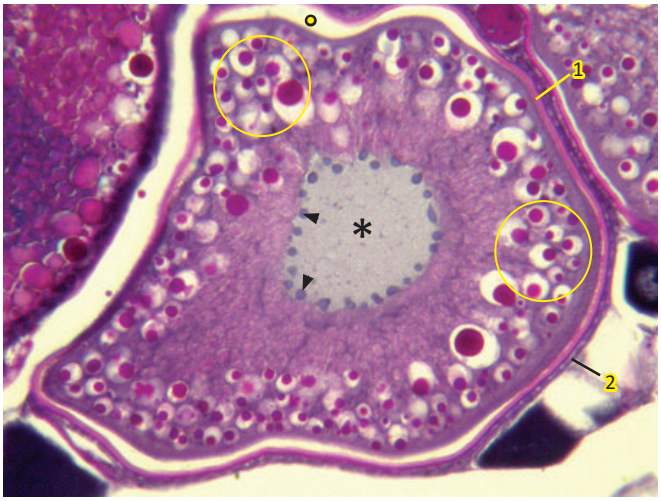


Fig. : 14.34 *Danio rerio* (AB-PAS-H / HM)

Early or mid-vitellogenic oocyte (stage IV). Young yolk globules (circles) are abundant in the peripheral ooplasm. The formation of the globules proceeds by and by towards the inner part of the cytoplasm. These yolk globules are stained magenta with the PAS method. The process of yolk deposition in oocytes is called vitellogenesis.

The centrally located germinal vesicle (∗) displays a large number of peripheral nucleoli (arrowheads) just beneath the nucleus envelope. This nucleoli multiplication is necessary for the formation of a population of ribosomes sufficiently large to last during the cleavage period.

The vitelline envelope or chorion (also called *zona radiata* - 1) is stained magenta and is surrounded by squamous follicle cells (*zona granulosa* - 2).

• : artefact = space between ooplasm and chorion.

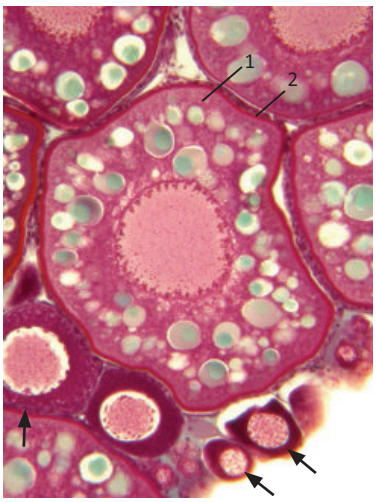


Fig. : 14.35 *Danio rerio* (MT / MM)

Zebrafish ovary showing previtellogenic oocytes (arrows) and, in the center of the picture, a stage IV oocyte (early vitellogenic) with yolk granules (green and pinkish) and a centrally located germinal vesicle containing peripheral nucleoli (red).

Under the light microscope, yolk appears as a fluid in which globules and granules of various sizes are dispersed.

1 : peripheral ooplasm - 2 : vitelline envelope.

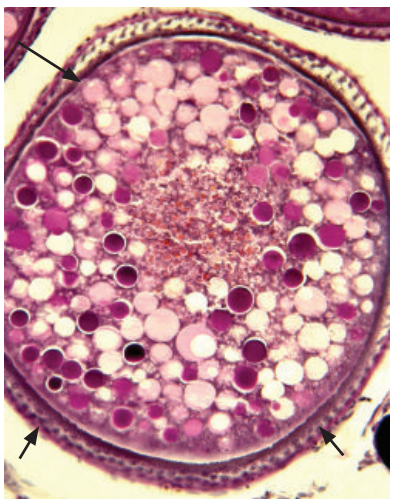


Fig. : 14.36 *Perca fluviatilis* (PAS-H / MM)

Large vitellogenic oocyte (stage V). At this stage, yolk granules (magenta) and yolk vacuoles (pink or white) have invaded almost all the ooplasm. The oocyte is surrounded by a well-developed *zona radiata* (long arrow) and the follicular epithelium is obvious (short arrows). The germinal vesicle has broken down.

In some fish, ovulation and spawning occur almost at the same time, whereas in others (*Salmonidae*) ovulated oocytes are retained in the peritoneal cavity and spawning takes place later.

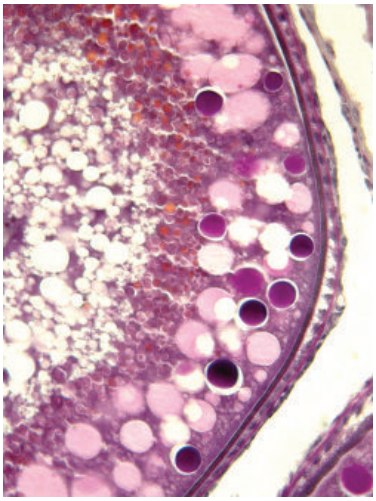


Fig. : 14.37 *Perca fluviatilis* (PAS-H / HM)

Vitellogenic oocyte (stage V). The cytoplasm is filled with yolk spheres, granules or vacuoles shown by wine staining with the PAS method. Oil droplets or globules (unstained) reside in the more central ooplasm. Oocytes are surrounded by an outer thecal layer and an inner *granulosa* layer. Here the *granulosa* and thecal cells are tightly stretched and flattened making these cell layers, particularly the *theca*, difficult to identify.

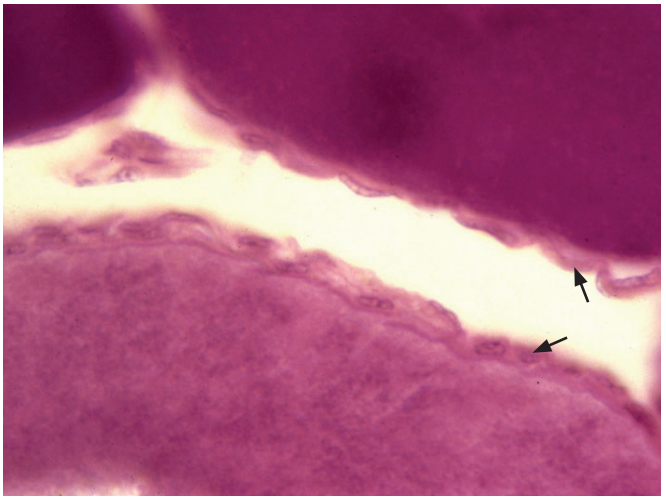


Fig. : 14.38 *Parachanna obscura* (H-E / IM)

Primary growth phase follicles (stage III). Detail of the follicular wall. The arrows point to the flattened *granulosa* cell layer. A distinct chorion (= vitelline envelope) does not appear yet at this previtellogenic stage.

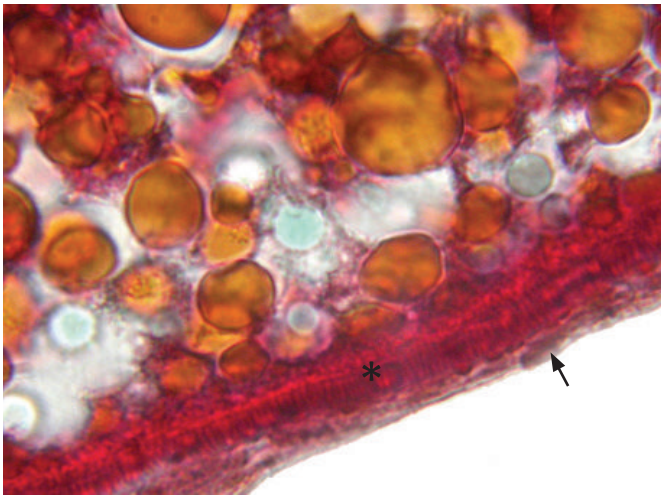


Fig. : 14.39 *Danio rerio* (MT / IM)

Part of oocyte with plenty of various-sized yolk globules. The arrow indicates an elongated follicular cell. At the end of vitellogenesis ovaries are packed with yolky oocytes which undergo maturation and ovulation under favourable conditions.

During growth of the ovarian follicle, the teleost oocyte becomes surrounded by an acellular coat, the vitelline envelope, or *zona radiata*, consisting of some major proteins. In this micrograph this coat is obvious (∗).

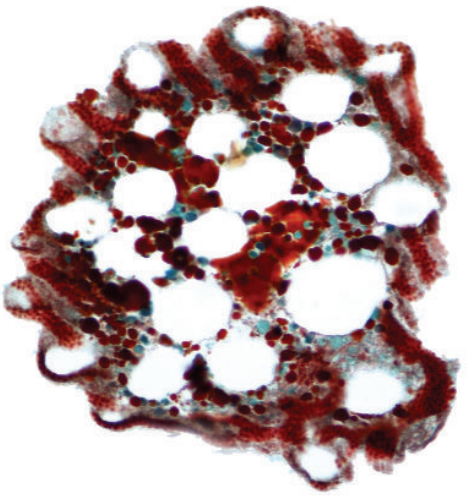


Fig. : 14.40 *Poecilia reticulata* (MT / MM)

Isolated yolk granules and droplets coming from an embryo's yolk sac content (pregnant female - remember that guppys are ovoviviparous fish). The yolk sac is an extraembryonic membrane which encloses the yolk and plays major roles (nutrition and respiration) in the development of the embryo. The latter receives digested yolk via the the vitelline blood vessels developed in the mesoderm of the yolk sac.

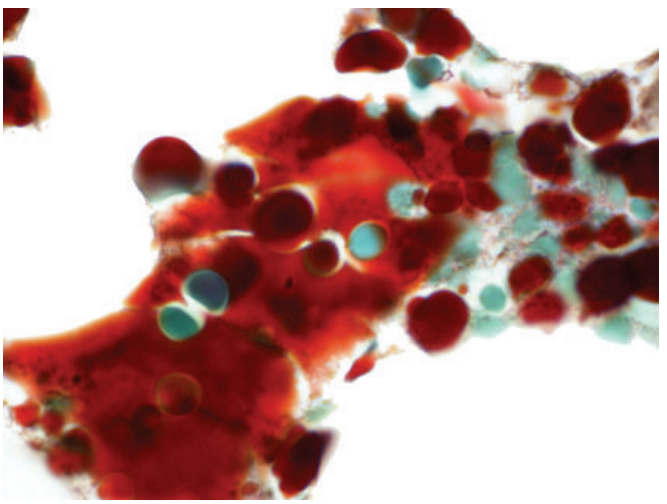


Fig. : 14.41 *Poecilia reticulata* (MT / HM)

Isolated yolk granules and droplets coming from an embryo's yolk sac content (pregnant female). Yolk is a global term for several chemical substances stored in the ooplasm. Its main constituents are proteins (lipovitellin and phosphovitin which form the membrane-bound yolk platelets), fats (phospholipids, triacylglycerols... in droplet form) and few carbohydrates (glycogen granules).

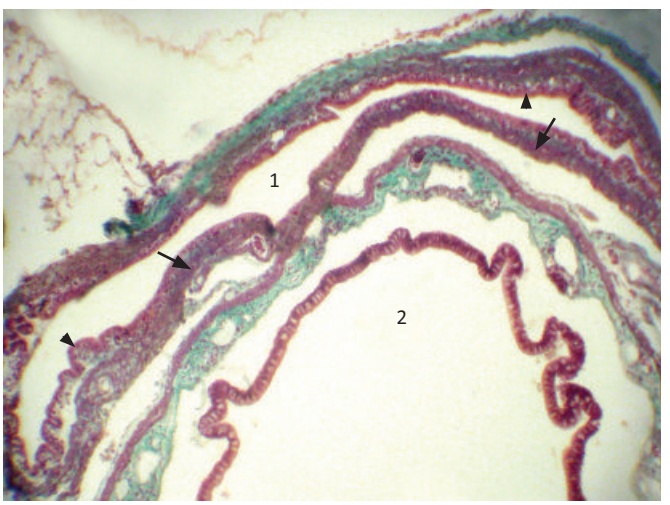


Fig. : 14.42 *Xiphophorus helleri* (MT / MM)

Oviduct. An oviduct (1), located above the rectum (2), generally leeds the eggs to the outside via an exit (genital pore) situated between the anus and the urinary pore. Ripe ova are released into the cavity of the ovary which is continuous with the simple ciliated oviduct epithelium (arrowheads). The oviduct wall is rich in muscular fibers (arrows). Collagen fibers are in green.

15

Sensory system and communication

Teleosts have various sense organs like an elaborate *linea lateralis* system, olfactory organs, taste buds, eyes, membranous labyrinth (inner ear), Weberian ossicles, electric organs.

AUDITORY AND LATERAL LINE SYSTEM

Inner ear

The inner ear in fishes is typical of that of other vertebrates, having three semicircular canals (oriented in one horizontal and two vertical perpendicular planes) and three otolith chambers or otolith organs : the *utriculus*, the *sacculus* and the *lagena* (Figs 15.1 to 15.3).

In the semicircular canals lined with simple squamous epithelium, local expansions named *ampullae* house sensory structures : the *cristae ampullaris* (Figs 15.10 to 15.14).

Each of the otolithic sacs contains a single hard, stony structure that is about three times denser than the rest of the fish body. These otoliths (Figs 15.3 to 15.7), the *lapillus* in the *utriculus*, the *sagitta* in the *sacculus*, and the *asteriscus* in the *lagena*, lie close to a sensory epithelium, named the *macula*. *Maculae* are patch-like collections of sensory hair cells and supporting cells (Figs 15.8 & 15.10). Like the semicircular canals, the otolithic sacs are filled with endolymph.

There are many aspects of the structures of the fish ear that vary between fish groups. Among these variations are the size and shape of the semicircular canals and of the otolith chambers. There is interspecific and intraspecific otolith variability (mainly the *sagitta* and the *asteriscus*) and the distribution of different types of sensory hair cells can also vary.

Fishes in general lack the various accessory devices by which in higher vertebrates sound waves reach the internal ear. However, the conduction of vibrations through the head skeleton to the internal ear can produce some degree of hearing (lateral line sensations are also closely allied to hearing). In most fishes water vibrations, to be perceived, must set up head vibrations, and these in turn produce endolymphatic vibrations which can be picked up by the hair cells of the inner ear.

Some bony fishes, however, have accessory structures which parallel in a way the "hearing aids" found in tetrapods, although evolved quite independently. In these fishes the gas bladder is utilized as a device for the reception of vibrations. In herring-like teleosts this gas bladder sends forward a tubular extension which comes to lie along side part of either membranous labyrinth and can thus induce vibrations in the endolymph. In a group of teleosts termed *Ostariophysi*, which includes the Siluriformes, Cypriniformes, Characiformes... in which hearing is well developed, another method is used. Neural processes of the four most anterior vertebrae are detached and develop independently to form the Weberian ossicles. These articulate in series to form a rigid connection between the anterior chamber of the gas bladder and the pars inferior (*sacculus-lagena* complex) of the stato-acoustic organ. The ossicles operate somewhat in the same way as the ear bones of a mammal, transmitting vibrations from the gas bladder to the liquids of the internal ear.

Lateral line organ

In fishes, as well as in larval amphibia, and some aquatic adult amphibia, a very complex sensory system exists, very polymorphic from the anatomical point of view, and generally known as the lateral line system (Figs 15.15 to 15.21). This term alludes to the existence in many fishes of a lateral line of sense organs on both sides of the trunk (Figs 15.17 to 15.19). However, the head region (Figs 15.15 & 15.16) is largely supplied with sensory organs of the

same kind; therefore the term «lateral system» must be accepted in a larger sense to designate the whole array of nerve endings belonging to this extensive group.

When the histological and the cytological aspects of the lateral line sense organs are examined, it appears that in the majority of them we find a typical structure : the neuromast.

The neuromasts (Figs 15.18 to 15.21) comprise the sensory hair cells interspersed with supporting cells, covered by the *cupula* extending into the water, and enclosed in canals lined by a squamous epithelium containing mucous cells (Fig. 15.21). Generally, the canals on the head consist of bony grooves covered or not by the skin (Figs 15.15 & 15.16), whereas the (usually single) trunk canal, stretching from behind the *operculum* to the tail, is formed by scales. In addition free sense organs are distributed more randomly.

As to its function, the lateral line system is generally considered as being able to detect delicate water movements.

CHEMORECEPTION

The distinction between smell (olfaction) and taste (gustation) is less clear in water than on land. It is generally accepted that olfaction is distance reception and gustation contact reception but in fishes it is possible for the taste organs to respond to distant sources of stimuli. But since both taste and smell require actual contact between a specific receptor cell and a dissolved chemical substance, they are merely different degrees of the same sense, called chemoreception. In fishes, taste receptors are not restricted to the mouth, as in terrestrial animals, but are found on the gills, the surface of the head, the fins, the barbels...

Taste buds (Figs 15.22 to 15.30) are typically ovoid or pear-shaped structures and vary between 30 and 80 μm in height and between 20 and 50 μm in width, with their longitudinal axis oriented vertically to the epidermal surface. They can be found elevated on epidermal «hillocks» (Figs 15.26 to 15.28), flush with the surrounding epidermis or sunken. They contain receptor or gustatory cells with apical *microvilli* and supporting cells innervated by cranial nerves (VII, IX and X). Unlike the neuromasts, the sensory cells stretch throughout the whole height of the organ. Solitary chemosensory cells also exist.

The olfactory organ. In sharks, the olfactory organs or olfactory rosettes (Figs 15.31 to 15.34) are highly developed. They are paired structures located in capsules on the ventral side of the snout, each covered by a simple flap of skin. They are generally spherical and composed of a series of (primary) *lamellae* and their secondary folds studded with numerous chemoreceptors. This arrangement greatly increases the surface area in contact with the water and thus the sensitivity. The *lamellae* are lined by an epithelium composed of sensory elements (ciliated, microvillar, and rod-like cells), as well as glandular and supporting cells. In the *center* of the *lamellae* is a central core, filled with loose vascularized connective tissue.

In teleosts, the olfactory organ can greatly differ according to the groups : it can be a simple, nasal tube with no or few *lamellae* (*Belonidae, Ammoditidae, Scombresocidae, Poeciliidae* - Figs 15.36 & 15.37) or a highly complex epithelial tissue with multiple folds (rosettes - Figs 15.38 to 15.43) and cell types. It is usually paired and contains many thousands of receptor cells located in the olfactory epithelium on the fish's rostrum.

The olfactory epithelium is a complex tissue composed of sensory and nonsensory elements. The nonsensory elements are ciliated and nonciliated cells, and underlying basal cells, above the basement membrane. Goblet cells are also found (Fig. 15.36). Nonsensory ciliated cells are columnar in shape and their motile *cilia* serve to ventilate the olfactory organ. The sensory epithelium is columnar, pseudostratified, and consists of receptor cells, either with several apical *cilia* or numerous *microvilli*, separated by supporting cells.

ELECTRIC ORGANS AND ELECTRORECEPTION

Electric organs

Fishes are the only animals that are sometimes equipped with organs specifically adapted to generate an electric discharge.

First of all, the following concepts must be remembered. The vast majority of fishes are non-electroreceptive animals : specialized receptors and electric organs are absent.

Electroreceptive fishes normally use electrical signals present in the environment, thus specialized receptors are present. Among these both non-electric and electric fishes are to be considered. In the former, electric organs are absent but passive electroreception is present (catfishes, polypterids); in the latter specialized electric organs and active electroreception are present (*Gymnarchidae, Mormyridae, Malapteruridae, Torpedinidae*).

With the exception of the freshwater sternachids, where the electric organs are modified motor neuron terminals, fish electric organs are modified striated muscle fibers (rhabdomyocytes - see chapter 3).

Location of the electric organs varies in different animals. In *Torpedo* (marine ray) they lie on either side of the head, between the gills and the anterior part of the pectoral fin. In electric eels, the organ extends laterally along each side from the trunk to the end of the tail. In *Raja*, *Gnathonemus* (Fig. 15.44) and *Gymnarchus* they are confined to the tail region. More unusually in African catfishes (*Malapterurus*) they are situated between the skin and muscles along the whole length of the body and in *Astroscopus* they are placed behind the eyes, in the form of patches, one on each side.

Typically each electric organ consists of a large number of columns held together by connective tissue (Figs 15.44 to 15.48). They may dispose themselves vertically from ventral to dorsal surface as in the marine ray (*Torpedo*) or may extend longitudinally from the tail to the head as in the freshwater eel (*Electrophorus*). Each column comprises a variable number of polynucleated, flattened and elongated cells called electrocytes, whose cytoplasm is filled with glycogen. All the cells of a column are stacked together in a manner that the nervous sides of all the electrocytes face the same direction. A vascular gelatinous material of mesodermal origin fills the gaps between the adjacent electrocytes. Electrocytes vary greatly in morphology but most are disc like cells with a smooth innervated face and a papiliform non-innervated face. In the electric ray *Torpedo*, there are over 1000 electrocytes in a column and 500-1000 columns per organ. In the electric eel *Electrophorus* there are about 60 columns on each side, with approximately 10,000 electrocytes per column. The electric organ of *Electrophorus* can generate a discharge of over 500 volts.

Electroreceptor organs

Electroreceptors (Fig. 15.54) occur in the teleost families of gymnotids (South America) and mormyrids (Africa), which use electrical impulses for orientation. The brain of these African fishes is remarkable by the monstrous development of the *valvula cerebelli* ("*mormyrocerebellum*"), which extends forward and backward so as to cover the dorsal encephalon. To this extraordinary hypertrophy of all centers in relation with the lateral line system, corresponds the existence of remarkable sensory endings in the skin, innervated by the thickly myelinated fibers of the lateral nerves. In adult mormyrid teleost fish, three types of electroreceptors are found in the specialized electroreceptive epithelium : the ampullary organs, the mormyromasts and the tuberous organs (knollenorgans). With this electroreceptive system, these fish are capable of recognizing congeners, predators and objects of varying conductivity.

Ampullary organs have their role in the sensing of low-frequency signals generated by a variety of biological and non-biological sources. Knollenorgans have their major role in electrocommunication (emission and detection of electric organ discharges of other fish, and of

their own species), whereas mormyromasts are active in electrolocation. Afferent nervous fibers from the three electroreceptors project into different parts of the *medulla*.

The ampullary organ (Figs 15.49 & 15.50) consists of a cavity extended by a canal which crosses the epidermis. This canal is generally obturated by an amorphous mucinous jelly-plug (polysaccharides). Multiple sensory cells are located at the base of the canal and are surrounded by different types of accessory supporting cells.

The mormyromasts (Fig. 15.51) have an intraepidermal cavity filled with acid polysaccharides and possess two sensory cell classes (type A and B) distinguished by morphology and location. It is the most abundant type of electroreceptors, mainly present in the epidermis of the mouth.

The tuberous organs or «Knollenorgans» (Figs 15.52 & 15.53) are composed of a variable number of modified epithelial cells which are sensitive to electric currents. Each sensory cell (neuron) is enclosed in a separate cavity and projects to the electrosensory lateral line lobe of the *medulla* via the lateral line nerve. There are also various supporting cells. The organs are embedded in the thickened epidermis and protrude into the dermis. The sense organ is surrounded by a basement membrane. «Knollenorgans» lack the jelly filled canal leading from sensory receptor cells to the external environment.

The *ampullae* of LORENZINI (Fig. 15.55) are special sensitive electroreceptors on the rostral part of the head in skates, rays, and sharks. They are jelly-filled canals opening to the skin surface by a pore plainly visible as a dark spot. The canals end in a cluster of small vesicles filled with special jelly enclosed in capsules of collagenous connective tissue. The deeper part of the *ampullae* is lined by a sensory epithelium containing thermo- and electroreceptive cells. Supporting elements are also found.

Each *ampulla* is innervated by a small bundle of fibers of the facial nerve (VII). The canal lengths vary from animal to animal, but the electroreceptor pores' distribution seems to be species-specific. The *ampullae* of LORENZINI can detect electrical fields generated by moving animals (prey detection in a sandy sea-bottom) and the Earth's magnetic fields thus helping their orientation during migrations.

VISION

The organization of the fish *eye* (Figs 15.56, 15.57 & 15.60) has the same general structure found in higher vertebrates. Eyes are large and round with a flattened corneal surface and no eyelids (but sharks can have elaborate eyelids : some of them can close their eyes completely and others have a third eyelid-nictitating membrane which protects the eyes when biting prey). Some deep-water species have disproportionately large eyes to optimize the limited light available, while in others, like cave fish, development of the optic primordia is arrested and subsequently degenerates.

Each *eye* is composed of three concentric layers : an external layer that consists of the sclera and the transparent cornea, a medial uveal layer, and an inner layer of nerve tissue, the retina. The additional components of the eyeball are the aqueous humor, the vitreous body, and the lens that are described as the refractile media of the *eye*.

Lens. The teleost lens (Figs 15.56, 15.58 to 15.59) is completely round and protrudes into the aqueous chamber almost to the cornea, providing a wide field of vision. The lens is an avascular tissue and is made up of an extracellular matrix (capsule) secreted by the cells contained within the lens. Lens cells are organized into two contiguous but morphologically very distinct cell sub-populations. The lens cells facing the anterior chamber of the *eye* are a monolayer of epithelial cells. The lens cells facing the posterior chamber, bathed in vitreous humor, are extremely elongated into fibers. The main function of the lens is, in combination with the cornea, to transmit and focus the incoming light onto the retina. Due to its rigidity, visual accommodation is accomplished not by deflecting the shape of the lens but by moving

it forward and backward via retractor muscles and suspensory ligaments.

Sclero-Corneal layer. The outermost layer of the *eye* is divided into an external cornea and an internal sclera. The cornea (Fig. 15.57) consists of an unpigmented squamous epithelium, a membranous stroma, and a thin endothelium. The corneal epithelium is multilayered and contiguous with the integument of the head. The wall of the posterior portion of the *eye* is made up of the thick fibroblastic sclera which is contiguous with the corneal stroma. While the cornea is unpigmented and therefore transparent, the sclera is non-refractile with an external sheath of hyaline cartilage in most fish (Fig. 15.64).

Uveal tract. The middle layer of the *eye* is composed of the choroid and the iris. The choroid is composed of three layers : a connective layer adjacent to the sclerotic, a *lamina* vasculosa with large blood vessels, and a third layer, the *lamina* choriocapillaris, rich in small vessels and adjacent to the retina. Generally, the choroid *rete*, surrounding the optic nerve, is a well developed and intricate array of arterioles and capillaries forming a *rete mirabile*. This organ is designed to satisfy the high oxygen demands of the poorly vascularized retina. The iris (Figs H, I and 15.56), a continuation of the choroid, projects in a thin layer over the anterior surface of the lens which often protrudes into the aqueous chamber. The iris separates aqueous and vitrous chambers with its leading edge defining the pupil.

The Retina. The retinal tunic (Figs 15.60 to 15.64) is composed of ten distinct layers. 1) The pigment epithelium, closely apposed to the choroid; 2) the elongated tips of cones and rods; 3) the external limiting membrane; 4) the outer nuclear zone containing the cell bodies and nuclei of the photoreceptors; 5) the outer plexiform layer; 6) the inner nuclear layer is that of the bipolar cells which transmit the impulses inward from the photoreceptors, and of associative glial cells; 7) the inner plexiform layer, a region of synapse formation; 8) the nuclei of the ganglion cell layer which pick up the stimuli from the bipolar elements and send fibers (9 - nerve fiber layer) along the optic nerve (Figs 15.56, 15.65 & 15.66) to the brain; 10) the inner limiting membrane composed of the expanded processes of glial cells.

Most fishes have a duplex retina, i.e. one containing both rods and cones. Both these visual cells share a common plan : an outer segment containing photosensitive pigment (rhodopsin or porphyropsin), an ellipsoid packed with mitochondria, an extensive myoid or foot-piece and a nuclear region. The rods and cones are held in position by the external limiting membrane. The rod cells are particularly longer (about 100 μm) and thinner with cylindrical outer segments and ellipsoids while in cones the outer segment is conical, and the ellipsoid rather bulbous. The ellipsoids and outer segments are connected by a ciliary neck with the characteristic nine filaments. Rods mediate the detection of light during dim photic conditions while different populations of cones are sensitive to the red, green or blue regions of the visible spectrum. The retina of salmonids also possesses specialised cones that are maximally sensitive to ultraviolet light, and this may also be true for other species. Twin cones are common in many bony fish. The ratio of these photo-receptive cells varies considerably between fish species, mainly in accordance with their habitat. For instance, most deep-sea fishes have retina only with rods.

The duplex retinae of many fishes have regions of specialization. For example, an *area temporalis* consists of a patch of closely packed cones (with few, or no rods) appropriately placed to receive light along the main axis of feeding, where light acuity would be an advantage. In pelagic feeders like herring, looking upwards and forwards to find food, the area is posteroventral on the retina. In horizontally feeding species such as the sailfish (*Istiophorus*), it is posterior on the retina, and in bottom feeders like the sea bream (*Sparus*), the area is posterodorsally located. The amphibious mudskipper (*Periophthalmus*) has a horizontal band of cones placed to perceive objects near ground level where both food and predators might be present. A *fovea* or depression with a high density of cones (as in many vertebrates) is pre-

sent in seahorses (*Hippocampus*) and pipefish (*Syngnathus*).

A fairly common special structure, the *tapetum lucidum* is located beneath the photoreceptors. It reflects light, thus making the «eyeshine» phenomenon common in many fish and carnivorous mammals. Its purpose is to enhance the light sensitivity of the eye. The position and the quality of the *tapetum* vary considerably between different species. Some species harbor a *tapetum* in the choroid, whereas in others, it is associated with the retinal pigment epithelium.

Remark: as one cannot be specialized in all organs and tissues, this last and very complex chapter may contain some errors or approximations. If so, do not hesitate to contact the authors to share your knowledge about these fabulous organs.

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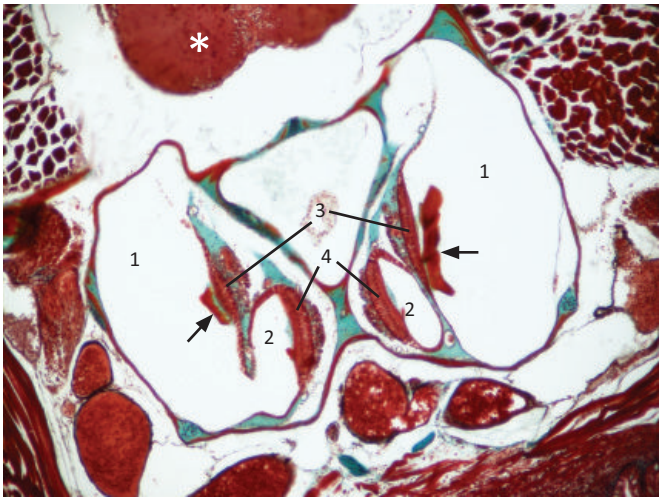


Fig. : 15.1 *Danio rerio* (MT / MM)

The inner ear (membranous labyrinth) in fishes is typical of that found in other vertebrates. Each half of the labyrinth consists of three semicircular canals (see Figs 15.10 to 15.14) and three otolith chambers (sac-like structures) : the *utriculus*, the *sacculus* and the *lagena*. All these sacs and canals are filled with endolymph.

Maculae are large flattened areas of sensory epithelium lying on the ventral surface of the chambers. *Maculae* are in contact with suspended compact structures, the otoliths. Each otolith chamber has its own otolith : the *lapillus* (otolith of the *utriculus*), the *sagitta* (otolith of the *sacculus*) and the *asteriscus* (otolith of the *lagena*).

This general view is a transverse section through the ventral part of the labyrinth showing the *lagena* (1) and the *sacculus* (2) with their respective sensory *maculae*, the *macula lagenae* (3) and the *macula sacculi* (4). The *macula lagenae* is covered by the *asteriscus* (arrows). The *sagittae* are not visible. ∗ : myelencephalon.

In *Danio rerio* as well as in most *Ostariophysi*, the *asteriscus* presents its greatest variability and development and is considerably larger than the *sagitta.*

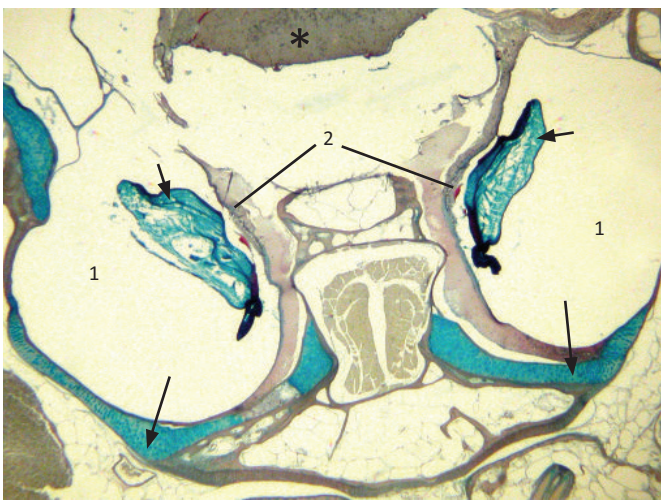


Fig. : 15.2 *Astatotilapia burtoni* (AB-PAS-H / MM)

Transverse section through the ventral part of the labyrinth showing the *sacculi* (otolith chambers - 1). The *sagittae* (the largest and most variable otoliths in non-ostariophysian fishes – short arrows) are suspended above the *maculae sagittae* (2). The long arrows point to cranium cartilage.

∗ : myelencephalon

Note : as we are not experts in otolith morphology, please refer to specialized literature for a reliable identification of the otoliths showed in the concerned figures.

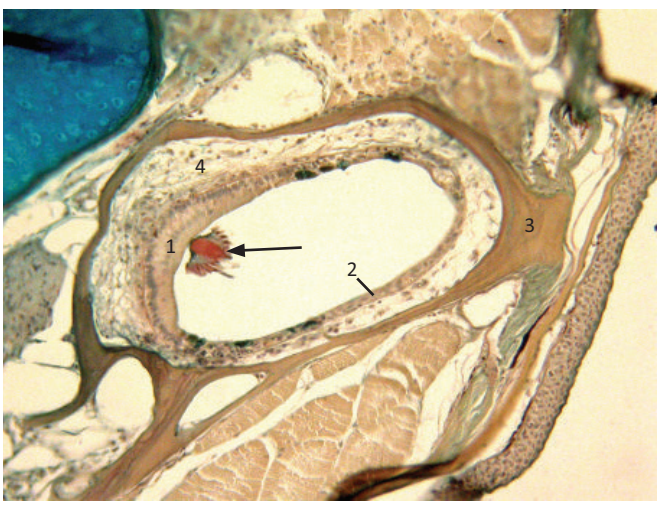


Fig. : 15.3 *Haplochromis multicolor* (AB-PAS-H / MM)

Transverse section through an otolith chamber. The arrow indicates the otolith overlying the sensory epithelium (*macula* - 1).

The otholith chambers are lined with squamous epithelium (2) and filled with endolymph.

3 : osseous labyrinth - 4 : fine connective strands running between the membranous and osseous labyrinths.

The present otolith could be the *asteriscus* which is small and shaped like a flattened hemisphere in non-ostariophysan fishes. It is highly variable morphologically and may have a crenelated aspect (like here) in some species.

Fig. : 15.4 *Haplochromis multicolor* (AB-PAS-H / HM)
Higher magnification of the previous image showing the crenelated aspect of the otolith and its relationship with the sensory epithelium (*macula*). The *macula*, in association with the auditory nerve VIII consists of sensory hair cells (1) surrounded by *microvilli*-covered supporting cells (2). ∗ indicates the endolymph of the otolith chamber.

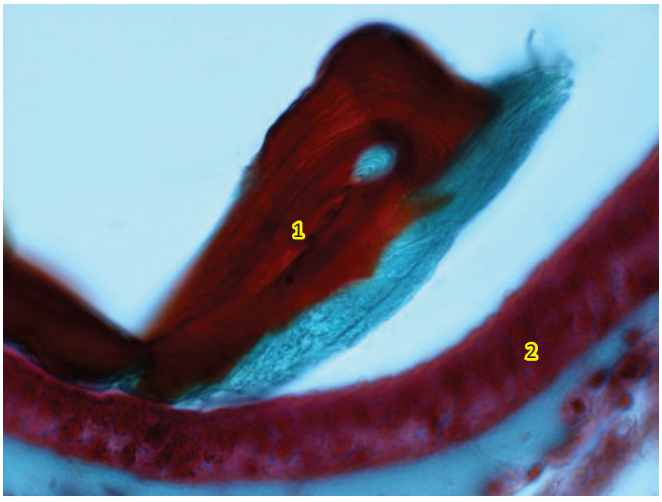


Fig. : 15.5 *Danio rerio* (MT / HM)

An otolith (1) linked to the sensory *macula* (2) by a thin otolithic membrane connecting the two structures.

Otoliths are free, compact, non bony structures composed of aragonite (a common calcium carbonate) deposited in a protein matrix. Three pairs are present in the labyrinths of each fish. Otoliths vary tremendously in shape and size in different species of fishes. The present one is a large otolith and is (probably) the *asteriscus* which presents its maximal development and variability in the *Ostariophysi*.

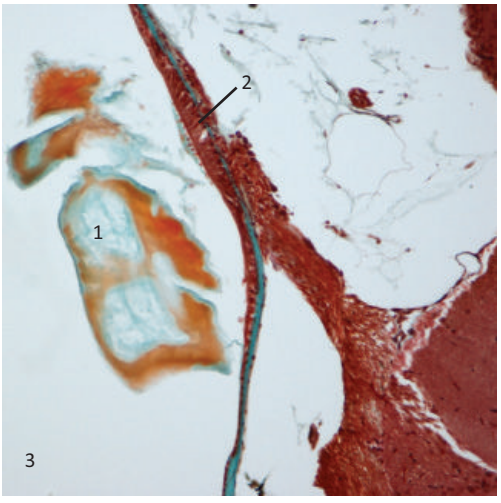


Fig. : 15.6 *Poecilia reticulata* (MT / MM)

In non-Ostariophysian fishes the saccular otolith (*sagitta* - 1) is generally the most developed. It is often larger than the *macula* (2). *Sagittae* are generally oval and laterally flattened and are major receptors of sound in fish. (3) indicates the *sacculus*, an otolith chamber which belongs to the ventral part of the labyrinth. Otoliths are usually strongly stained.

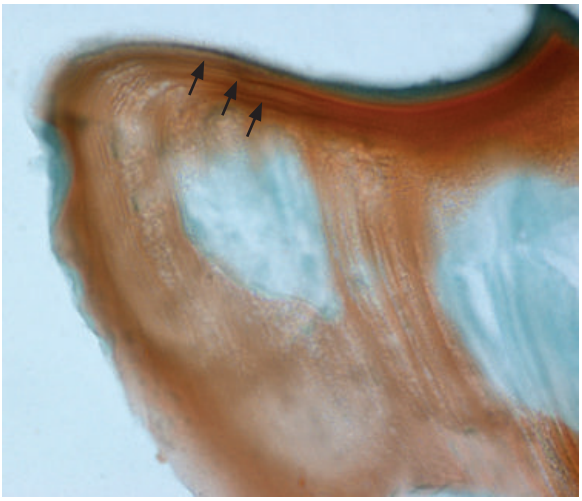


Fig. : 15.7 *Poecilia reticulata* (MT / IM)

Otolith formation involves rhythmical variations in the deposition and size of organic matrix fibers and aragonite crystals, resulting in the formation of zonations. Such zonations are shown here (arrows). By measuring the thickness of individual rings it is thus possible to obtain information on the age (daily and annual increments) of some teleost fishes by using a microscope.

Remember that fish never really stop growing, though growth rate in adult fish is very reduced.

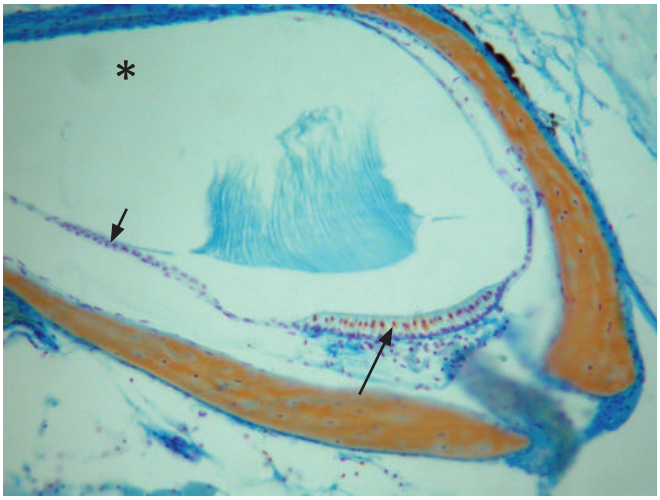


Fig. : 15.8 *Gnathonemus petersii* (FR-HB / MM)

Otolith chambers (lower labyrinth) are lined with squamous epithelium (short arrow) and filled with endolymph (∗). The parts of the lower labyrinth (*pars inferior*) each contain a sensory *macula* (long arrow). The *maculae* respond to sound, gravity and linear accelerations of the fish body. Cellular bone is stained in orange.

Fig. : 15.9 *Poecilia reticulata* (α–HSP70 Ab/ HM)

Lower labyrinth wall bearing an oval sensory *macula* consisting of sensory hair cells and supporting cells. The sensory cells of the *maculae* are similar to the neuromasts of the lateral line system (see Figs 15.18 to 15.21). The hair bundle on the apical surface of the hair cells is indicated by arrows.

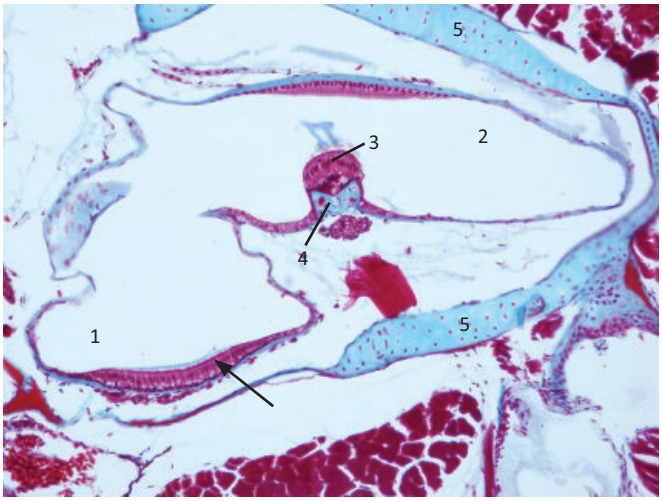


Fig. : 15.10 *Poecilia reticulata* (MT / MM)

The utricular part of the labyrinth is formed by the *utriculus* itself (1) on which are implanted three semicircular canals, having at one end an ampullar dilatation (here the *ampulla* of the horizontal canal - 2). Each *ampulla* contains a sensory area, usually elevated called the *crista ampullaris* (3). Each *crista ampullaris* is an elongated epithelium situated on a ridge of supporting tissue (4) rising from the wall of the *ampulla*. The arrow indicates the *macula* of the *utriculus* (*lapillus* not visible). Hyaline cartilage (5) surrounds the semicircular canal.

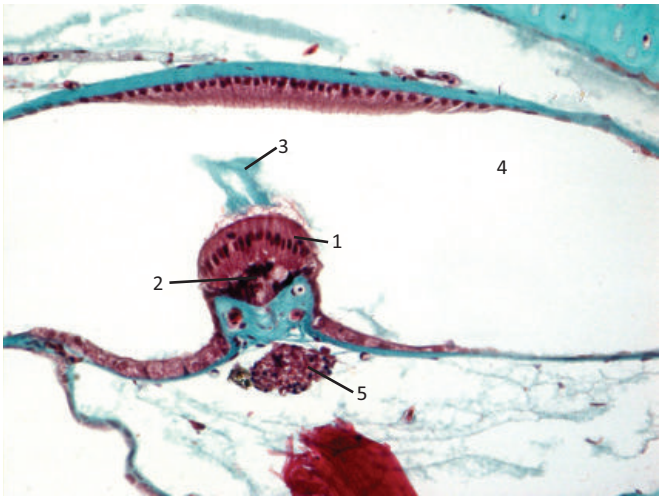


Fig. : 15.11 *Poecilia reticulata* (MT / HM)

Section through a semicircular canal in the inner ear of the guppy. This photomicrograph shows the main components of the *crista ampullaris*. It is covered by neuroepithelium, with hair cells (1) and supporting cells (2). From this sensory organ rises a gelatinous cupula (3) that normally contacts the opposite wall of the *ampulla* (4). Fibers (5) from the eighth nerve are also seen.

Here again the sensory cells are comparable to the neuromasts and are sometimes called so.

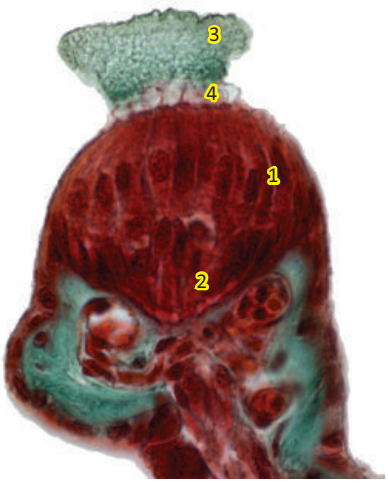


Fig. : 15.12 *Danio rerio* (MT / IM)

Crista ampullaris in the *ampulla* of a semicircular canal. The elongated ciliated sensory cells (neurons) are obvious (1) and are supported by sustentacular cells (2). The cupula (3) overlies the sensory (or neuromast) cells whose *cilia* (4) are embedded in the gelatinous substance. Connective tissue is stained green and contains blood vessels.

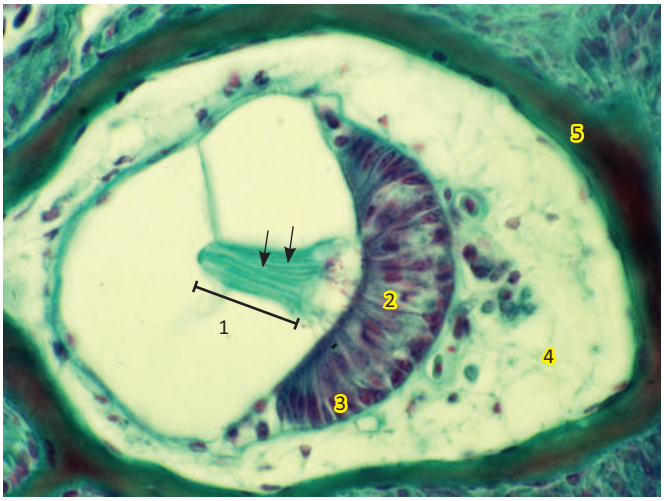


Fig. : 15.13 *Gnathonemus petersii* (MT / HM)

Crista ampullaris in the *ampulla* of a semicircular canal. The *cupula* (1) overlies the sensory cells whose well-visible *cilia* (thin arrows) are embedded in the gelatinous mass. The elongated sensory neurons (2) are supported by the supporting cells (3). Loose connective tissue (4) and cellular bone (5) are also found.

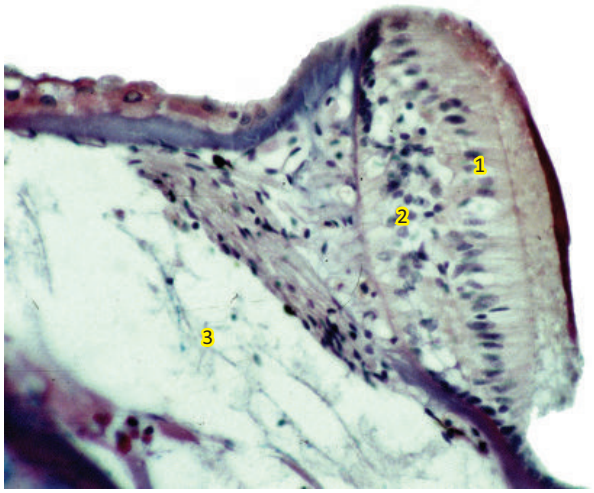


Fig. : 15.14 *Astatotilapia burtoni* (AB-PAS-H / HM)

Another micrograph of a *crista ampullaris* with the sensory cells (1), the supporting cells (2), and connective tissue (3).

Linear accelerations are detected by bending of columnar hair cells, which then become depolarised and send action potentials via nerve VIII to the brain.

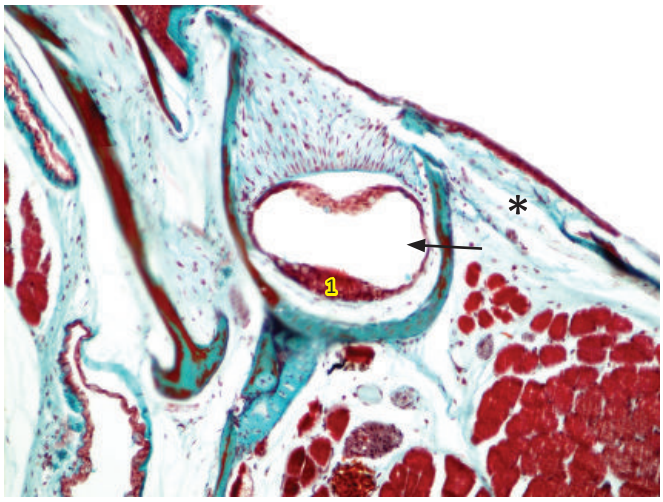


Fig. : 15.15 *Poecilia reticulata* (MT / MM)

In addition to the inner ear fishes possess a system, called the lateral line system, that plays a key role in the detection of movements, pressure changes and vibrations in the environment. The lateral line apparatus consists of numerous sensory units (mechanoreceptors) called neuromasts arranged in a network along the head and the body.

Lateral line organs may be free neuromasts lying on the whole surface of the skin or within small or large pits. Neuromasts of canal organs consist of a series of receptors in tunnel-like canals (arrow) embedded in the dermis (∗). The canals open at intervals to the exterior. 1 : neuromast.

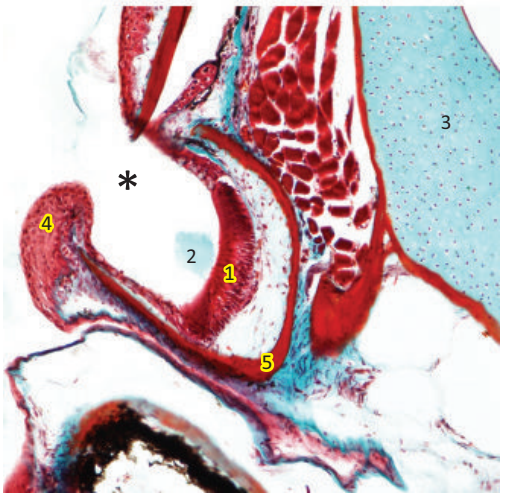


Fig. : 15.16 *Poecilia reticulata* (MT / MM)

Neuromasts (group of sensory hair cells) may occur within pits or grooves (∗) which are not covered by the epidermis. Several types of lateral line organs may thus exist in the same fish. This image shows a large neuromast (1) present within a groove located in the head region, behind the eyes.

Each neuromast in the groove organs bears a jelly-like cupula on its summit : remnants of this gelatinous cupula (2) can be seen here.

3 : hyaline cartilage - 4 : epidermis - 5 : bone surrounding the groove.

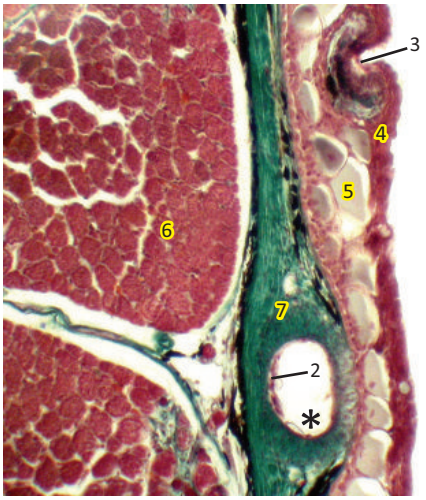


Fig. : 15.17 *Pangasius micronemus* (MT / MM)

Transverse section through the medial region of the trunk. Teleosts usually have lateral line canals (∗), embedded in the dermis (7), in which the neuromasts (2) are not directly exposed to the environment. They communicate with the outside world via a series of openings (pores) in the skin. Canals of the lateral line system are filled with fluid (water and mucous secretion) which transmits vibrations to the neuromasts through the skin pores.

A superficial neuromast within a small pit (3) is found on the top of the image.

4 : epidermis with alarm cells (5) - 6 : skeletal muscles - 7 : connective tissue of the dermis.

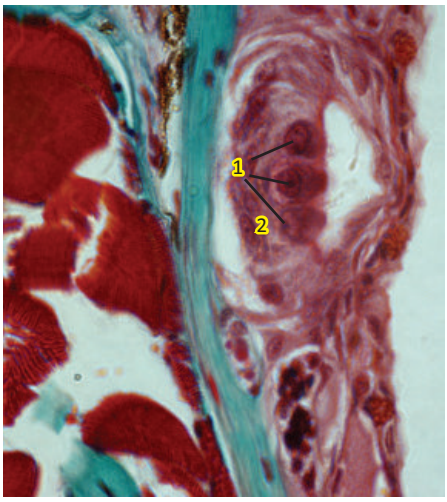


Fig. : 15.18 *Kryptopterus bicirrhis* (MT / HM)

Transverse section in the posterior trunk. The neuromasts are present on the skin (superficial neuromasts) or are distributed in lateral line canals (canal neuromasts) running along the head (cephalic canals) and the body. This micrograph shows a superficial and typical neuromast consisting of three sensory cells (1) supported by sustentacular cells (2). Collagenous fibers are in turquoise and skeletal muscles in red.

The lateral line system or «sixth sense of fish» is a collection of small mechanoreceptive structures or neuromasts located superficially on the skin or just under the skin into a series of canals.

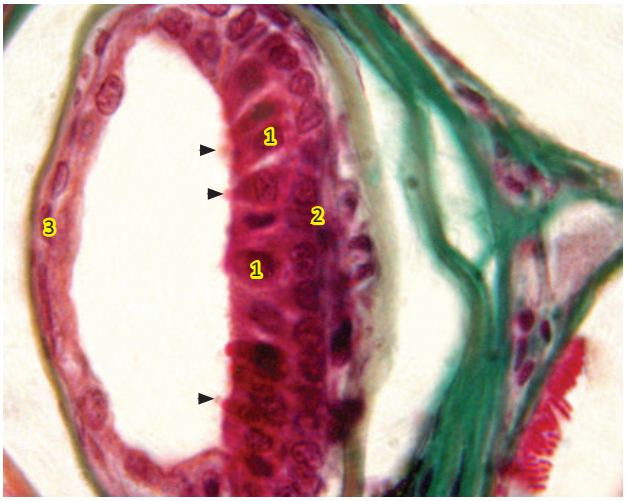


Fig. : 15.19 *Kryptopterus bicirrhis* (MT / IM)

Transverse section. The receptors (= neuromasts or «sense-hillocks») of the mechanosensitive lateral line system are typically composed of two types of cells : the sensory (hair) cells and the sustentacular (supporting) cells. The sensory cells (1) are pear-shaped cells located in the upper half of the organ and are covered by a gelatinous cupula (not visible here). They possess a large centrally located nucleus. The sustentacular cells (2) occupy the whole height of the organ and their thin apical portions extend between the hair cells. The supporting cells have a basally located nucleus.

The sensory cells of the neuromast have hair-like structures (arrowheads - *stereocilia* and *kinocilia*) that are connected to nerve cells and project into the cupula that bends in response to water currents. Note that the opposite site of the canal is lined with a simple squamous epithelium (3).

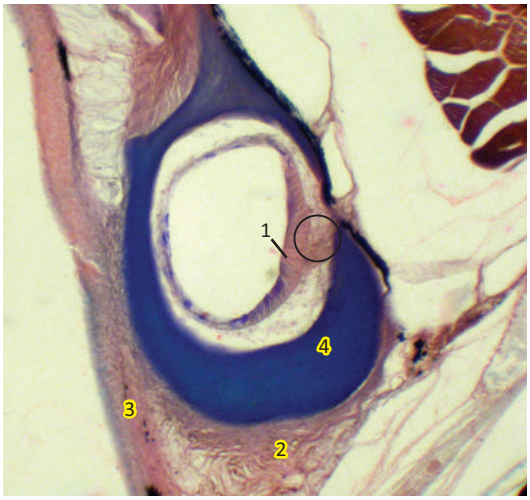


Fig. : 15.20 *Pelvicachromis pulcher* (AB-H-E / MM)

Transverse section through an encapsulated neuromast of the head part of the lateral line system. The latter runs along the sides of the body but also onto the head, where it divides into branches to the snout and to the lower jaw. Neuromasts (1) of canal organs are present in tunnel-like canals embedded in the dermis (2) and opening at intervals to the exterior. Acellular bony canal (4 - deep blue) is covered by the epidermis (3).

Bundles of nerve fibers (circle) lie below the neuromast. The lateral line organs are supplied by fibers of the VIIth (facial) plus the Vth (trigeminal) cranial nerves in the head region and by fibers of the Xth (vagus) nerve at the trunk level.

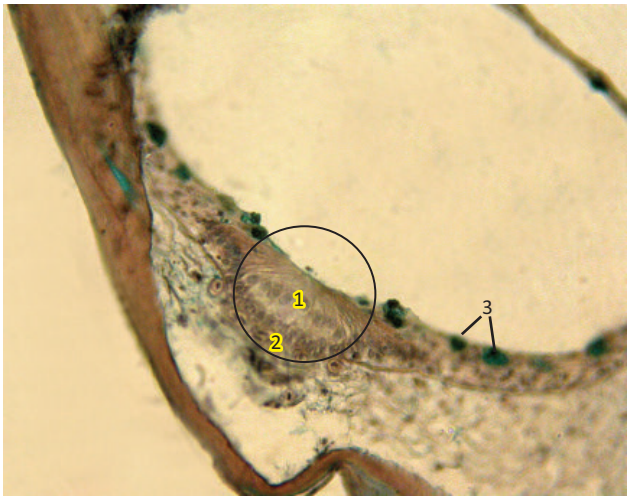


Fig. : 15.21 *Pelvicachromis pulcher* (AB-H / HM)

The lateral line system is a collection of neuromasts located superficially on the skin or just under it in fluid-filled canals in all fishes (and in larval as well as adult aquatic forms of amphibians).

A well-defined neuromast is here visible (circle). It is made up of a cluster of hair cells (1) and supporting cells (2). Some mucous cells (dark green - 3) are often present in the adjacent simple epithelium close to the neuromast.

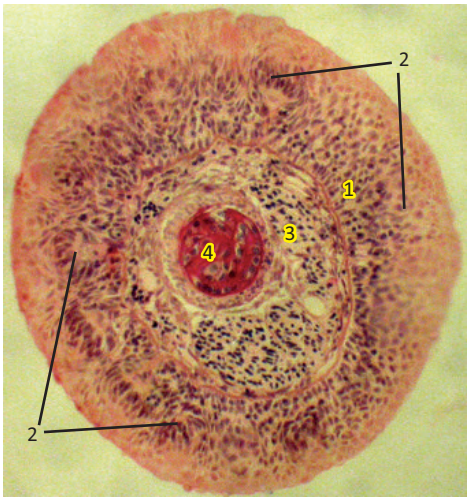


Fig. : 15.22 *Corydoras paleatus* (PAS-H / HM)

Transverse section of the barbel of this siluroid fish. Catfishes have generally a poor vision and their barbels ("whiskers") serve as additional taste organs. This general view shows a thick stratified epidermis (1) housing numerous taste buds (2). The dermis is made up of connective tissue containing fibroblasts, some chromatophores and a large number of nerve fibers (3) surrounding a cartilaginous support (4).

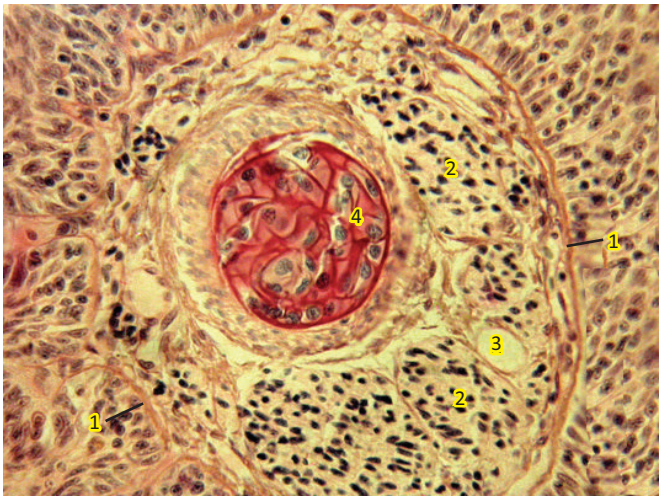


Fig. : 15.23 *Corydoras paleatus* (PAS-H / HM)

Transverse section of the barbel of the peppered corydoras. This micrograph shows the central portion of the previous picture and particularly the extensive terminal nerve network penetrating the base of each taste bud.

Delicate collagenous fibers (1), numerous bundles of nerve fibers (2) in relation with the taste buds, blood vessels (3) and the conspicuous cartilaginous rod (4) are easily recognized.

The primitive form of cartilage found in the catfish barbel (see chapter 2) consists of closely-packed cells with relatively abundant chromophobic (pinkish) cytoplasm. Chondrocytes are separated by the extracellular matrix (deep magenta).

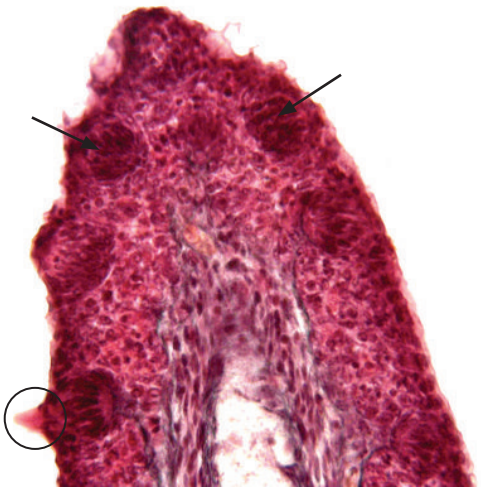


Fig. : 15.24 *Pangio kuhlii* (MT / HM)

Taste buds. This photomicrograph shows numerous external taste buds (arrows) which abound within the epithelium covering the coolie loach barbels. The circle indicates apical *microvilli* at the receptor area level.

Unlike olfactory sense receptors, taste receptors are not restricted to a single location : they may occur in the epithelia of the oral cavity, of the pharynx, of the gill-rakers (see Fig. 15.30) and in some species taste buds are found within the epidermis over the heads and parts of the body.

Solitary chemosensory cells also occur.

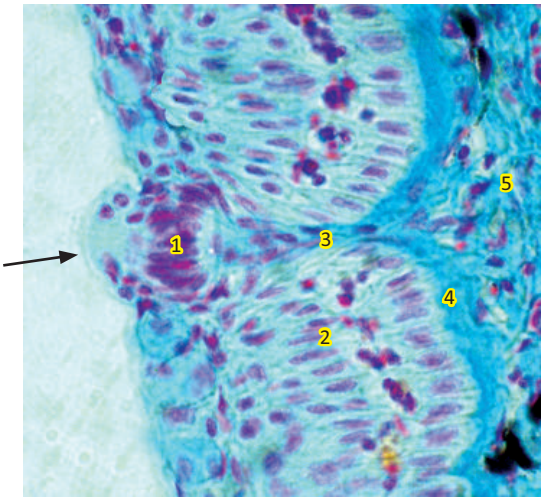


Fig. : 15.25 *Gnathonemus petersii* (FR-HB / HM)

Oral cavity showing a pear-shaped taste bud (arrow) within the stratified epithelium. The sensory cells (1) possess elongated nuclei and are grouped in the central zone of the bud. The buds can occupy the entire height of the epithelium (2). But their height is often less than that of the epithelium and in this case the bud is on a long stalk (3) of basement membrane and connective tissue. Note the thick basement membrane (4) and the dermis (5).

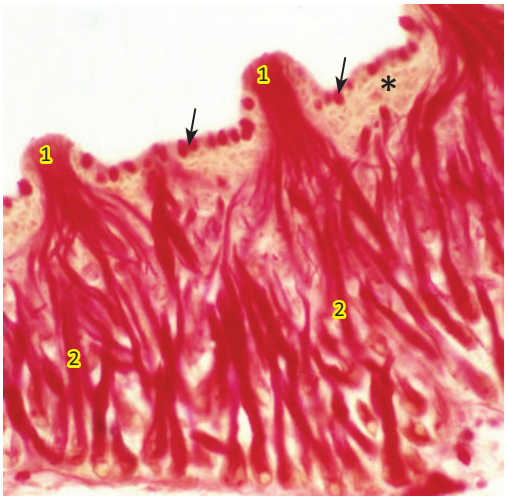


Fig. : 15.26 *Esomus malayensis* (PAS-H / MM)

Taste buds innervation. This photomicrograph shows taste buds (1) and their rich innervation (nerve fibers in bright red - 2). Taste buds, here, are found elevated on epidermal hillocks. The epithelium (∗) is rich in mucus-secreting cells (fine arrows).

Taste buds transmit information to enlarged lobes of the *medulla oblongata* via the nerves VII (facial nerve), IX (glossopharyngeal nerve) and X (vagal nerve). These three cranial nerves form a plexus at the base of the gustatory cells.

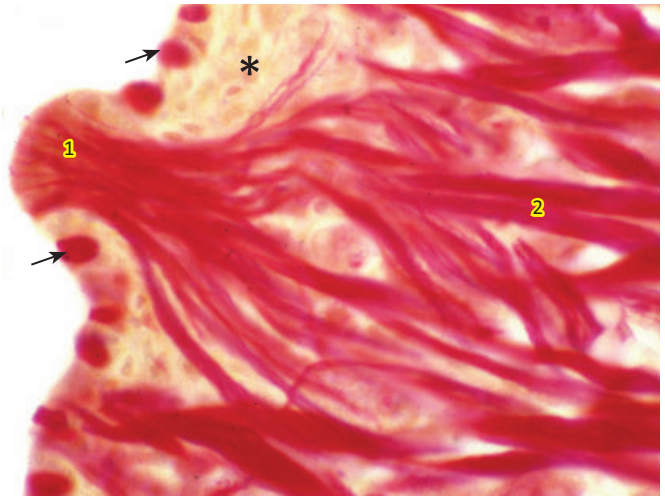


Fig. : 15.27 *Esomus malayensis* (PAS-H / HM)

Higher magnification of the previous document. A taste bud (1) located on an epidermal hillock is shown. This structure is richly innervated and bundles of nerve fibers (2), in magenta (PAS+), are well demonstrated. Numerous mucus-secreting cells (fine arrows) are found at the surface of the epithelium (∗).

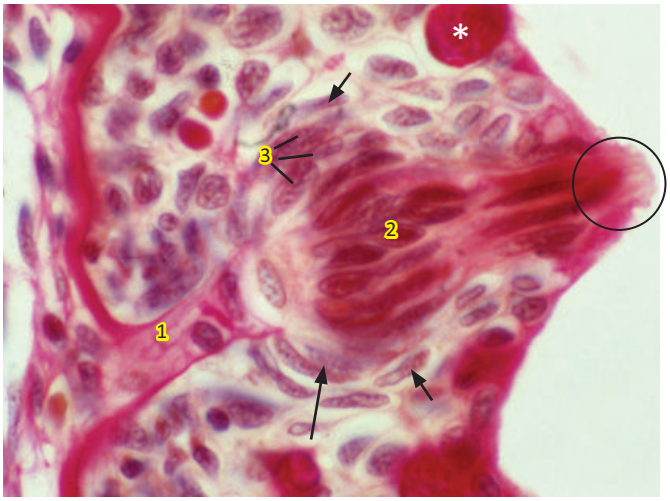


Fig. : 15.28 *Corydoras paleatus* (PAS-H / HM)

Taste buds are typically pear-shaped structures. The present taste receptor (long arrow) is located on an epidermal hillock and is supported by a connective stalk (1). Its longitudinal axis is oriented vertically to the epidermal surface. Fish taste buds consist mainly of two types of cells, sensory and supporting. The taste cells (2) are elongated and each of them terminates by sensitive hairlike structures, the receptor *microvilli* (circle) which project to the outside or into the organ's lumen. Sustentacular cells (3) are usually located at the periphery of the central sensory cells. Some authors have also described two types of sensory cells and basal cells located below the taste buds. Marginal epithelial cells (short arrows) delimit the taste organ.

∗ : mucous cell

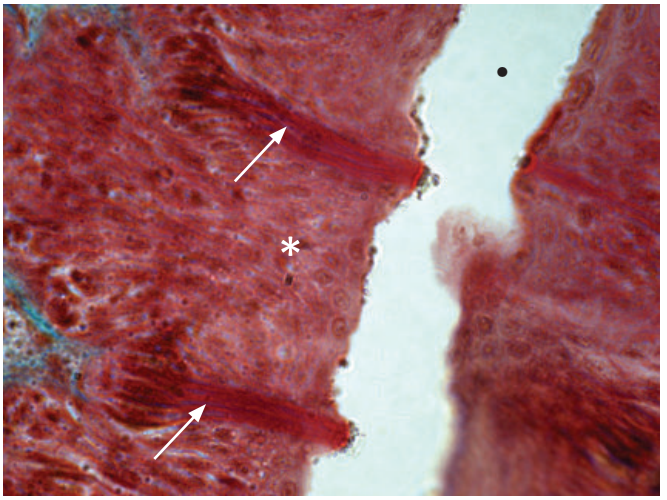


Fig. : 15.29 *Poecilia reticulata* (MT / HM)

Taste buds usually consist of barrel-like or pear-shaped collections of elongated cells sunk within ectodermal epithelia. However they can take a tubular form (arrows) like in this stratified epithelium (∗) of the guppy's oral cavity (•).

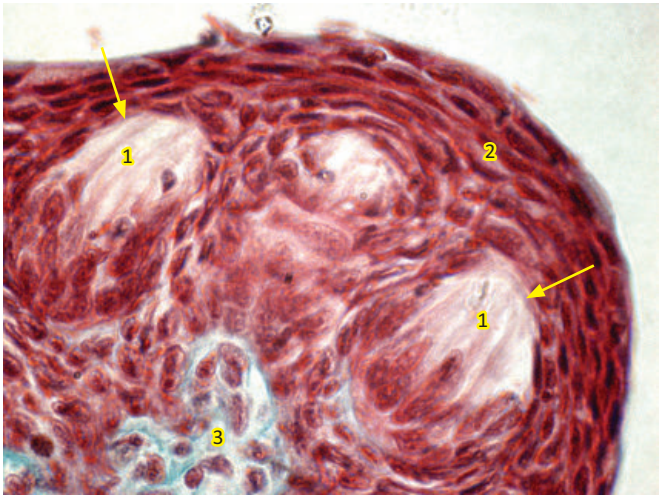


Fig. : 15.30 *Cyprinus carpio* (MT / HM)

Taste buds (arrows) located within the epithelium of a carp's gill raker. Their pear-shaped structure is evident. Such a taste organ is made up to a few tens of mostly elongated epithelial cells.

1 : sensory cells - 2 : stratified squamous epithelium of the gill raker - 3 : supporting connective tissue

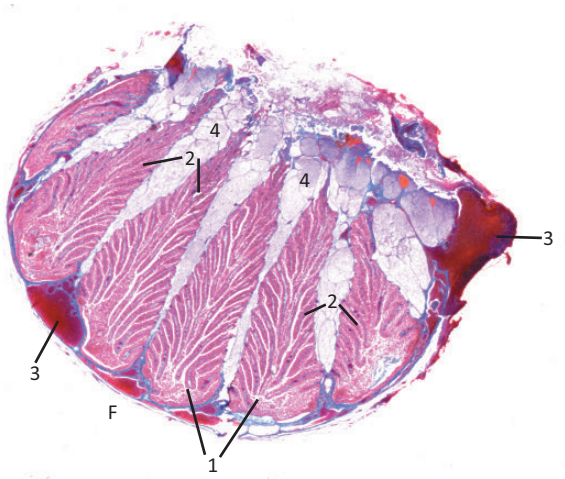


Fig. : 15.31 *Scyliorhinus canicula* (MT / LM)

Right olfactory sac of the lesser spotted dogfish. The olfactory ability in sharks is well known and sharks possess amongst the largest olfactory organs (rosettes) of any vertebrate.

The fish olfactory system is composed of olfactory sacs or rosettes in contact with the olfactory bulbs which transmit information to the olfactory lobes of the telencephalon via the olfactory tract. The latter contains nerve fibers of the cranial nerve I (olfactory nerve).

Olfactory rosettes are situated on the floor of nasal chambers. In elasmobranchs, each nostril is covered by a simple flap of skin dividing it into an inhalant and an exhalant aperture.

This micrograph represents a section of the olfactory sac (rosette) which consists of primary *lamellae* (1) arranged longitudinally and bearing on either side numerous secondary folds or secondary *lamellae* (2).

3 and 4 respectively indicate large blood vessels and nerve bundles. F : floor of the rosette

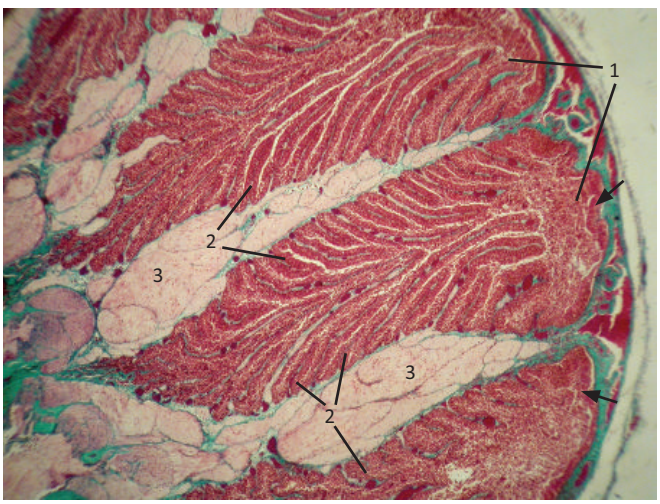


Fig. : 15.32 *Scyliorhinus canicula* (MT / LM)

Shark olfactory rosette. Higher magnification of the floor side showing two primary *lamellae* (1) and their numerous secondary *lamellae* (2). Abundant nerve fascicles (3) run between the primary *lamellae* and give precise smell information to the fish. Collagenous fibers are stained green. The arrows point to the epithelium located on the floor side and containing mucus-secreting cells (non visible at this magnification).

The olfactory rosette is a specialized area of epithelium capable of detecting very low levels of chemical substances (as low as 1 ppb in sharks but also in eels and salmons !). Indeed the large surface provided by the olfactory *lamellae* dramatically increases the sensitivity and efficacy of the olfactory system.

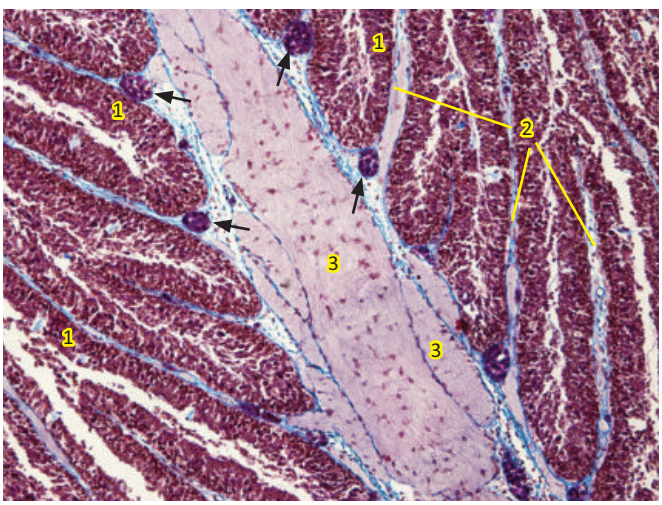


Fig. : 15.33 *Scyliorhinus canicula* (MT / MM)

Section through the olfactory organ. The picture illustrates a part of one olfactory *lamella* bearing on either side numerous secondary *lamellae* (1) of the sensory epithelia. Thin nerve fibers (2) arising from the secondary folds aggregate to form large nerves bundles (3) which altogether will constitute the olfactory nerve in relation with the olfactory lobe.

Delicate connective fibers (in blue) and regularly disposed blood vessels (arrows) are seen.

This arrangement with both primary and secondary epithelial folds reminds the arrangement of the gill's folds.

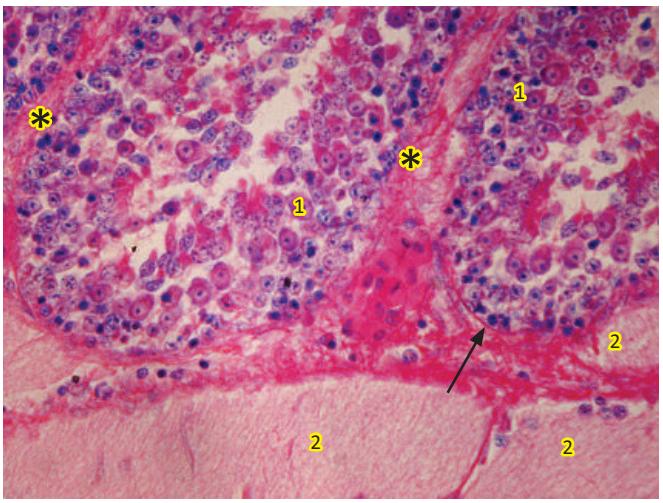


Fig. : 15.34 *Scyliorhinus canicula* (TM / HM)

Section through the olfactory organ showing portion of two secondary folds. This high magnification allows to see the olfactory epithelium (1) lining the *lamellae*. The epithelium is a continuous thick sheet of pseudostratified columnar epithelial cells and is composed of numerous chemoreceptors (ciliated, microvillar, microridged and rod-like cells), basal cells as well as supporting cells. Mucus-secreting cells are also present in the inner epithelium (see arrows in the Fig. 15.32).

Each secondary *lamella* has a central core (∗) housing collagenous fibers (red) and a great number of capillaries. A basal *lamina* separate the sensory epithelium from this supporting tissue (arrow). Nerve fibers (2) fill the bottom of the image.

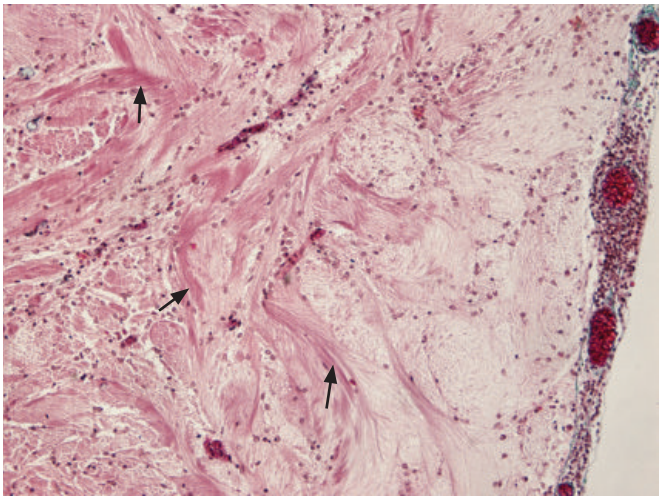


Fig. : 15.35 *Scyliorhinus canicula* (MT / MM)

Section through the olfactory lobe. In the lesser spotted dogfish olfactory bulbs and olfactory lobes are very close to the olfactory sacs (no apparent tract). The two olfactory lobes of the telencephalon receive and analyze nerve impulses from the nostrils via the two olfactory nerves.

The present olfactory lobe shows the inextricable network of fascicles (arrows) which belong to the olfactory nerve I. These nerve bundles are supported by an extensive mass of glial cells and neuropil (neurites and other neuron components). Note the absence of connective tissue.

The olfactory lobes are particularly developed in fish (sharks, catfish ...) that find their food principally by smell.

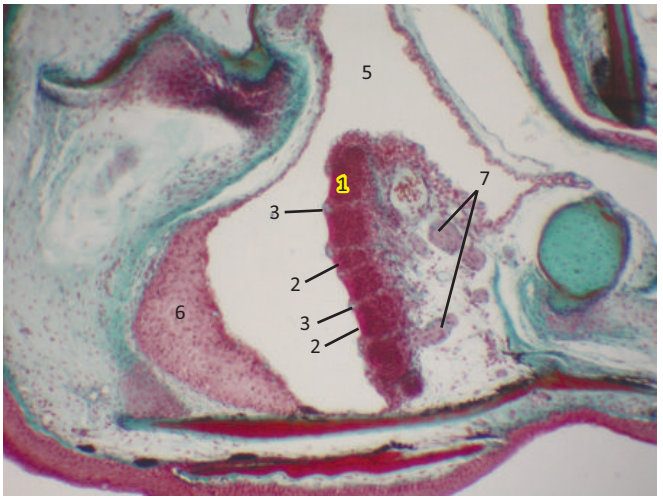
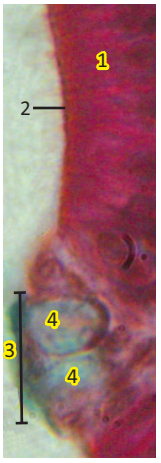


Fig. : 15.36 *Poecilia reticulata* (MT / MM)



Not all fish have olfactory rosettes. For instance the guppy's olfactory organ is simple and in transverse section the sensory epithelium (1) is practically flat. The olfactory epithelium consists of hair (*cilia* and *microvilli*) sensory cells, supporting and basal cells. Olfactory cells are concentrated in special concave areas (2) separated by non-sensory epithelium (3) containing some mucous cells (4). Water is pumped into the olfactory organ by beating of the *cilia* and with the help of one (or more) accessory nasal sac (5). A stratified epithelium (6) lies at the lateral surface of the nasal cavity in front of the sensory epithelium. Small fascicles (7) of the nerve I are observed.

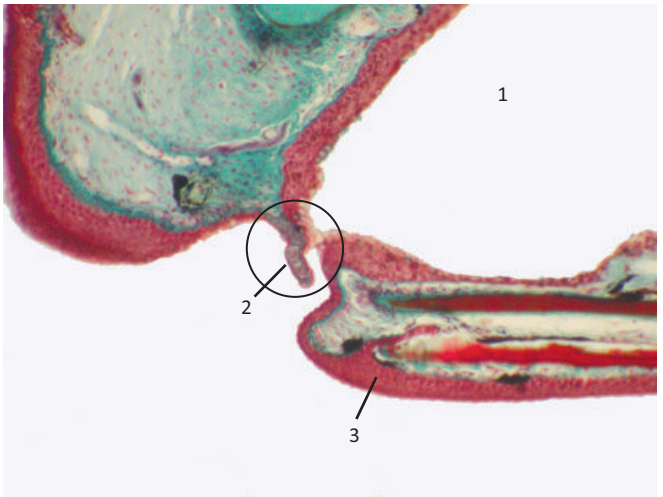


Fig. : 15.37 *Poecilia reticulata* (MT / MM)

The nasal cavity (1) of the guppy is connected to the external environment by the nostril (circle) protected by a cartilage-like fold (2). It is clearly visible that the epithelium lining the cavity (and the accessory nasal sac) is continuous with the epidermis (3).

The simple organization of the olfactory organ in guppy resembles that of early stages of development of adult fish provided with a well-developed olfactory organ.

All these observations suggest that this small ovoviviparous fish, originating from Central America, relies secondarily on olfaction.

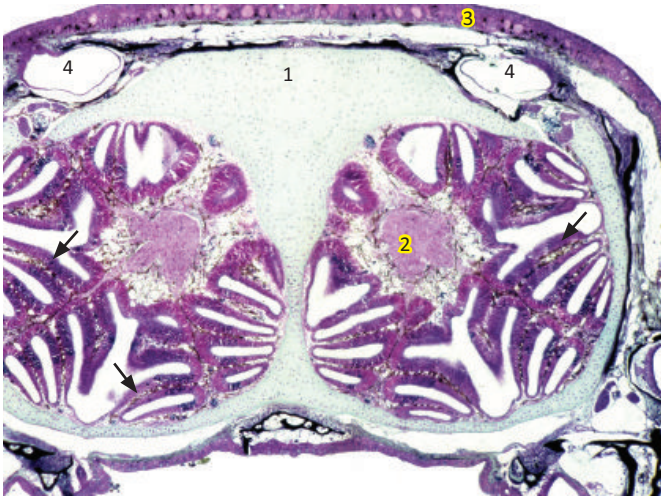


Fig. : 15.38 *Polypterus senegalus* (MT / LM)

Transverse section through the paired olfactory organs showing the olfactory chambers lined with olfactory *lamellae* (arrows). One can clearly see that the olfactory sacs are located in cartilaginous capsules (hyaline cartilage - 1) on the ventral side of the snout. Nerve bundles (2) of nerve I (olfactory nerve) aggregate in the centre of each of the sensory chambers.

This lamellar arrangement, although less extended than that of sharks, greatly increases the surface area in contact with water and thus its sensitivity.

The skin (3) with large glandular cells and two cephalic canals of the lateral line system (4) are also present.

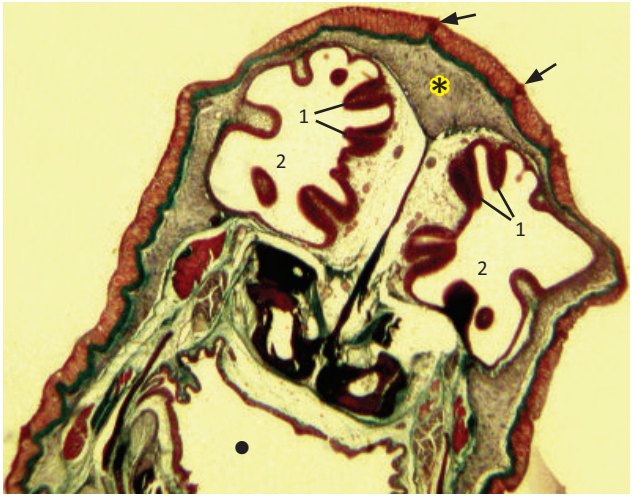


Fig. : 15.39 *Pangio kuhlii* (MT / LM)

Transverse section through the paired olfactory sacs. The latter exhibit a few low folds or *lamellae* (1) which protrude into the olfactory chambers (2). The (primary) *lamellae* are lined by a ciliated sensory epithelium containing some mucus-secreting cells. A fibrocartilaginous tissue (∗) surrounds the sacs. The oral cavity (•) is seen on the bottom of the image and some taste buds (arrows) seat in the epidermis.

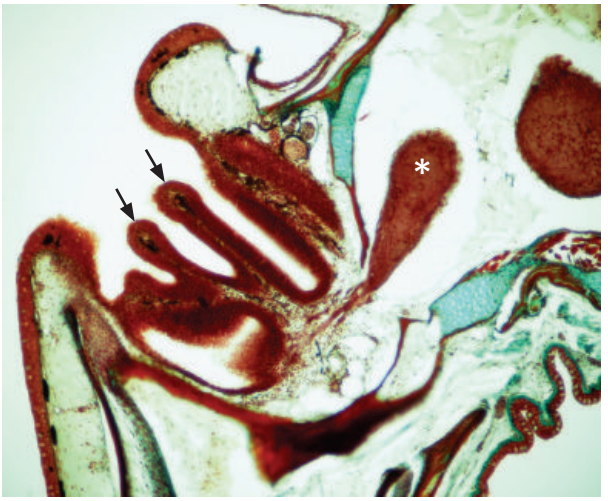


Fig. : 15.40 *Danio rerio* (MT / MM)

Transverse section through the anterior part of a zebrafish olfactory organ. Folds (similar to the dogfish's primary *lamellae* - arrows) are attached to the floor of the olfactory chamber and have their dorsal free margin extending into the chambers.

On the right : part of the olfactory tract (∗) going to the olfactory lobe.

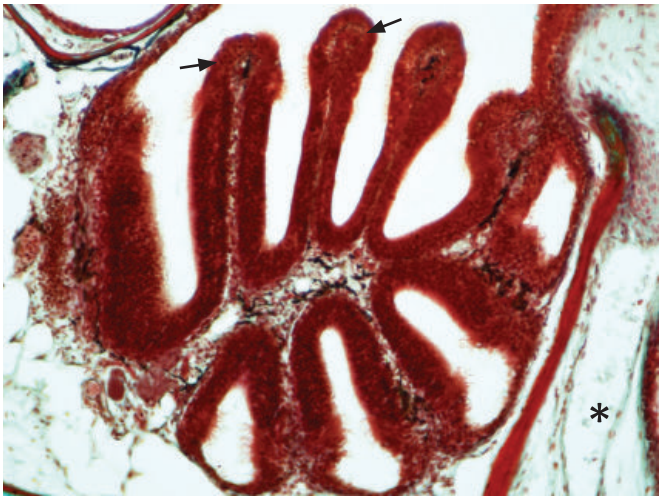


Fig. : 15.41 *Danio rerio* (MT / HM)

Transverse section through the medial part of a zebrafish olfactory organ. The sensory epithelium covering the *lamellae* (arrows) is highly folded allowing a large number of olfactory cells to be packed into the small area of the sac. ∗ : loose connective tissue.

The olfactory epithelium lines a lamellar olfactory rosette in many teleosts. Acanthopterygian fish can possess flat, single, double or even triple folded olfactory epithelium, but extremely rare are the species which possess olfactory organs as efficient as the elasmobranch olfactory rosettes.

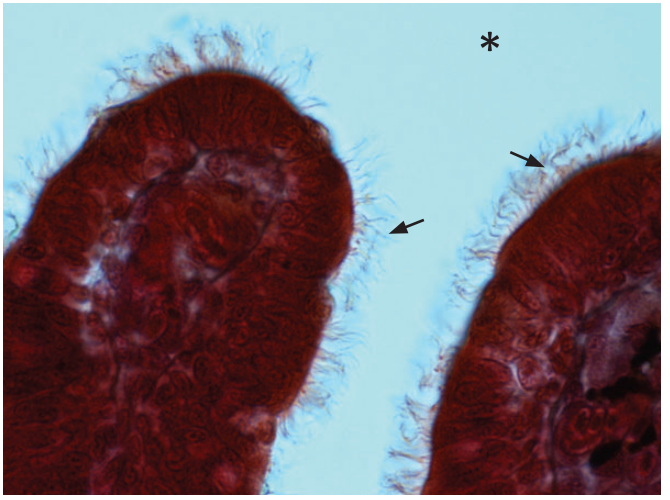


Fig. : 15.42 *Danio rerio* (MT / IM)

Free margin of two olfactory folds covered by the olfactory epithelium. This epithelium contains sensory ciliated cells interposed with supporting cells ; mucous cells can be encountered only in a few fish species. This high magnification (immersion) allow to see the *cilia* (arrows) bathing in the water currents circulating through the olfactory chamber (*).

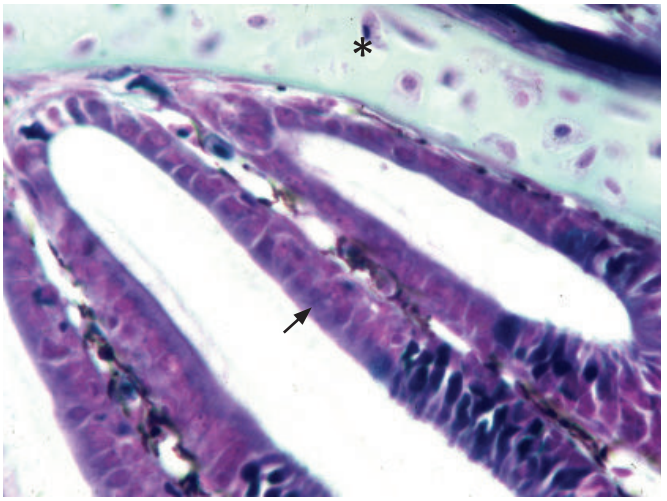


Fig. : 15.43 *Polypterus senegalus* (MT / HM)

Higher magnification of the *Polypterus* olfactory epithelium (see Fig. 15.38). The sensory epithelium (arrow) is columnar pseudostratified and is composed of sensory cells (microvillar olfactory neurons and ciliated olfactory neurons), non-sensory cells, supporting cells and basal cells. Here, these cell types are difficult to distinguish. ∗ : hyaline cartilage.

A remarkable feature of olfactory cells is that a long nerve *fiber* extends from each cell and makes a connection to the forebrain !

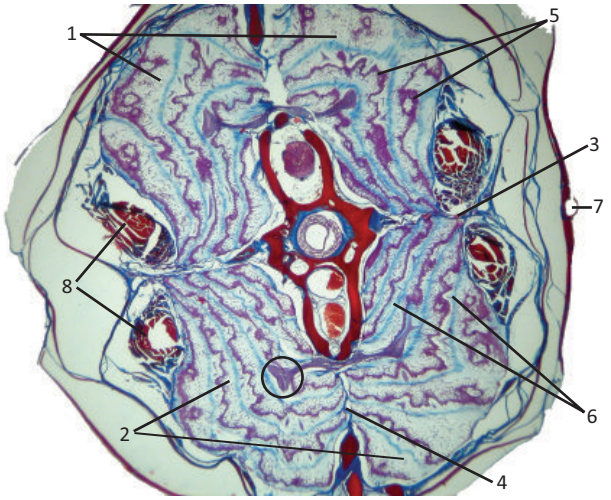


Fig. : 15.44 *Gnathonemus petersii* (MT / LM)

Transverse section through the caudal peduncle. The principal groups of weakly electric fish include gymnotiform fish of South America and mormyrid fish of Africa. The elephant nose fish has an electric organ lying in the thin caudal peduncle of the posterior part of the body. The electric organ consists of four longitudinal parts, a dorsal (1) and a ventral (2) pair separated horizontally by connective *septa* (3) and vertically by vertebral apophyses and connective tissue (4).

The electrocytes (purple – 5) are the elementary units of the electric organ. They are polynucleated, very elongated and flat cells separated by collagenous *septa* (sky blue – 6).

The spinal nerves supplying the organ divide to form four main electric nerves : two dorsal and two ventral. The circle shows the end of a ventral electric nerve.
A canal (7) of the the lateral line system and lateral fascicles (8) of rhabdomyocytes are seen. The center of the image represents the vertebra with the spinal cord, the notochord and caudal vessels (for a detailed description see Fig. 2.1).

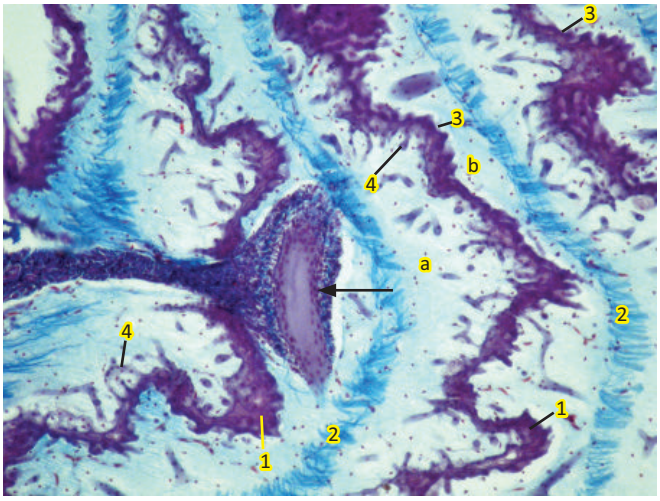


Fig. : 15.45 *Gnathonemus petersii* (MT / MM)

Electric organ of the elephant-nose fish. The electrocytes (purple – 1) are the morphological and functional elementary units of the electric organ which generate electric signals. They are modified rhabdomyocytes found in the medial part of the deep lateral muscles. Electrocytes are surrounded by two gelatinous layers (pale blue - a and b) and are separarated from each other by collagenous *septa* (sky blue – 2).

Each electrocyte has a relatively smooth anterior face (3) and a posterior one with elongated *papillae* (4).

The arrow points to a large electric nerve. Mormyrids have a brain / body weight ratio much higher than in any other fish.

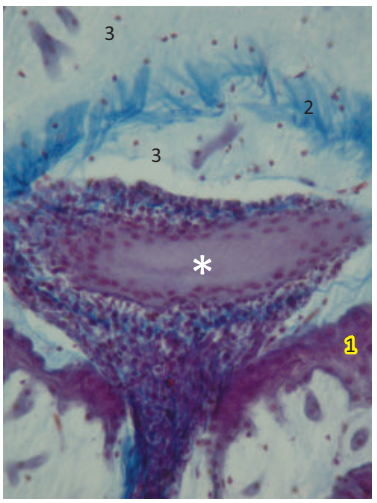


Fig. : 15.46 *Gnathonemus petersii* (MT / MM)

Main branch (∗) of an electric nerve lying between the electrocytes. The weakly electric fish *Gnathonemus petersii* has small eyes and lives in muddy waters. During active electrolocation, the fish produces a series of electric organ discharges and can detect, localize and identify the preys and the predators. This electric sense may also be used for communication and territorial interactions with congeners.

Therefore the nerve supply to this organ is quite complicated : it is innervated by numerous spinal neurons whose motor fibers form four (two ventral and two dorsal) main branches or electric nerves.

1 : electrocyte / 2 : collagenous *septum* / 3 : gelatinous layers

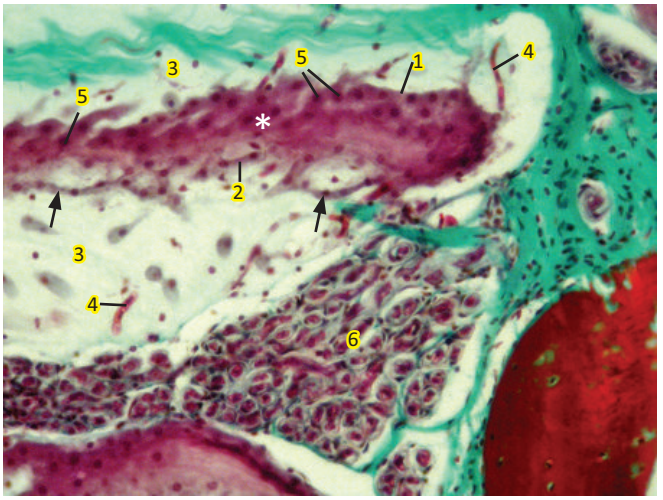


Fig. : 15.47 *Gnathonemus petersii* (MT / HM)

Electric organ of the elephant nose fish. Each electrocyte (∗) is a *syncitium* (large mass of protoplasm containing several nuclei) limited by an anterior (non-innervated - 1) and a posterior (innervated – 2) face. The latter exhibits numerous papillae (arrows) which actually are plasma membrane invaginations.

Gelatinous layers (3) surround the electrocytes and contain some capillaries (4). The syncitial mass shows nuclei (5) located preferentially at the periphery of the electrocyte and in the *papillae*.

Many fine caliber axons from the electric nerve (6) are observed.

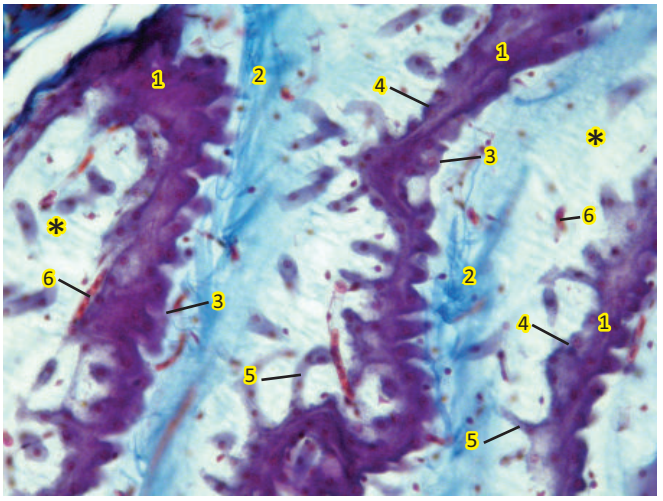


Fig. : 15.48 *Gnathonemus petersii* (MT / HM)

Section through the electric organ of the elephant nose fish showing three electrocytes (1). Each of them is encased in a diffuse collagenous capsule (blue - 2).

3 and 4 respectively indicate anterior, non-innervated and posterior, innervated faces of the electrocyte - 5 : *papillae* with nuclei - 6 : capillaries containing red erythrocytes - ∗ : gelatinous layers with fragments of *papillae*.

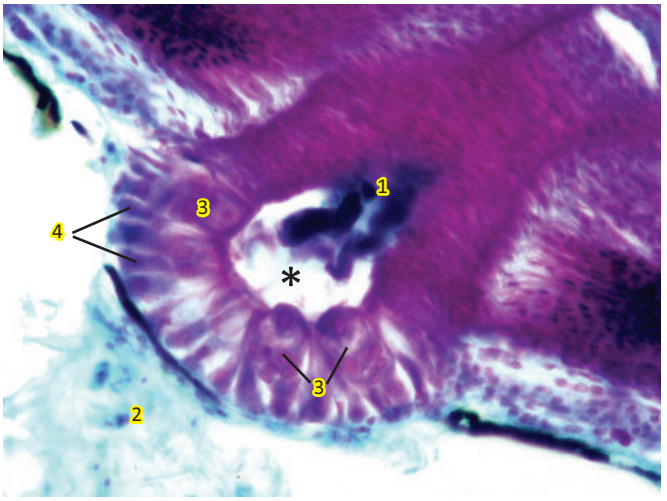


Fig. : 15.49 *Gnathonemus petersii* (MT / HM)

Ampullary organ. The elephant-nose fish possesses three types of electroreceptors : the ampullary organs (Figs 15.49 & 15.50), the mormyromasts (Fig. 15.51) and the tuberous organs («Knollenorgans» - Figs 15.53 & 15.54). These electroreceptors are found in the specialized electroreceptive epidermis covering the head as well as the ventral and dorsal parts of the body. Impulses emitted by the electric organ are picked up and analyzed by the sensory cells of these electroreceptors.

The ampullary organ (also called type-I mormyromast) is an intraepidermal structure consisting of a cavity (∗) from which a canal leads to the surface of the skin. This canal (more visible on the next figure) is generally filled with polysaccharides forming a jelly-plug (1). This electroreceptive organ, supported by connective tissue (2) consists of numerous sensory cells (3) surrounded by various types of accessory cells (4).
Ampullary organs play a key role in the sensing of low-frequency electric signals.

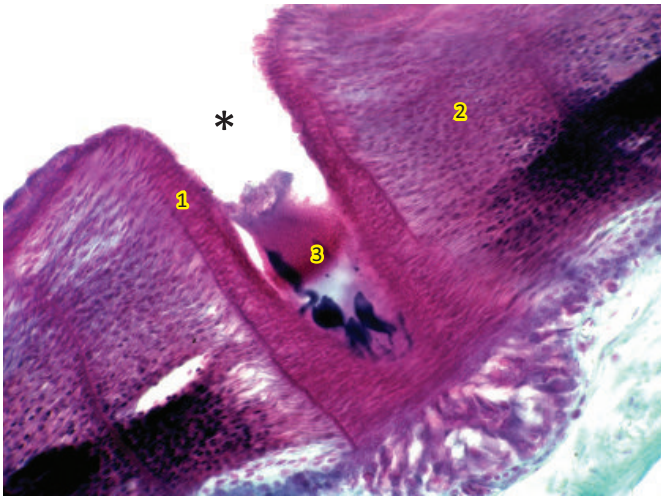


Fig. : 15.50 *Gnathonemus petersii* (MT / HM)

Ampullary organ. The canal (∗), lined by cuboidal cells (1), is well-visible and crosses the conspicuous specialized electroreceptive epidermis (2). The supporting cells produce a highly conductive jelly-like substance (3) which fills the canal linking the sensory cells to the surrounding water. Sensory and supporting cells are difficult to differentiate here. In green connective tissue.

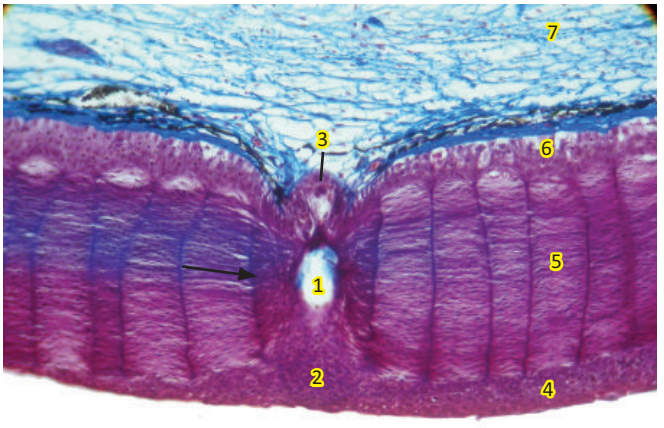


Fig. : 15.51 *Gnathonemus petersii* (MT / HM)

Section through the skin containing a mormyromast electroreceptor organ (arrow). The mormyromast has a large and spherical cavity (1) filled with acid mucopolysaccharides and covered by a cap of epithelial cells (2). A cellular clump (3) lies at the base of the cavity and contains sensory cells surrounded by a great number of small supporting cells. The thick electroreceptive epidermis consists of three layers : the superficial polyhedral cells (4), the flat cells of the intermediate layer (5) forming hexagonal pillars and the basal cylindrical or polyhedral layer (6). This epithelium seems to be unique amongst fishes. Note loose (7) connective tissue of the dermis.

The mormyromast (also called type II mormyromast) is particularly abundant in the epidermis around the buccal cavity. It is mainly used for detecting the deformation of the electric field generated by the mormyrid's own discharges.

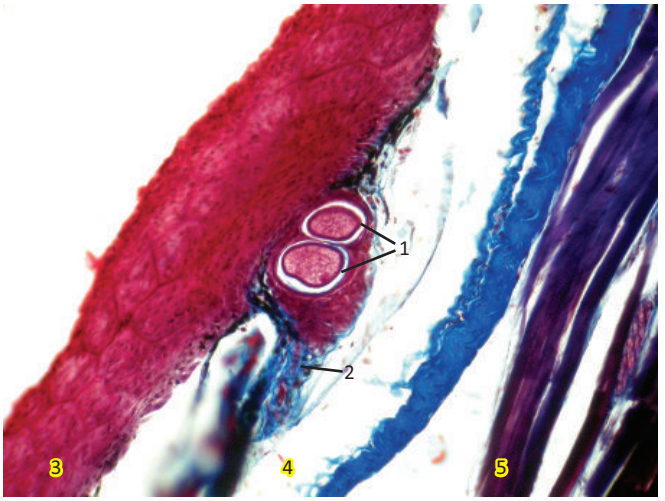


Fig. : 15.52 *Gnathonemus petersii* (MT / MM)

Tuberous organs («Knollenorgans»). The bulbous organs are specific cutaneous electroreceptor organs of the lateral line system. They lack both cavity and canal and are formed by epithelial masses that protrude into the dermis. The organ has a few number of large sensory cells (1) each enclosed in its own cavity. The whole organ is surrounded by a fibrous capsule (2). Electroreceptive epidermis (3), dermis (4 - collagen in blue) and skeletal muscle (5) are also observed.

Tuberous organs play their major role in electrocommunication (detection of conspecific electric signals) and are used for the detection of high-frequency signals.

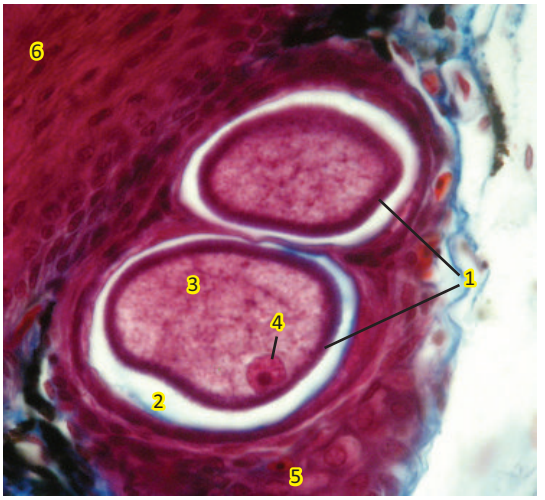


Fig. : 15.53 *Gnathonemus petersii* (MT / IM)

Tuberous organ in a mormyrid fish skin. The organ exhibits here two giant globular sensory cells (1) each surrounded by a free space (unstained substance - 2). The cytoplasm (3) contains glycogen granules and the nucleus (4) is eccentrically located. There are also supporting cells (5) at the base of the tuberous organ and sensory neurons which project to the electrosensory lateral line lobe (Figs 12.8 & 12.9) of the *medulla* via a branch of the X^{th} nerve.

The epithelial cells (6) over the sensory receptors allow electric signals to pass from the external environment to the electroreceptive cells.

Note that the substance surrounding each sensory cell may stain in red by toluidine blue.

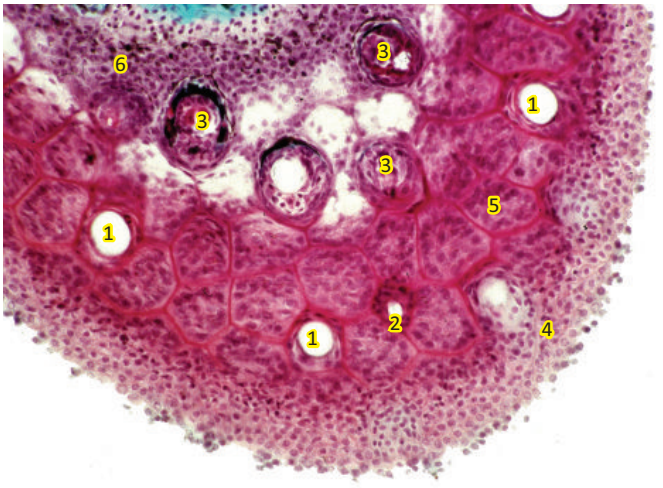


Fig. : 15.54 *Gnathonemus petersii* (MT / HM)

Preparation showing the three types of electroreceptors of the mormyrids: the ampullary organs or type I mormyromasts (1), the mormyromasts or type II mormyromasts (2) and the tuberous organs or «Knollenorgans» (3). These electroreceptors are found in a specialized electroreceptive epidermis and are supplied by lateral line nerves. One can see the three layers of the epidermis : the superficial polyhedral cells (4), the hexagonal pillars of the intermediate layer (5) and the basal stratified layer (6).

The enormous development of the *mormyrocerebellum* can be undoubtedly correlated with the abundance of these sensory terminations. In mormyrids the latter can be found over the whole body, except on the caudal peduncle which is the region of the electric organ.

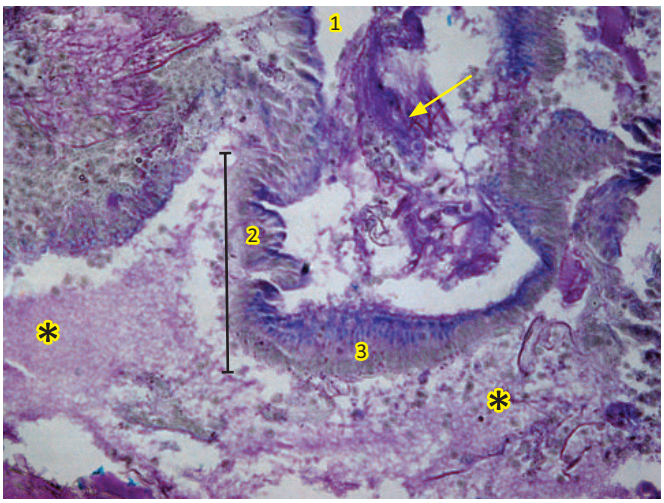


Fig. : 15.55 *Scyliorhinus canicula* (PAS-H / MM)

Ampulla of LORENZINI. The cephalic region of elasmobranchs contains modified lateral line sensory organs located in the dermis and called *ampullae* of LORENZINI. They are jelly-filled canals in open communication with the skin surface by pores visible to the naked eye (dark spots). The canal (1) ends in a cluster of small vesicles enclosed in capsules of collagenous connective tissue (∗). This photomicrograph shows the terminal dilatation (*ampulla* - 2) of such canal. The deeper part of the *ampulla* consists of a sensory epithelium (3) containing thermo- and electroreceptive cells alternating with supporting cells. The arrow points to the gelatinous substance.

The primary function of the *ampullae* of LORENZINI is electroreception and each *ampulla* is innervated by afferent fibers of the facial nerve (VII).

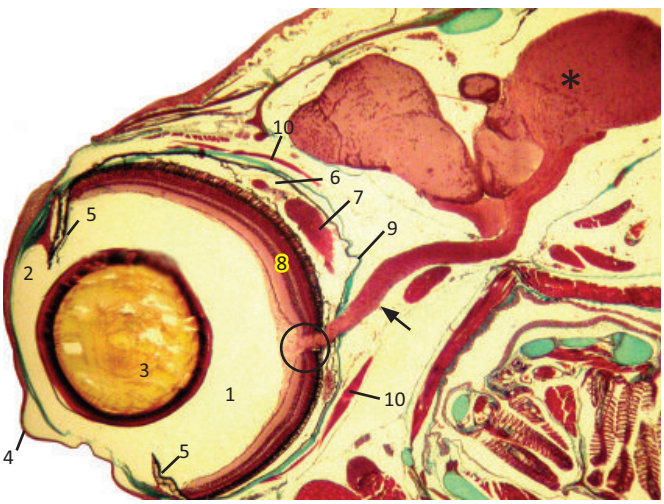


Fig. : 15.56 *Poecilia reticulata* (MT / LM)

Cover's picture. This general view emphasizes the connection between the optic nerve (arrow) with the brain (∗). The eye (left) conforms to the general vertebrate plan and numerous structures are well-visible in this micrograph.

Eyes are organized into the large chamber of the vitreous body (1) and aqueous cavities (anterior and posterior chambers - 2) located in front of the lens (3). The anterior portion of the eye's outermost layer is modified into a transparent cornea (4) with a central hole, the pupil, surrounded by the iris (5). The lens (3) is completely round and protrudes into the aqueous chamber. The middle layer of the eye is composed of a vascular tunic, the choroid (6) which contains the choroid *rete* (7 – see Fig. 13.37). The retina (8) is the light sensitive part internal to the choroid and disappearing near the lens. Finally the posterior part of the outermost layer constitutes the sclera (9) on which ocular muscles (10) insert.

The circle shows the optic disc (see last figure).

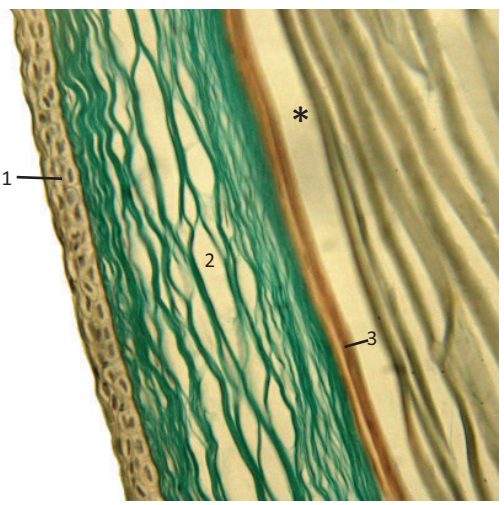


Fig. : 15.57 *Pelvicachromis pulcher* (AB-H / MM)

Section through the cornea (lateral side of the eye). In this micrograph the layers of the cornea can be seen.

1 : the squamous corneal epithelium : it is an unpigmented, transparent stratified epithelium contiguous with the integument ;

2 : the corneal stroma : this layer is the main constituent of the cornea and consists of tightly bound collagen fibers ;

3 : the thin corneal endothelium that forms the inner surface of the cornea.

∗ : aqueous anterior chamber.

Most fish have no eyelids, except the Chondrichtyans.

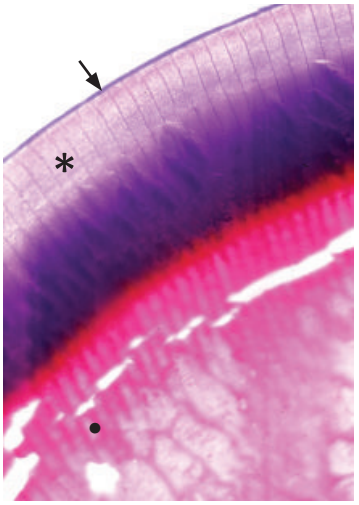


Fig. : 15.58 *Astronotus ocellatus* (MT / HM)

The lens is transparent and almost entirely composed of living cells. It is surrounded by a capsule (arrow) consisting of carbohydrates and glycoproteins. The lens is an avascular tissue and contains two morphologically different cell populations. The cells close to the aqueous chambers form a monolayer of epithelial cells (∗) whereas those facing the chamber of the vitreous body are very elongated (•).

To accommodate for close objects the lens can move forward and backward by small muscles and suspensory ligaments.

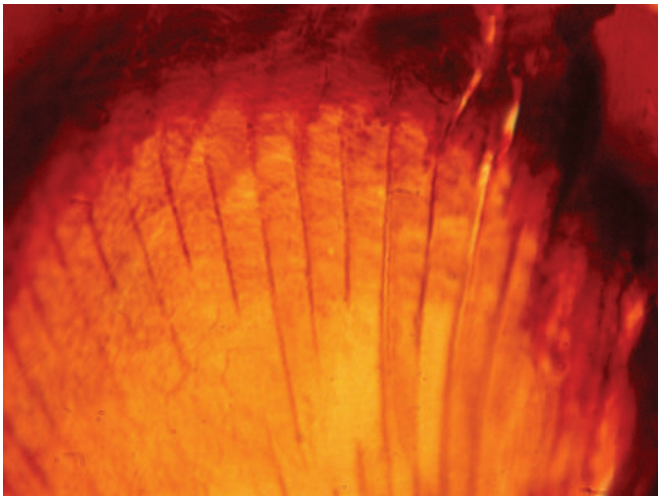


Fig. : 15.59 *Poecilia reticulata* (MT / IM)

The lens is a transparent disc whose main function is to bring images into critical focus on the retina. The picture illustrates fully parallel differentiated lens fibers having lost their nuclei. Crystalline protein is the major protein found in the lens cells : the latter are very elongated and separated by very little extracellular matrix.

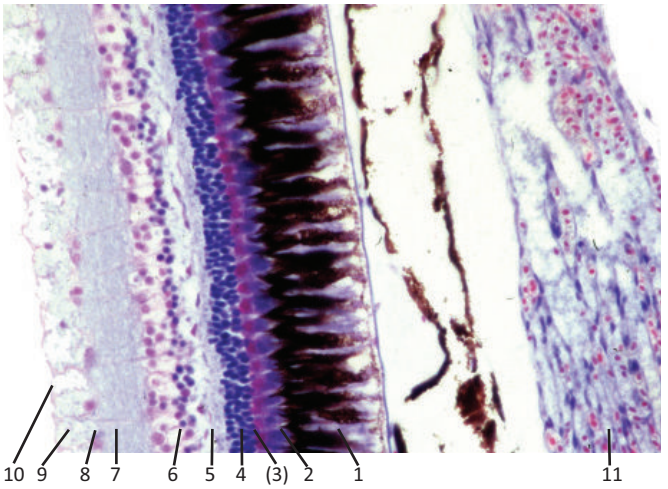


Fig. : 15.60 *Astronotus ocellatus* (MT / HM)

This photomicrograph shows the entire depth of the retina and the uveal layer on the right.

The retina is divided into ten distinct layers.

1 : pigment epithelium - 2 : photoreceptor layer (rod and cone processes) - 3 : outer limiting membrane (non visible) - 4 : outer nuclear layer consisting of the nuclei of the photoreceptors - 5 : outer plexiform layer - 6 : inner nuclear layer - 7 : inner plexiform layer - 8 : ganglion cell layer - 9 : layer of ganglion cell axons forming the optic nerve or nerve fibers layer - 10 : the inner limiting membrane.

The choroid plexus (11) is a well developed and intricate array of arterioles and capillaries forming a *rete mirabile* (choroid *rete*).

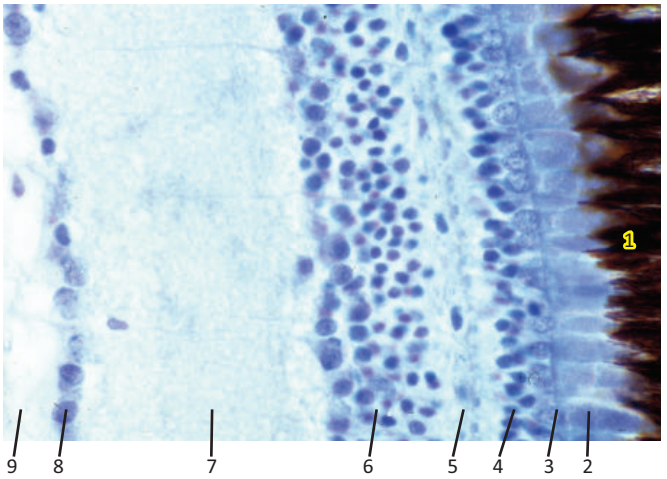


Fig. : 15.61 *Astronotus ocellatus* (AB-H / HM)

Retina. Practically the same legend as in the previous picture except for the tenth layer (10) which is not illustrated.

1 : pigment epithelium - 2 : photoreceptor layer (rod and cone processes) - 3 : outer limiting membrane - 4 : outer nuclear layer consisting of the cell bodies of the photoreceptors - 5 : outer plexiform layer - 6 : inner nuclear layer - 7 : inner plexiform layer - 8 : ganglion cell layer - 9 : layer of ganglion cell axons forming the optic nerve or nerve fiber layer

As compared to the previous image the outer limiting membrane is clearly visible.

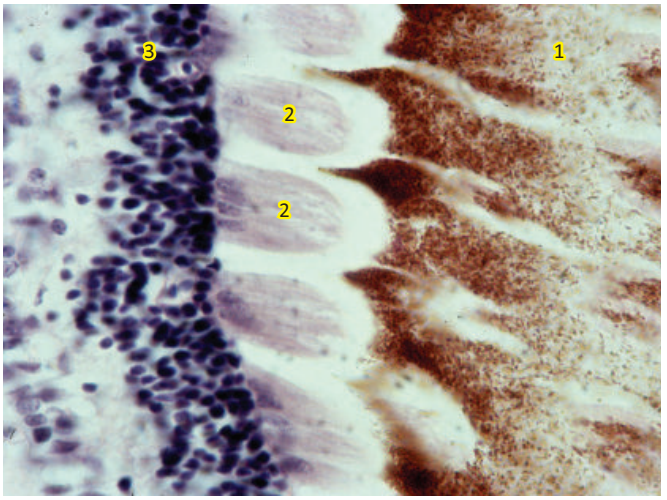


Fig. : 15.62 *Gnathonemus petersii* (MT / IM)

Posterior part of the retina. The pigment epithelium (1 – melanin in brown), the photoreceptor cell processes (inner and outer segments - 2) and the cell bodies of the cone and rod cells (outer nuclear layer – 3) are clearly visible. The outer limiting membrane is not distinguishable.

Teleosts often have several twin cones.

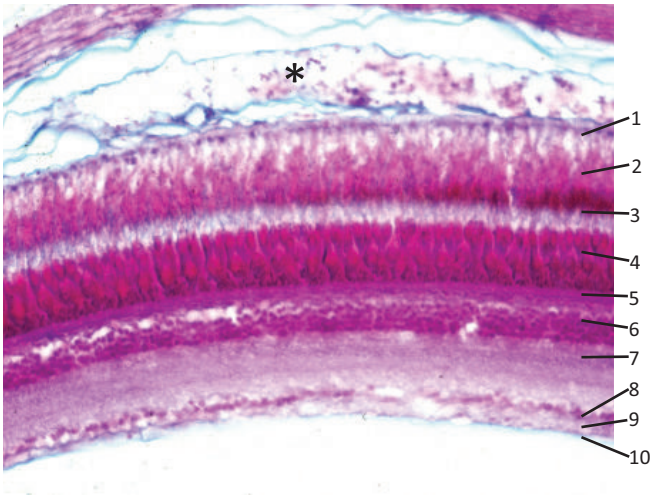


Fig. : 15.63 *Barbus caudovittatus* (H-E / HM)

This picture shows the retinal layers backed by a heavily vascularized choroid (∗). For the last time here is the legend of the ten layers of the retina.

1 : pigment epithelium mostly devoid of melanin - 2 : photoreceptor layer (rod and cone processes) - 3 : outer limiting membrane - 4 : outer nuclear layer consisting of the nuclei of the photoreceptors - 5 : outer plexiform layer - 6 : inner nuclear layer - 7 : inner plexiform layer - 8 : ganglion cell layer - 9 : nerve fiber layer - 10 : the internal limiting membrane (blue)

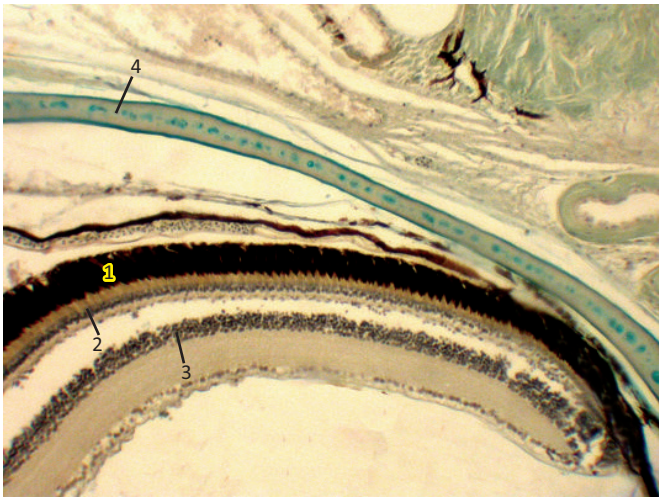


Fig. : 15.64 *Pelvicachromis pulcher* (AB-H / MM)

Posterior part of the eye. Some layers of the retina, including the pigment epithelium (1), the outer (2) and the inner (3) nuclear layers, are obvious. The wall of the posterior portion of the eye is made up of the thick fibroblastic sclera which is reinforced with an external sheath of hyaline cartilage (4) in most fish.

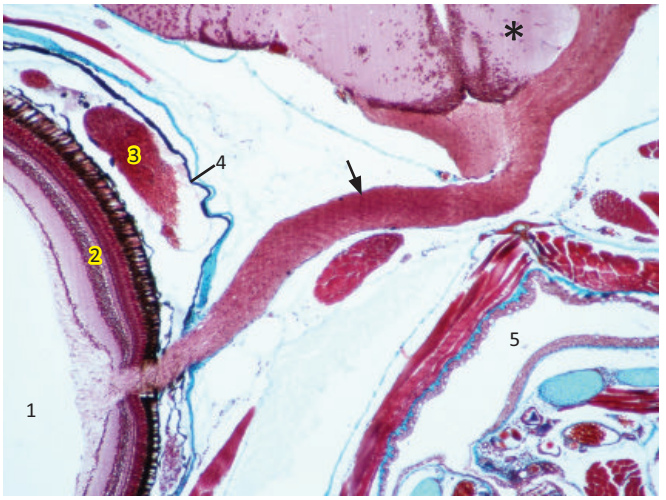


Fig. : 15.65 *Poecilia reticulata* (MT / MM)

This micrograph is a higher magnification of the figure 15.56 and displays the optic nerve (arrow – see also Fig. 12. 40) connecting the eye with the brain (∗).

The roof (*tectum*) of the mesencephalon as well as diencephalic structures are related with the optic nerve and receive sensory input from it.

1 : chamber of the vitreous body - 2 : retina / 3 : choroid *rete* - 4 : sclera - 5 : pharynx

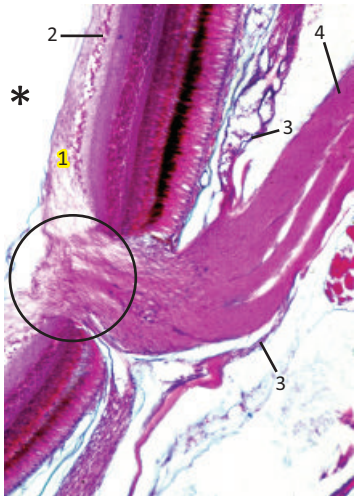


Fig. : 15.66 *Barbus caudovittatus* (H-E / HM)

Posterior region of the eye. The afferent fibers (1) from the ganglion cells (2) penetrate the sclera (3) to form the optic nerve (4).

∗ indicates the chamber of the vitreous body.

The circle shows the optic disc (or optic papilla) which is the point where the optic nerve leaves the retina («blind spot»). Note the absence of photoreceptor cells in this spot.

List of fish names

Scientific name	Family	Common name
Acipenser gueldenstaedtii - Brandt & Ratzeburg, 1833	*Acipenseridae*	Russian sturgeon
Anguilla anguilla - (L., 1758)	*Anguillidae*	European eel
Astronotus ocellatus - Cuvier, 1829	*Cichlidae*	Oscar
Atherina boyeri - Risso, 1810	*Atherinidae*	Big-scale sand smelt
Barbus caudovittatus - Boulenger, 1902	*Cyprinidae*	None
Carapus acus - (Brünnich, 1768)	*Carapidae*	Pearlfish
Carassius auratus - (L., 1758)	*Cyprinidae*	Goldfish
Chromidotilapia guentheri - (Sauvage, 1882)	*Cichlidae*	Guenther's mouthbrooder
Corydoras paleatus - (Jenyns, 1842)	*Callichthyidae*	Peppered corydoras
Cyclopterus lumpus - L., 1758	*Cyclopteridae*	Lumpsucker / Kiark-varrey
Cyprinus carpio - L., 1758	*Cyprinidae*	Common carp
Danio rerio - (Hamilton, 1822)	*Cyprinidae*	Zebra danio
Dicentrarchus labrax - (L., 1758)	*Moronidae*	European seabass ; white salmon
Distichodus sexfasciatus - Boulenger, 1897	*Citharinidae*	Six-banded or six-bar distichodus
Esomus malayensis - Ahl, 1923	*Cyprinidae*	Malayan flying barb
Garra congoensis - Poll, 1959	*Cyprinidae*	None
Gnathonemus petersii - (Günther, 1862)	*Mormyridae*	Elephant nose fish
Haplochromis burtoni - (Günther, 1894)	*Cichlidae*	Burton's haplo
Heteropneustes fossilis - (Bloch, 1794)	*Heteropneustidae*	Stinging catfish
Kryptopterus bicirrhis - (Valenciennes, 1840)	*Siluridae*	Glass catfish
Microctenopoma congicum (Boulenger, 1887)	*Anabantidae*	Congo ctenopoma
Oncorhynchus mykiss - (Walbaum, 1792)	*Salmonidae*	Rainbow trout
Pangasius micronemus - Bleeker, 1847	*Pangasiidae*	Shortbarbel pangasius
Pangio kuhlii - (Valenciennes, 1846)	*Cobitidae*	Coolie loach
Parachanna obscura - (Günther, 1861)	*Channidae*	Snake-head
Pelvicachromis pulcher - (Boulenger, 1901)	*Cichlidae*	Rainbow krib ; purple cichlid
Perca fluviatilis - L., 1758	*Percidae*	European perch
Petrocephalus microphthalmus - Pellegrin, 1908	*Mormyridae*	None
Pimelodus pictus - Steindachner, 1876	*Pimelodidae*	Pictus cat
Poecilia reticulata (*var.* Endler) - Peters, 1859	*Poeciliidae*	Guppy ; million fish
Polypterus senegalus - Cuvier, 1829	*Polypteridae*	Gray bichir
Rutilus rutilus - (L., 1758)	*Cyprinidae*	Roach
Schilbe mystus - (L., 1758)	*Schilbeidae*	African butter catfish
Scyliorhinus canicula - (L., 1758)	*Scyliorhinidae*	Lesser-spotted dogfish / Small-spotted catshark
Sparus aurata - (L., 1758)	*Sparidae*	Gilthead seabream
Stomatorhinus puncticulatus - Boulenger, 1899	*Mormyridae*	None
Symphysodon aequifasciatus - Pellegrin, 1904	*Cichlidae*	Discus
Thalassoma pavo - (L., 1758)	*Labridae*	Ornate wrasse
Trisopterus luscus - (L., 1758)	*Gadidae*	Pouting
Xiphophorus helleri - Heckel, 1848	*Poeciliidae*	Green swordtail

Please refer to the index for page references.

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