

Fish Pathology

THIRD EDITION

Edited by

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Edinburgh • London • New York • Oxford • Philadelphia • St Louis • Sydney • Toronto 2001

The Anatomy and Physiology of Teleosts

INTRODUCTION

The purpose of the present chapter is to give a brief outline of the aspects of the anatomy and physiology of teleost fish which are necessary for an appreciation of the pathological changes which can occur in fish and for the understanding of the mechanisms underlying clinical disease manifestations.

Many basic functions are similar to those of other vertebrates, and knowledge of mammals is not entirely irrelevant. However, the teleosts must not be regarded as primitive forebears of the mammals; they are advanced, evolutionarily recent and expanding into a multitude of niches. There are more species of teleost than any other class of vertebrate (Bone *et al.* 1995), so that many generalizations are obviously of dubious value, but the principal exploited species are of a restricted range so that useful statements can still be made. The main emphasis in this account will be on specific differences from the more familiar mammalian anatomy and physiology.

A major consideration is the aquatic environment and the constraints it imposes on fish. It is against the physical and biological degradative influences of this medium that the *milieu interieur* of the fish must be maintained and with which necessary exchanges of materials must take place. An overriding factor is the high specific heat of water, which imposes, on most fish, ectothermy (poikilothermy), i.e. the body temperature conforms to the environmental temperature. No simple physiological constant values can be given, e.g. for heart rate, rate of digestion or rate of growth; all of these are subject to temperature and this must always be borne in mind when studying teleosts. The same animal is often unrecognizable at different temperatures, e.g. whether adrenaline increases or decreases heart rate in rainbow trout depends on the temperature (Randall 1970).

THE INTEGUMENTARY SYSTEM

The skin is the primary barrier against the environment, allowing normal internal physiological function, so its condition is important in many disease processes. The layers of teleost skin, comprising cuticle, epidermis, basement membrane, dermis and hypodermis, are indicated diagrammatically in Fig. 2.1.

Cuticle

The external layer, the cuticle or glycocalyx, was first described in detail by Whitear (1970) as a mainly mucopolysaccharide layer approximately 1 μm thick. It is normally formed largely

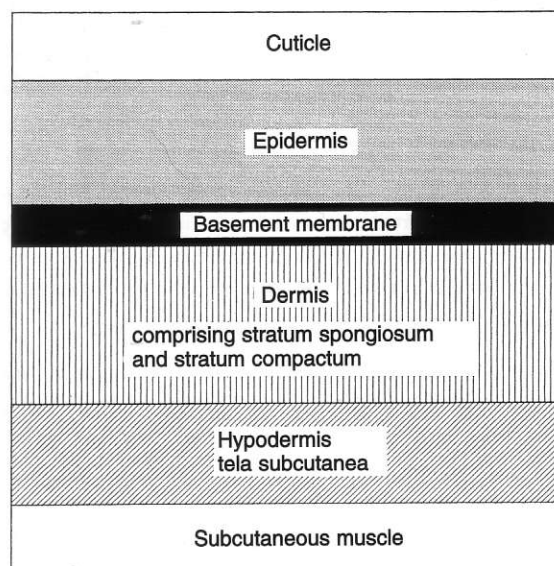


Fig. 2.1. Schematic diagram of normal teleost skin layers. (From Bullock & Roberts 1975.)

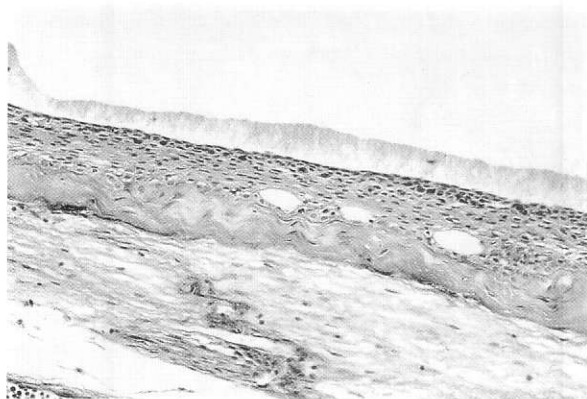


Fig. 2.2. Tail skin of whiting, showing cuticle, epidermis and dermis. PAS $\times 142$.

from epithelial surface cells rather than by secretion from goblet mucous cells and is a complex of cell protoplasm, sloughed cells and any goblet cell mucus that has been secreted onto the surface (Fig. 2.2). The physical consistency of the cuticle varies considerably between species, being especially developed in rock pool and benthic species. The cuticular layer contains specific immunoglobulins and lysozyme (although the amount of the latter varies very much between species), and free fatty acids. These are believed to have anti-pathogen activity, as part of the mucosal defence system of the skin, working in conjunction with cellular proliferation kinetics to continuously remove microorganisms from the surface (Speare & Mirasalimi 1992). Normally, however, small numbers of bacteria will still occur on such surfaces and there are obvious limits with regard to the efficiency of such systems when pathogen loading of the environment is high.

Epidermis

As in all vertebrate species, the fundamental unit of the epidermis of the teleost fish is the fibrous malpighian cell. This is, however, the only consistent feature, as there is great diversity in all of the other cell types occurring there (Bullock & Roberts 1975). In adults the epidermis is a stratified squamous epithelium covering the body surface and investing the tail and fins. Unlike its mammalian counterpart, it is living and capable of mitotic division at all levels, even at the outermost, squamous layer. The surface of the outermost layer is arranged in a whorling pattern of micro-ridges (Fig. 2.3).

The thickness of the epidermis varies with species, age, site and often, stage of the reproductive cycle. It is usually thicker in those species with negligible scale cover (e.g. the eel) and

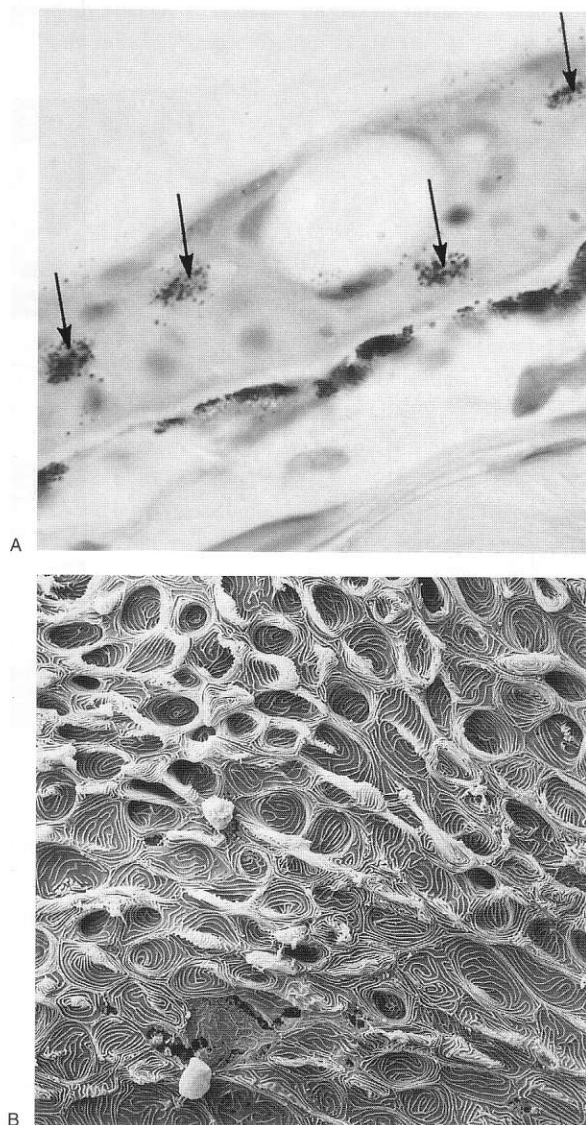


Fig. 2.3. A. Autoradiograph of section of plaice skin from a young fish inoculated with tritiated thymidine 12 hours previously. The specifically labelled nuclei of dividing cells (arrowed) are found at all levels of the epidermis. H + E $\times 500$. B. Scanning electron micrograph of the surface of the epidermis of guppy showing the characteristic arrangement of micro-ridges. $\times 2200$. (B, by courtesy of Dr D.K. Cone.)

also over the fins, where it is particularly well endowed with nerve end organs and mucous cells (Fig. 2.4).

The malpighian cells are always present in teleost epidermis. They are rounded cells very similar in structure at all levels except the outermost. Here they are flattened horizontally, with a cytoplasm composed largely of an accumulation

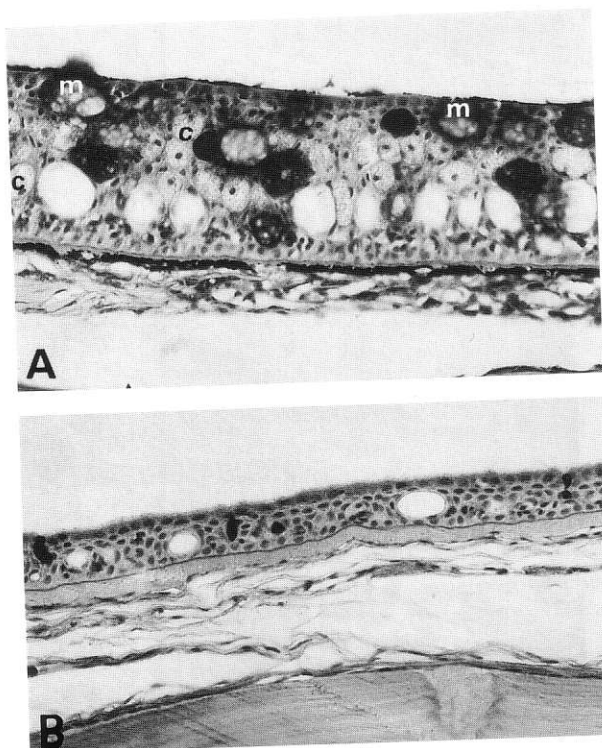


Fig. 2.4. The epidermis of the gurnard. A, Body surface, mucous cells (m), club cells (c) and cystic spaces similar to those found in gadoid species. B, The pectoral fin. PAS $\times 224$.

of elongated vesicles, degenerating mitochondria and some dense bundles of fibres, instead of the more typical widely distributed bundles of fibres and mitochondria around a generally ovoid nucleus.

Mucus-secreting cells are found in the epidermis of all teleosts but numbers vary greatly with site and species. These goblet cells usually originate in the middle layers of the epidermis, although in a very thin epidermis, a mucous cell may be seen to have its base on the basement membrane. They increase in size and elaborate secretions (mainly glycoproteins) as they approach the surface.

Club cells are large, usually round, cells, found in the lower and middle layers of the epidermis of certain teleost groups. The classical club cells are the *Shreckstoffzellen* found in the epidermis of cyprinids, which secrete a potent alarm substance, but many other species possess morphologically similar large clear non-mucoid cells in their epidermis, which do not appear to be related to such fright reactions.

Granule cells are found in a wide variety of teleost epidermes but as yet no function has been ascribed to them. Other cells found in the epidermis include lymphocytes,

macrophages and large clear cyst-like structures, of putatively cellular origin, which are especially prominent in the Gadidae.

Dermis

The dermis is composed of two layers. The upper layer, the *stratum spongiosum*, is a loose network of collagen and reticulin fibres, contiguous with the epidermal basement membrane. It contains the pigment cells (chromatophores), mast cells and cells of the scale beds and also the scales. The lower layer, the *stratum compactum*, is the collagenous dense matrix which provides the structural strength of the skin. The capacity for colour change to match the environment or due to sexual activity or disease is very highly developed in many teleosts and is induced by controlled modulation of the interplay of absorptive and reflective properties of the chromatophores. Melanophores, the dark, pigment-containing cells, are asteroid cells containing large numbers of membrane-bound electron-dense granules of melanin pigment which can be moved within the cytoplasm of the cell to give the desired effect. Lipophores are chromatophores containing organic solvent-soluble pigments and are subdivided into erythrophores, containing red pigments, and xanthophores, containing yellow pigments (Bullock & Roberts 1974).

The pigments are mainly carotenoids, which cannot be synthesized *de novo* by the fish and must of necessity come from food. Leucophores and iridophores are responsible for white and silver colours and contain purines, usually guanine, which exist as plates of reflecting material up to 10 μm thick and arranged within the cell in parallel arrays, rather like a venetian blind (Denton & Nicol 1966) (Fig. 2.5).

The scales of teleosts are calcified flexible plates which lie partly within shallow 'scale pockets', oriented posteriorly. Two main types, differing in surface sculpture, are described. Ctenoid scales bear stiff spicular processes on their posterior external edge which are absent from cycloid scales. Both types have growth rings on their surface, which, in many species, allow determination of the individual's age. Ultrastructurally, scales consist of collagen fibres interspersed with a matrix of albuminoid materials in which are deposited hydroxyapatite crystals.

Hypodermis

The hypodermis is a looser, adipose tissue, which is more vascular than the overlying stratum compactum of the dermis and a frequent site of development of infectious processes.

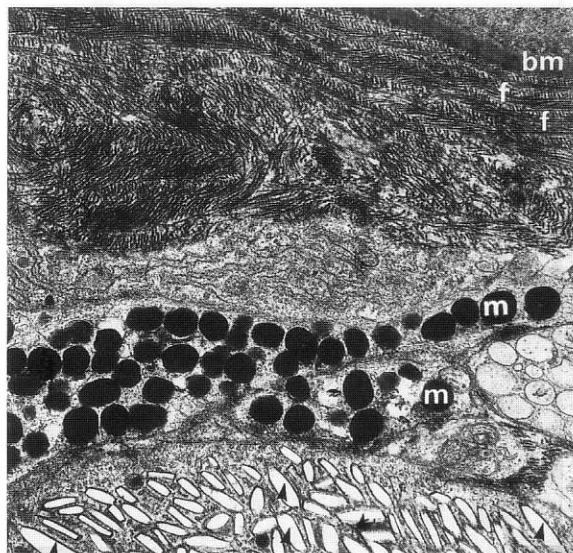


Fig. 2.5. Electron micrograph of the stratum spongiosum of the dermis of the plaice. bm = basement membrane; f = collagen fibres in layers; m = melanin granules. Arrows indicate iridophore platelets. EM $\times 2800$.

THE MUSCULOSKELETAL SYSTEM

The fusiform shape of the typical fish is determined by the requirements of swimming. The streamlined exterior minimizes drag and the main muscle blocks (myomeres) are arranged on either side of the axial skeleton in order to bend

the body laterally to generate propulsive forces by oscillation of body and tail (Gray 1968, Videler 1993).

Axial skeleton

The layout of the skeleton can be seen in Figs 2.6, 2.7 and 2.8. The skull consists of a rigid cranium to which are articulated the bones of the jaws and branchial and opercular apparatus. The structure is very complex and much of the skull moves during feeding and breathing movements. All the components are interdependent and the structure of the skull is best understood in the context of a description of breathing movements such as that of Ballintijn and Hughes (1965).

The number of vertebrae is not constant in a given species and is affected by environmental conditions during larval development. Each vertebral centrum is a simple cylinder, the 'cross' seen on radiographs reflecting the conical recesses enclosing the intervertebral pad. The edges of adjacent centra are connected by ligaments and the whole column is held together by longitudinal elastic ligaments which run dorsal and ventral to the vertebrae. All the vertebrae have a neural arch and a neural spine, the caudal vertebrae also having a ventral haemal arch and haemal spine. In the thoracic region, instead of the haemal arch, there are pleural ribs which support the lateral walls of the body cavity. In many species, intermuscular bones of various arrangements also radiate out from the vertebral column in the septa between the myotomes.

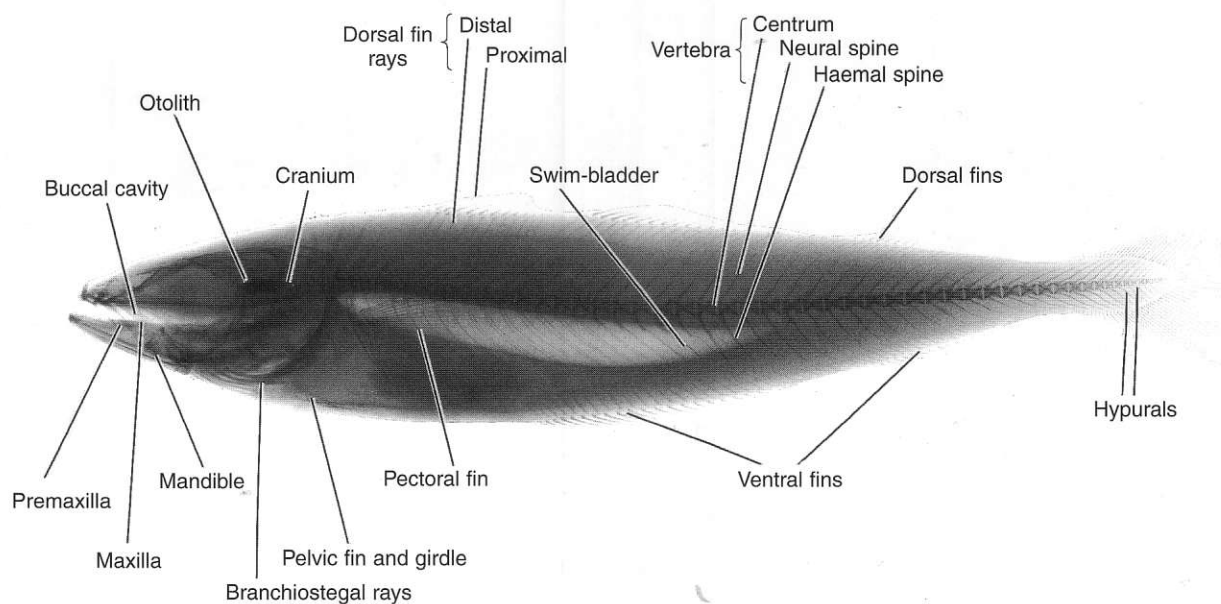


Fig. 2.6. Radiograph of a typical round fish, the saithe.

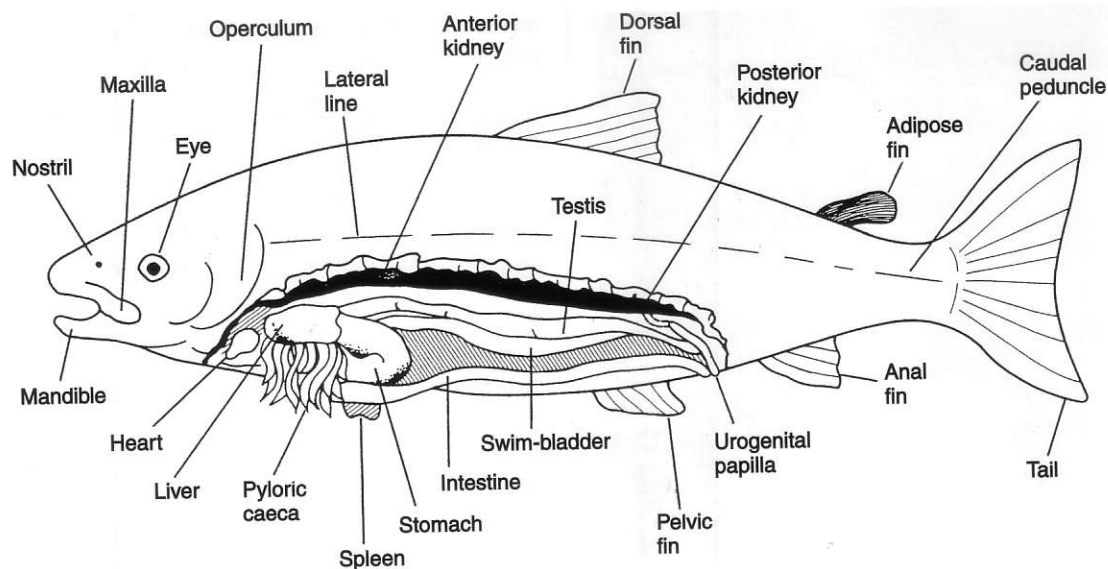


Fig. 2.7. Diagram of the basic anatomy of a salmonid fish.

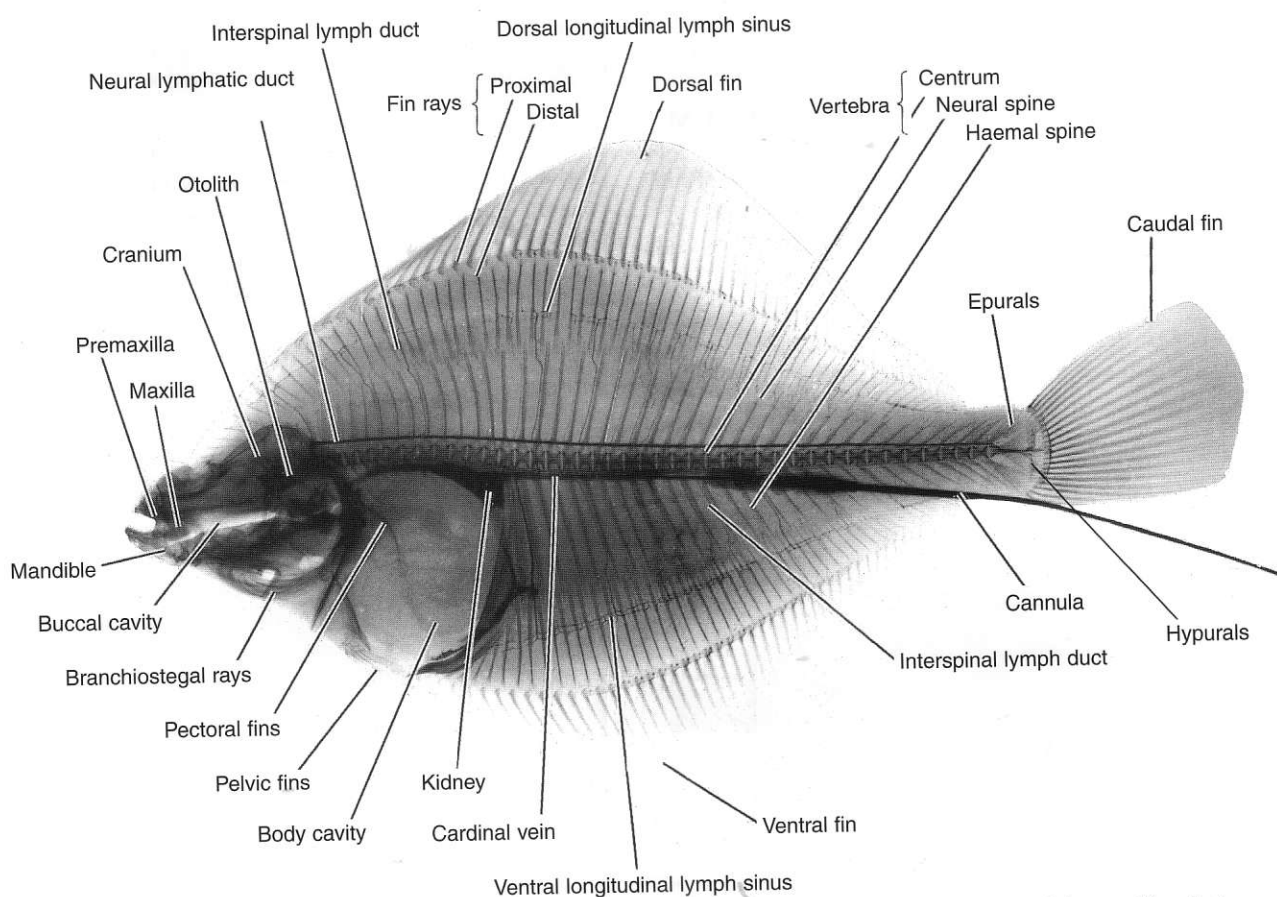


Fig. 2.8. Radiograph of a typical flatfish, the plaice. The injection of a radiopaque substance via a cannula has delineated the neural lymphatic duct and the renal portal vein. (By courtesy of Dr C.R. Wardle.)

Fins

The pelvic girdle in lower teleosts (e.g. salmonids) is embedded in the ventral body musculature. In more advanced types it is in a more anterior position resting against the pectoral girdle. The pectoral girdle is suspended immediately behind the opercular region of the skull.

The median dorsal and ventral fins are articulated to the pterygiophoral muscles which continue the line of the neural and haemal spines. The caudal fin is articulated on a series of flat plates, the dorsal epural and ventral hypural bones.

The fin rays can be of two types: spiny or soft. The structure of the fin rays of the teleosts was used at one time to separate them into two major groups: the *Malacopterygii* (which is roughly equivalent to the *Isospondylii* of present systematics) or soft-rayed species, and the *Acanthopterygii* (roughly equivalent to the present day *Perciformes*). The spiny rays are simple single bones as in the first dorsal fin of *Perciformes*. The caudal fin rays in all teleosts are of the soft kind, as are also all the other fins in *Isospondylii* (e.g. *Salmonidae*, *Clupeidae*). The soft rays are segmented, often branched and formed of two identical lateral components either side of the midline. The fin web of wild fish is clear and very fine but in farmed fishes it is often thicker.

Bone

The microscopic structural elements of fish bones are similar to those of other vertebrates and generally two types of bone are found, cellular and acellular. The former contains osteocytes and is confined to lower orders, e.g. *Clupeidae*, *Salmonidae* and *Cyprinidae* (Fig. 2.9). Acellular bone is unique in vertebrates; it contains no osteocytes and is found in advanced teleosts such as *Percidae* and *Centrarchidae*, often having a solid featureless matrix (Moss 1965).

The lack of cells has been shown to preclude resorption of calcium from the bones so that acellular bones cannot function as a calcium reserve. Repair of fractures under acalcaemic conditions in advanced teleosts is therefore hindered (Moss 1965).

Despite the presence of vascular canals and 'marrow' spaces in some bones of both main types, no haemopoietic tissue is present in such spaces. It is evident that in teleosts there are major departures from other vertebrates in bone structure and physiology.

Muscles

Most fish swim by passing a wave of increasing amplitude posteriorly along the body. This is most evident in the movement

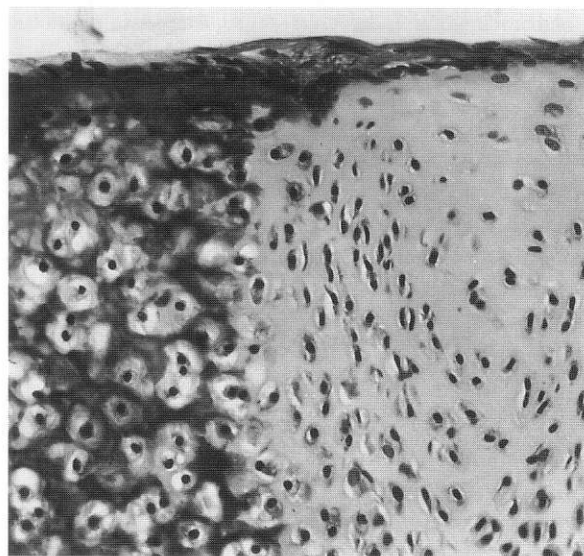


Fig. 2.9. Section through a cranial cartilage of a young rainbow trout at the edge of a centre of ossification. The darker area is the calcified component. H + E $\times 300$.

of eels (anguilliform movement) in which the wave is generated by sequential contraction from head to tail of the muscle blocks or myomeres. In shorter-bodied, more typical, fish, the mechanism is the same, but during swimming the flexure of the body shows less than a complete wave and only the oscillation of the tail is really apparent (carangiform locomotion). Some fish swim by sculling or waving motions of certain fins, in which case the appropriate muscles are highly developed and the main myomeres may be considerably reduced (e.g. sea horse).

The most obvious feature of the muscle of a round fish is the folding and interlocking of the myomeres (Fig. 2.10). Externally, the body muscles occupy the quadrants of the body, separated from each other by the median septum and the transverse horizontal septum. The two blocks of muscles dorsal to the horizontal septum are called *epaxial muscles*, while those ventral are called *hypaxial muscles*. Superficially, both epaxial and hypaxial myomeres are folded in the vertical plane. However, dissection of the myomeres shows that they are secondarily folded in the horizontal plane so that below each flexure in the epaxial and hypaxial myomeres the muscle extends backwards and towards the median septum as posterior cones. At the horizontal septum the muscle projects forwards and inwards as a single anterior cone. Thus contraction within an arrangement of epaxial and hypaxial muscles causes the body to bend (Fig. 2.11).

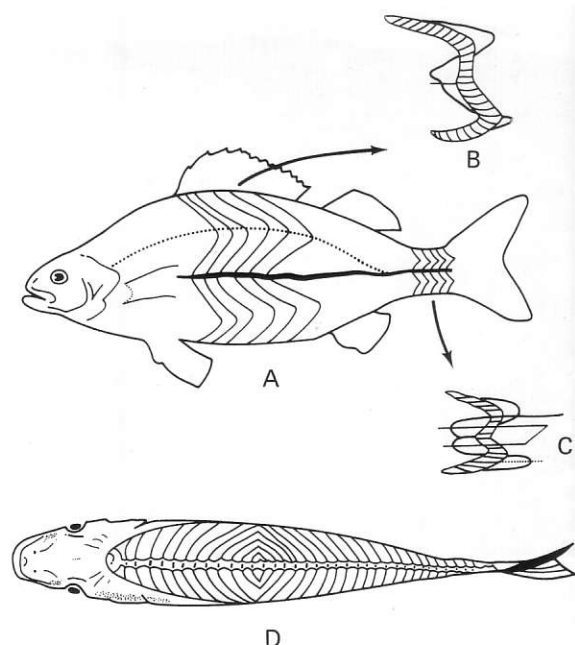


Fig. 2.10. Patterns of folding of myomeres of a typical teleost. A, Lateral view with details of superficial folding of epaxial (1) and hypaxial (2) muscles. B, C, Secondary folding of the myomeres from trunk and caudal peduncle respectively. D, Plane view of section through epaxial muscles to show the arciform appearance of the myomeres about the median septum. (Redrawn from Greer-Walker 1970.)

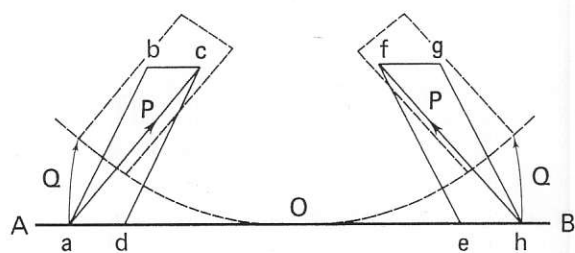


Fig. 2.11. Action of forces applied by a myomere to bend the body. The line AB represents the median septum and axial skeleton. The parallelograms *abcd* and *efgh* represent the anterior and posterior cones of a single myomere in the relaxed condition. The broken lines indicate the positions and shapes of these components after contraction of the myomere. The diagonal lines within the anterior and posterior cones indicate the direction of the resultant forces acting within during initial contraction and tend to bend the median septum and axial skeleton through the arcs Q. (After Nursall 1956.)

The folding and interlocking of myomeres produces smooth sequential contraction along the body, since the contraction of an individual myomere also influences several overlying and adjacent myomeres. The muscle fibres in the superficial layers of the myomere run in an anteroposterior direction, parallel to the median septum. Contraction therefore occurs in the plane of the axis of the fish. However, the deeper down within the myomeres the fibres are, the more they are angled to the axis of the fish. Thus the extent of shortening in this plane will be reduced the closer the fibres lie to the median septum. This clearly compensates for the changes in absolute velocities with distance from the centre of the bend and prevents folding of the skin.

Histological and biochemical examination of the myomeres has revealed a range of fibre types which are organized in many species as distinct zones. In most teleosts there are two main subdivisions: the *muscularis lateralis superficialis*, consisting of the so-called red muscle fibres, and the *muscularis lateralis profundus*, which consists of white fibres (Fig. 2.12). From mechanical, electrophysiological and biochemical differences it has been shown that the red fibres are aerobic, slow-contracting, fibres, similar to their counterparts in mammalian muscle, and that the white are anaerobic, fast-contracting and fast-fatiguing fibres (Jayne & Lauder 1993). In the Salmonidae and to an extent in Cyprinidae, sandwiched between the red and white are found the pink fibres which appear to be intermediate in function between the red and white fibres. Also, in salmonids, the bulk of the myomere is made up of a mosaic of white and pink fibres rather than white fibres only (Bilinski 1974).

In gadoids the red fibres are about 50 μm in diameter and the white about 100 μm , but this varies considerably with development, exercise and starvation (Greer-Walker 1970, 1971). Electromyographic studies have shown that the red muscle is active during low cruising speeds and the white is recruited as mosaic during strenuous swimming, particularly in sprints or bursts of activity.

There are also differences in vascularization between the fibre types, the red muscle being generously supplied with blood and providing a good site for the injection of drugs, anticoagulants and anaesthetics, since it only takes a few seconds for material to reach the central nervous system and other parts of the body.

The innervation of the types of myofibrils is also quite different. In the red muscle the nerve endings occur *en grappe* in the middle of the muscle fibres, while in white muscle the nerve endings are *en platte* and are terminal where the white fibres arise from the myosepta. In higher teleosts, however, white muscle has multiple, *en grappe* innervation. The pink

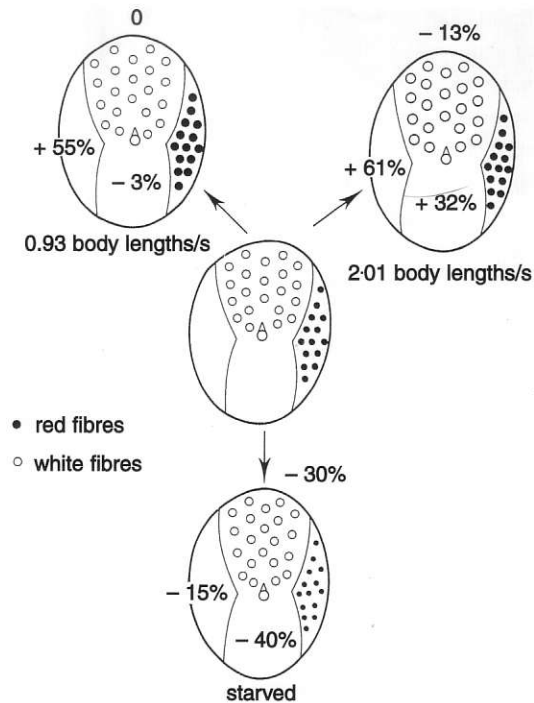


Fig. 2.12. Transverse sections of saithe at point 0.34 of the body length from the tail. The open circles represent the larger diameter, fast-contracting white muscle fibres, while the closed circles represent the slow, red fibres. The percentage change in diameter of red and white muscle fibres following exercise at two different swimming speeds and starvation, compared with the control, are indicated within each muscle mass. The percentage decrease in bodyweight (indicated above each section) is represented as a decrease in total cross-sectional area. (Redrawn from Greer-Walker 1971.)

fibres have an innervation intermediate between these two extremes. Although the transmitter in all cases is acetylcholine, there appear to be differences in the receptor sites, as shown by the varied responses to drugs like dexamethonium, which blocks red receptors preferentially. The neurones of white motor units tend to be of large diameter and myelinated, while the neurones of red muscles are slightly or non-myelinated and thus seem thinner. The form of action potentials in these neurones also differs in time-course and amplitude. This distinction between red, pink and white fibres is also found in the respiratory and fin muscles according to the nature of their mechanical function.

THE RESPIRATORY SYSTEM

The area of epithelium of the gills is comparable to the total area of the skin and in many species is considerably larger,

making its structure a major consideration in the homeostasis of the *milieu interieur* of the fish. The epithelium is thin to allow gas exchange and this also renders it particularly vulnerable to invasion by pathogens. As well as having a respiratory function, the gills are responsible for regulating the exchange of salt and water and play a major role in the excretion of nitrogenous waste products. Even slight structural damage can thus render a fish very vulnerable to osmoregulatory as well as respiratory difficulties (Hughes & Morgan 1973).

The structure of the gills

The gills of a typical teleost comprise two sets of four holobranchs, forming the sides of the pharynx (Fig. 2.13). Each holobranch consists of two hemi-branches projecting from the posterior edge of the branchial arch or gill arch in such a way that the free edges diverge and touch those of the adjacent holobranchs. Close examination of the hemi-branches of a fresh gill shows that they consist of a row of long thin filaments, the primary lamellae, which project from the arch like the teeth of a comb. The surface area of each primary lamella is increased further by the formation of regular semilunar folds across its dorsal and ventral surface—the secondary lamellae. The dorsal and ventral rows of secondary lamellae on each primary are staggered so that they complement the spaces in the rows of lamellae of adjacent filaments (Fig. 2.14). This arrangement of arches and lamellae forms the sides of the pharynx into two sets of corrugated sieves through which the water must flow.

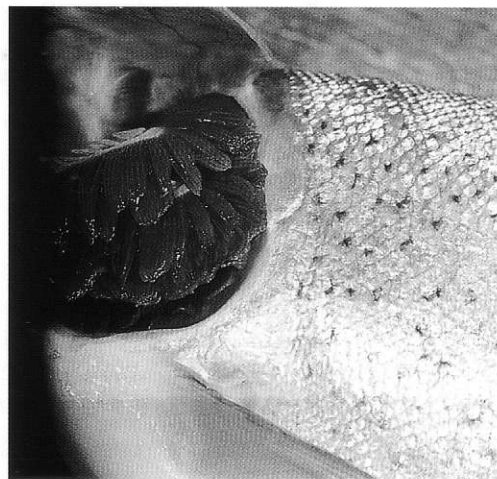


Fig. 2.13. The opercular cavity of a rainbow trout showing the gill arches, with rakers, and primary lamellae.

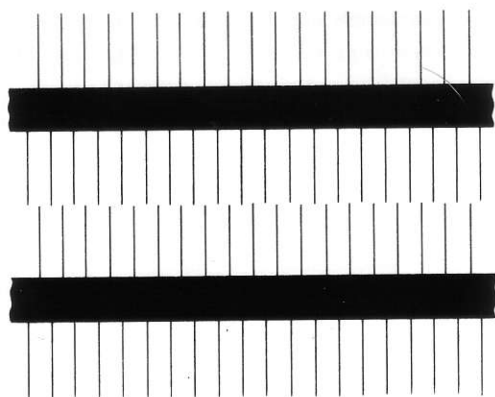


Fig. 2.14. The interdigitating profile presented by the arrays of adjacent secondary lamellae to the direction of water flow. (After Hughes 1961.)

The gill arch

The teleost gill arch is a curved osseous structure from which radiate the bony supports (the gill rays) of the primary lamellae. The angle of these lamellar rays can be altered by a set of adductor muscles to adjust the amount of ventilation of the lamellae (Fig. 2.15). Also contained within these arches are the afferent branchial arteries from the ventral aorta and the efferent branchial arteries serving the dorsal aorta. The ventral aorta divides into numerous fine branches as it passes up the holobranchs. The afferent filament arteries run along the

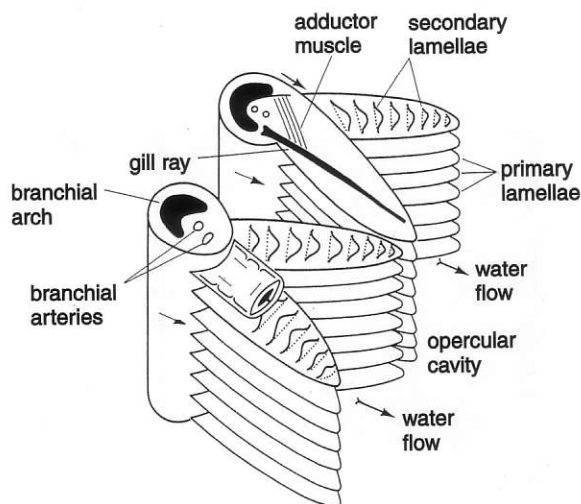


Fig. 2.15. Diagrammatic representation of segments of two adjacent gill arches, showing the flow patterns of water and blood. In the upper segment the configuration of the branchial arch, the gill ray and the adductor muscle for a single primary lamella is indicated. (After Hughes 1961.)

opercular edge of the primary lamellae, which is downstream of the water flow. Blood enters the blood spaces of the secondary lamellae by short afferent lamellar arteries. This deoxygenated blood flows in the opposite direction to water pumped through the gill sieve. The resulting countercurrent exchange leads to 60–80% of the oxygen in water being transferred to the blood. Oxygenated blood leaves the secondary lamellae by efferent lamellar arteries to feed the dorsal aorta by way of efferent filament and efferent branchial arteries. Within the primary lamellae oxygenated blood is shunted through a nutritive circuit to the active tissue of the gills.

The gill arch is covered by typical teleost epidermal tissue but at the origin of the primary lamellae the epidermis is much thicker and usually extremely well endowed with mucous cells. Below this epidermis there is usually an array of lymphoid tissue, comprising lymphocytes and, in many species, large cells containing eosinophilic granules. These latter are also frequently found along the length of the primary lamella and may occur in large numbers at its tip. The primary lamella is covered by a mucoid epidermis which may have within it, in euryhaline species, the pale-staining saline, or salt-secreting, cells and, beneath these, lymphocytes, and eosinophilic granule cells, and phagocytic cells, varying in number between species.

The secondary lamellae

Gaseous exchange takes place across the surface of the secondary lamellae (Fig. 2.16). Essentially these consist of an envelope of epithelial cells, usually one layer thick, supported and separated by pillar cells, which are arranged in rows 9–10 μm apart. Where the pillar cells impinge on the basement membrane of the epithelial envelope they spread to form flanges which coalesce with those of neighbouring pillar cells to complete the lining of the lamellar blood channels which connect the afferent and efferent lamellar arteries (Hughes 1975).

The pillar cells have been shown to contain columns of contractile protein similar to that found in amoebae. They also invest columns of extracellular connective tissue within their cell membrane. Since the blood entering the lamellar blood spaces comes directly from the ventral aorta at high pressure, the presence of fibrous and contractile elements in the supports of these spaces will serve to resist their distension under normal circumstances (cf. lamellar telangiectasis) (Fig. 2.17). Although it has been suggested that pillar cells are used to control lamellar perfusion, there is, as yet, no experimental evidence to support this hypothesis. It has been shown,

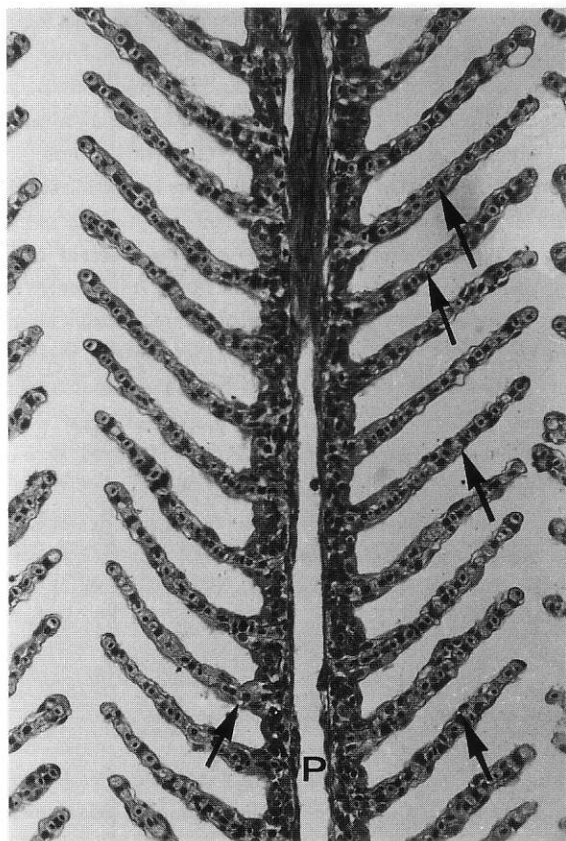


Fig. 2.16. Section through the gill lamellae of a rainbow trout showing the primary lamella (P) with its arrays of delicate secondary lamellae (arrowed). H + E $\times 100$.

however, that from standard to maximum aerobic metabolic rate the functional area increases by a factor of 6, mainly by recruitment into activity of lamellae from the base of the primary lamellae distally. Regulation of branchial blood flow is not fully understood. Involvement of autonomic control has been shown through the action of neuro-transmitters and endocrines on branchial resistance to blood flow. Adrenaline and noradrenaline both reduce gill resistance, acting through β receptors. Acetylcholine acts antagonistically to increase blood pressure by way of muscarinic receptors. The sites of these receptors have not been identified but the afferent and efferent lamellar arteries and the arteriovenous connections to the branchial nutritive circuit have been suggested as likely locations (Perry *et al.* 1992).

Restriction of blood flow through the lamellae has three main effects:

1. Reduction in rate of gaseous exchange.

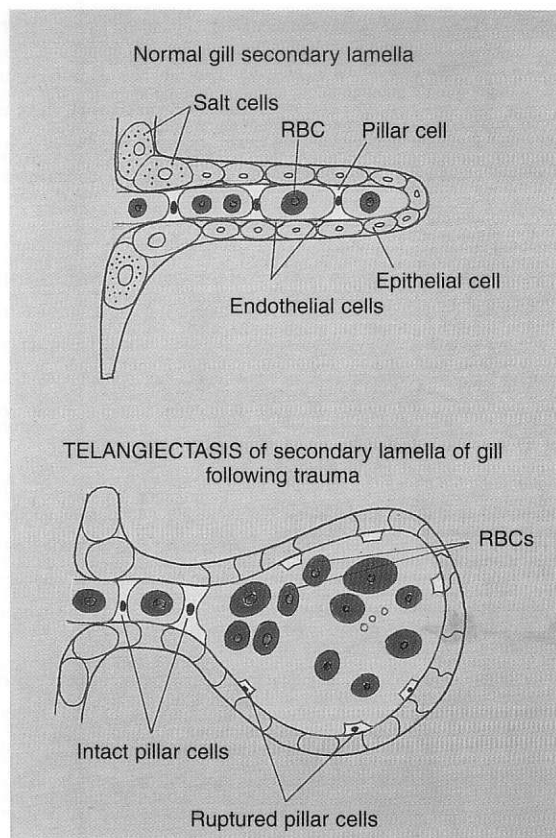


Fig. 2.17. Diagrammatic representation of section through secondary lamella of gill showing location of pillar cells and effect of pillar cell rupture to produce lamellar telangiectasis.

2. Reduction in exposure of body fluids to ionic exchange.
3. Reduction of the size of the pressure loss *en passage* through the gills, possibly by shunting blood directly from afferent to efferent lamellar arteries, thus enhancing the efficiency of the systemic circulation.

The surface of the lamellar epithelium is irregular, although not, as with the epidermal surface, thrown into such distinctive microvilli. These irregularities serve to aid attachment of the cuticular mucus, which, in addition to its role in reducing infection and abrasion, has a significant role in regulating the exchange of gas, water and ions.

The combined thickness of cuticle, respiratory epithelium and flanges of the pillar cells ranges from 0.5 to 4 μm , and represents the total diffusion distance for respiratory exchange, since the diameter of the lamellar blood channel is virtually the same as the diameter of the teleost erythrocyte.

The heart

The heart in teleosts is situated inside the pericardium anterior to the main body cavity and usually ventral to the pharynx. It has four chambers through which blood flows in simple succession (Fig. 2.19). Deoxygenated venous blood enters the sinus venosus from the ductus cuvieri and main veins. There are no inlet valves and the sinus is so small that it can hardly be recognized as a discrete cardiac chamber. The wall is thin, composed mainly of collagenous connective tissue, although in some species it is muscular and contractile. It is in the wall of the sinus venosus that the pacemaker, which initiates the cardiac contraction, is located.

Through two sino-atrial valves the blood passes into the atrium, which lies dorsal to the ventricle. The atrium has a thin wall, and muscular trabeculae traverse the lumen in a loose meshwork. The endothelial lining is therefore large in area and in some species has a phagocytic activity as part of the reticuloendothelial system. Contraction of the atrium forces the blood through valves into the ventricle.

The ventricle has a much thicker wall than the atrium, and in normal histological sections only a minimal lumen is apparent. There is a distinct outer compact layer of muscle and an inner spongy layer with numerous trabeculae. The thickness of the compact layer is related to the scope for activity, being almost absent in less active species such as pleuronectids. Coronary vessels run over the outside of the ventricle, supplying the compact muscle, the spongy muscle obtaining most

of its oxygen supply from the 'venous' blood in the lumen. Fishes with high activity levels, such as the tunas, may have coronary supply to the spongy layer as well (Tota 1989).

Individual cardiac muscle fibres are approximately 6 μm in diameter, about half that of mammalian muscle. In other respects the fibres are similar to mammalian ones, with intercalated discs between individual cells. From the ventricle the blood is passed into the bulbus arteriosus through a pair of valves. The bulbus has a thick wall composed of a mixture of elastic tissue and smooth muscle (Priede 1976). It has a complex structure but acts basically as a passive elastic reservoir which smooths the pressure pulse from the ventricle and maintains blood flow during ventricular diastole. The elastic tissue of the bulbus is very different in structure from that of the elastica of arteries.

The whole of the heart is enclosed in the visceral pericardial sac, which is intimately associated with the cardiac surface. The parietal or outer pericardial sac lines the pericardial cavity. The pericardial space is filled with serous fluid, separating the two membranes.

The electrocardiogram (ECG) of the teleost heart is usually similar to that of other vertebrates (Fig. 2.20), peaking at about 70 mV on the QRS wave. The conduction velocities are slower than in mammals, being temperature-dependent.

Typical ventricular systolic pressures are 30–70 mmHg (4–9 kPa) and diastolic 10–30 mmHg (1.5–4 kPa). Heart rates vary considerably according to temperature, falling as low as 15 beats/min for trout at 5°C. At 15°C the maximum heart rate is about 100/min.



Fig. 2.19. The heart of an Atlantic salmon showing the muscular ventricle (v), the fibroelastic bulbus and the dark, soft atrium (a).

Arteries

The ventral aorta runs forward from the heart and distributes blood to the gills via the afferent branchial arteries. The

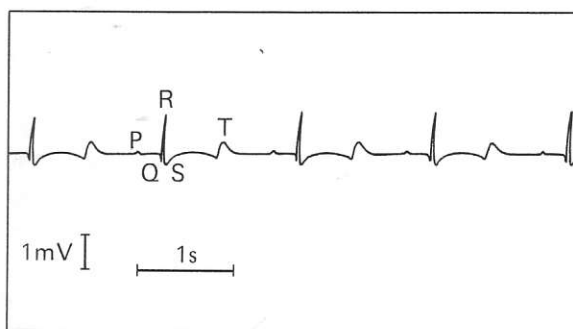


Fig. 2.20. A typical electrocardiogram of a teleost as recorded by external contact electrodes. (By courtesy of Dr R.L. Oswald.)

arteries afferent to the gills have a normal vertebrate arterial structure with three layers in the wall: adventitia on the outside, media and intima.

The endothelium comprises flattened cells, which can usually be distinguished only by their dark-staining nucleus, which bulges into the lumen. Contiguous cells interdigitate so that the endothelium forms a continuous surface. There is a fine basement membrane beneath the endothelium, but this is visible only with the electron microscope. The intima is largely elastic tissue and the media is composed of elastic tissue laminae with smooth muscle cells in between. The wall of the ventral aorta is highly resilient and can contract to make adjustments in blood flow (Kirby & Burnstock 1969). The outer adventitia is thin and composed mainly of collagen fibres.

The efferent branchial arteries join, dorsal to the pharynx, to form the dorsal aorta, the precise pattern varying in different species. From the first efferent branchial some blood flows through the pseudobranch and thence to the eyes and cranium. Also in this region, arteries branch ventral to the pharynx to supply the hyoidean and coronary systems.

There is a considerable pressure drop across the gills so that a typical mean blood pressure in the ventral aorta of trout is about 50 mmHg (6.5 kPa) and in the dorsal aorta 25 mmHg (3 kPa). This is reflected in the structure of the arteries in that the efferent vessels have thinner walls with a smaller amount of elastic tissue and muscle. The dorsal aorta in fish can be regarded as being intermediate between artery and vein in structure. In the dorsal aorta of lower teleosts, including salmonids and clupeids, there is an elastic ligament stretched along the length of the lumen of the dorsal aorta which can act as an auxiliary 'heart', automatically increasing circulation to the muscles during swimming movements (Priede 1975). Along the length of the dorsal aorta there are lateral branches to the body musculature, the viscera being supplied mainly by the anterior mesenteric artery.

Veins

The veins of fish, as with other vertebrates, are relatively indistensible and have walls composed mainly of collagen. The major veins are large in diameter, and pressures are low, being less than 10 mmHg (1.5 kPa), although there is no evidence of negative pressures such as occur in the sharks.

There is renal portal drainage through the kidneys, mainly from the caudal region, and from the viscera there is a typical vertebrate hepatic portal system. Valves are not common in the teleost venous system.

Control of circulation

Cardiac output can be varied considerably, changes in stroke volume being more pronounced than changes in heart rate. The heart has a vagal inhibitory innervation and in some species an adrenergic stimulatory innervation has also been demonstrated (Gannon & Burnstock 1969). The heart also responds positively to increase in venous return in accordance with Starling's law. General increase in circulation during exercise can be accounted for by the action of circulating catecholamines on α -receptors in various parts of the body. Vasomotor nerves have also been demonstrated and, although it is apparent that there are many interspecific differences in the teleosts, many of the mechanisms familiar to mammalian physiologists have their counterparts in teleosts.

Capillaries

In mammals the capillary blood (hydrostatic) pressure opposes an equal blood colloidal osmotic pressure across the capillary wall. Due to the low arterial pressure this would seem to be impossible in fish but the capillaries are highly permeable so that the osmotic pressure across the wall is much lower than in other vertebrates (Hargens *et al.* 1974). The interstitial fluid has a high protein concentration and, effectively, plasma runs quite freely through the capillary walls. Thus fluid balance in teleosts is fundamentally different from that in mammals so that, for example, quite large changes in plasma concentration can be readily tolerated.

Lymph

The lymph drainage system of fish is very extensive, probably because of the high capillary permeability. The lymph volume is about four times the blood volume (Wardle 1971) and its composition is almost identical to that of blood plasma. In the main bulk of the myomeres the lymphatic circulation is the only circulation available since there are no significant blood vessels in the white muscle. There are various lymph propulsors or 'lymph hearts' along the length of major lymphatic vessels which aid lymph return during breathing movements (Kampmeier 1969).

A unique feature of fish circulatory systems is the presence of a secondary circulation, arising as narrow coiled arterial vessels from the gill primary vessels and also from arterial supply to various surface areas such as skin and gut. Because the majority of the blood cells are directed via the primary

circulation system at the bifurcations of the two systems, the blood in the secondary circulation is normally of lower haematocrit and lower pressure. Its circulation time may therefore be of the order of hours rather than the minutes required for the primary circulation (Steffenson & Lomholt 1992; Iwama & Farrell 1998).

Haemoglobin and gas transport

Most teleosts have haemoglobin in their erythrocytes as do other vertebrates. Since blood temperatures are often low, much oxygen can be carried in simple solution in the plasma and so certain polar fishes have no haemoglobin. There is considerable variation in fish haemoglobins, up to four types occurring within an individual, each with its own characteristics. Also, many fish have different haemoglobins at different stages of development. Species can be adapted to different environmental oxygen tensions, and acclimatization to different temperatures entails modification in oxygen dissociation characteristics.

The Bohr effect, whereby increase in PCO_2 or reduction in pH lowers the affinity for oxygen, occurs particularly in fish adapted to conditions of high oxygen and low carbon dioxide content. Fish living in acidic waters of low oxygen content would not benefit from a Bohr shift.

In many fish there is an additional phenomenon where low pH lowers the total oxygen-carrying capacity of the haemoglobin as well as shifting the dissociation curve. This so-called Root effect is unique to the teleosts. It greatly facilitates unloading of oxygen to the tissues and is of great importance in secretion of oxygen at high pressure in the gas gland of the swim-bladder and in the choroid plexus of the eye. The overall exchange of respiratory gases at the gills and tissues is greatly influenced by the rate of loading and unloading of oxygen to the haemoglobin of fish blood. In the gills the pH is about 7.4 and here the rate of oxygenation is approximately four times faster than the rate of deoxygenation. But at low pH, typical of actively metabolizing tissue, the process is reversed and the rate of deoxygenation is 400 times faster than that of oxygenation (Forster & Steen 1969; Hughes & Koyama 1974).

In the transport of carbon dioxide the turnover occurs mainly in the bicarbonate component of the blood, and direct combination with haemoglobin to form carbamino compounds is small. To this end the fish erythrocyte contains the enzyme carbonic anhydrase, which facilitates the conversion of carbon dioxide to bicarbonate. The capacity for carrying carbon dioxide is significantly higher in deoxygenated than in

oxygenated blood and is reduced considerably with increase in temperature. At low temperatures the slope of the carbon dioxide dissociation curve is also greater. The amount of carbon dioxide, which can be carried by the blood, and the potential turnover at the gills is therefore greater at low temperatures (Eddy 1971).

BLOOD COMPOSITION

Blood volumes of teleosts are small compared with all other classes of vertebrates, being in the region of 5% of body weight.

Plasma

The composition (mg/100 ml) of brown trout serum is given by Wolf (1963) as chloride 424, sodium 358, magnesium 2.3, potassium 20.1, calcium 12.5, phosphorus 9.3, sulphate 0.8 and whole blood glucose 71; freezing point depression is about 0.57°C . This is remarkably similar to mammalian serum and indeed mammalian salines have been successfully used for fish tissue culture. Frog Ringer solution is low in NaCl and KCl, is hypotonic and is unsuitable for fish work.

Plasma protein concentrations are lower than in man (7 g/litre), values from 1.68 to 6.19 g/litre having been recorded in different species of teleosts. The immunological and other functions of the proteins are broadly similar to those in higher vertebrates but many interspecific differences remain to be investigated.

Cellular components of blood

The cellular components of fish blood differ from those of higher animals principally in relation to the nucleation of the red cells and the presence of nucleated thrombocytes. These, rather than the anuclear platelets of higher animals, are the source of prothrombin. There are also differences between species with regard to particular elements and the roles of the neutrophil and eosinophil are less obvious in the teleost. An excellent comparative review of the blood cells of teleosts and their tinctorial properties is given by Yokote (1982).

Erythrocytes

The teleost erythrocyte is similar in size, tinctorial properties and ultrastructure to that of the other vertebrates but, like the avian and reptilian erythrocyte, it is nucleated (Fig. 2.21).

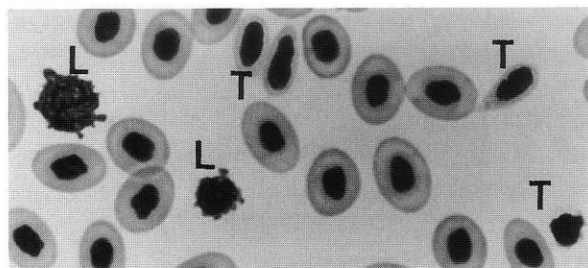


Fig. 2.21. Erythrocytes and various white cells from blood smear of plaice. L = lymphocytes, T = thrombocytes. Leishman $\times 1000$.

Numbers vary with species and are also affected by stress and environmental temperature, but they usually range between $1.05 \times 10^6/\text{mm}^3$ and $3.0 \times 10^6/\text{mm}^3$. Immature erythrocytes, known as polychromatocytes, represent approximately 1% of the total number and are rounder and bluish-grey in Giemsa-stained smears. Haemoglobin is, as in other vertebrates, the main vehicle for transport of oxygen and to a lesser extent carbon dioxide, but unlike in mammalian erythrocytes, where anaerobic metabolism predominates, in the teleost erythrocyte, cell metabolism is primarily oxidative phosphorylation, resulting in the production of ATP.

Neutrophils

The term neutrophil, or polymorphonuclear leucocyte, is drawn from human histology. Since the granules are not necessarily neutral-staining, and the nucleus may not be multi-lobed, in other species of animal the terms heterophil or, in fish, 'type I leucocyte', have been suggested, but in view of its wide usage the term neutrophil will be used in this description.

Neutrophils have been identified in teleosts on ultrastructural and histochemical grounds. Evidence of phagocytic activity, such as is found in mammalian neutrophils, is also available, and they are commonly found at sites of inflammation (Figs 2.22 and 2.23). Neutrophils in fish are present in about the same numbers as in mammals ($3-6 \times 10^3/\text{mm}^3$) but they comprise a much smaller proportion of the blood leucocyte population (about 6-8% in fish compared with 60-70% in mammals).

Morphologically, fish neutrophils closely resemble their mammalian counterparts though the degree of nuclear polymorphism in teleosts varies considerably. The histochemical characters of plaice neutrophils have been extensively studied by Ellis (1975) and in most respects they bear close resemblance to mammalian neutrophils, being positive with periodic acid-Schiff (PAS), Sudan black B and the benzidine-peroxidase, acid

and alkaline phosphatase tests. Ultrastructurally, the specific granules are oval in shape and exhibit a fibrous appearance. They bear a resemblance to one of the less commonly found granule types of mammalian neutrophils (Ferguson 1975a).

Release of neutrophils into the blood, causing a neutrophilia, is known to occur as a non-specific response to a variety of stress stimuli in mammals and fishes. This is probably mediated through the pituitary-adrenal axis.

The origin of teleost neutrophils is most probably the haemopoietic tissue of the kidney, though the spleen may play a minor role. In smears of teleost kidney, granuloblasts are seen in large numbers and may be characterized by their histochemical properties. They are similar in morphology and staining properties to their counterparts in mammalian bone marrow, the myeloblasts and myelocytes. There is little information on the lifespan of teleost neutrophils but they probably have a rapid turnover time of about 5 days, as in mammals.

Monocytes

Monocytes are partially differentiated end cells, which, under appropriate circumstances, will develop into mature cells of the mononuclear phagocyte system but are not capable of further division. In teleost fishes this system is organized as in other vertebrates, with circulating monocytes arising from renal haemopoietic tissue and being readily able to take up a functional tissue role.

Monocytes of fishes form about 0.1% of the circulating leucocyte population, though they increase in number for a short time (about 48 hours) after injection of foreign particulate matter like colloidal carbon. Morphologically they are very similar to mammalian monocytes, which they also resemble histochemically, possessing a few fine scattered granules, which stain positively with PAS and acid phosphatase.

Ultrastructurally the cell membrane is thrown into pseudopodia and the chromatin of the eccentric nucleus dispersed marginally. The lysosomes vary in size and are usually very electron dense. The Golgi apparatus is especially prominent. Monocytes in fishes have been observed to take up foreign particulate material such as carbon and thorotrast, and in pathological processes where melanosomes are released, these are avidly phagocytosed. Their powers of phagocytosis are, however, limited compared with those of higher animals.

Thrombocytes

Thrombocytes are responsible for blood clotting and are important in preventing the loss of tissue fluids from a surface

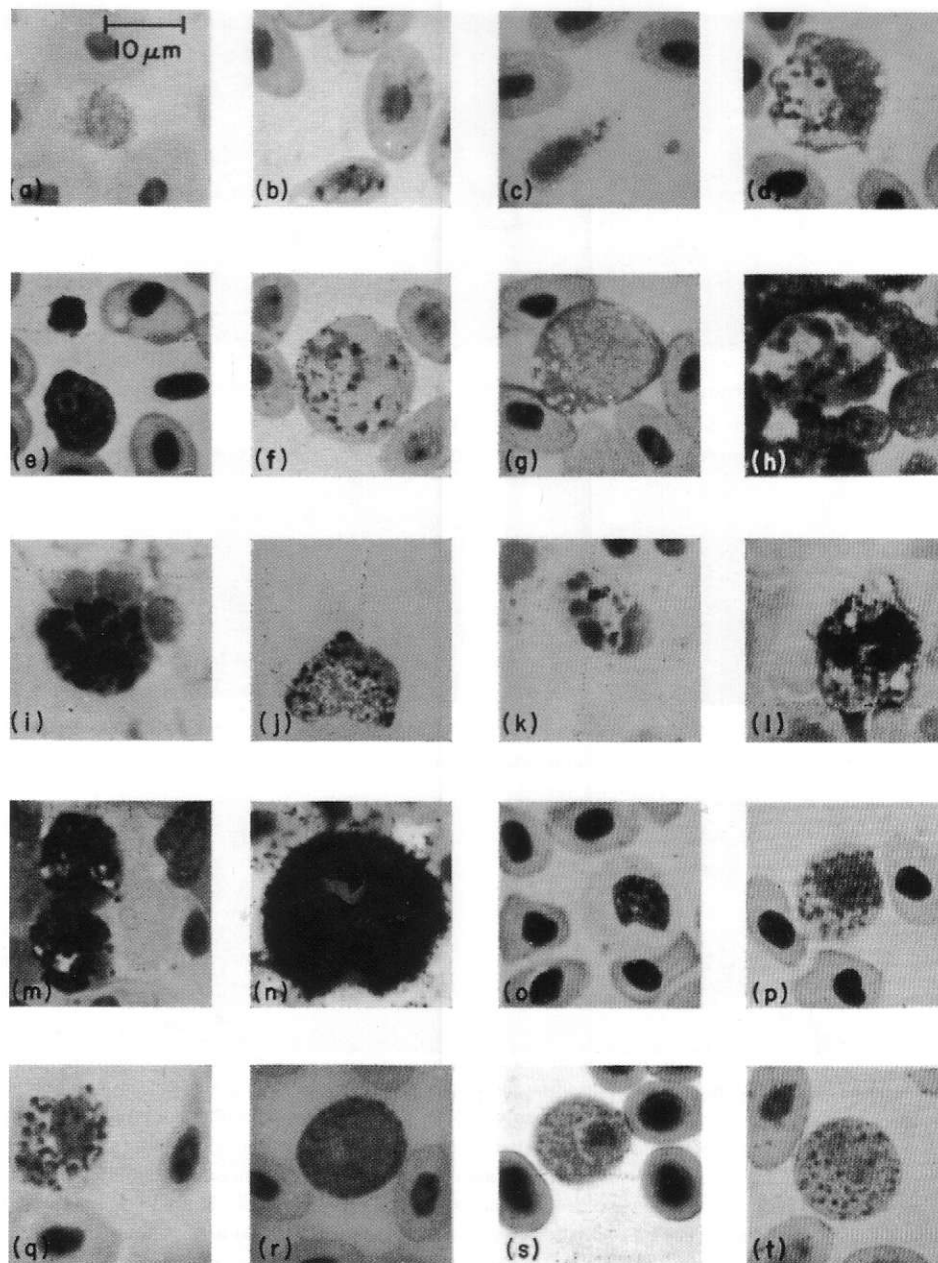


Fig. 2.22. Blood cells of the plaice. (a) Blood lymphocyte with slight PAS positivity of the cytoplasm. PAS $\times 900$. (b) Thrombocyte with several acid phosphatase-positive granules. $\times 900$. (c) Thrombocyte with characteristic PAS-positive granules at the base of the 'spike'. $\times 900$. (d) Blood monocyte containing several carbon particles. Leishman $\times 900$. (e) Blood monocyte in an area of smear disallowing proper spreading of the leucocytes which consequently appear smaller. $\times 900$. (f) Blood monocyte with acid phosphatase-positive granules. $\times 900$. (g) Blood monocyte showing characteristic tinge of PAS-positivity of the cytoplasm. $\times 900$. (h) Macrophage (from thymus smear) containing cellular debris. Leishman $\times 900$. (i) Tissue macrophage containing large granules with acid phosphatase activity. $\times 900$. (j) Peritoneal macrophage with a granular PAS-positivity. $\times 900$. (k) Splenic macrophage filled with free iron compounds. Perl's potassium ferrocyanide $\times 900$. (l) Splenic melanomacrophage containing Perl's-positive material. $\times 900$. (m) Splenic melanomacrophage. PAS $\times 900$. (n) Kidney melanomacrophage with acid phosphatase-positive cytoplasm. $\times 900$. (o) Blood neutrophil. Leishman $\times 900$. (p) Blood neutrophil. Sudan black B and Leishman $\times 900$. (q) Blood neutrophil. Benzidine peroxidase test of Sato & Sekiya and safranin $\times 900$. (r) Blood neutrophil. PAS $\times 900$. (s) Blood neutrophil. Alkaline phosphatase $\times 900$. (t) Blood neutrophil. Acid phosphatase $\times 900$.

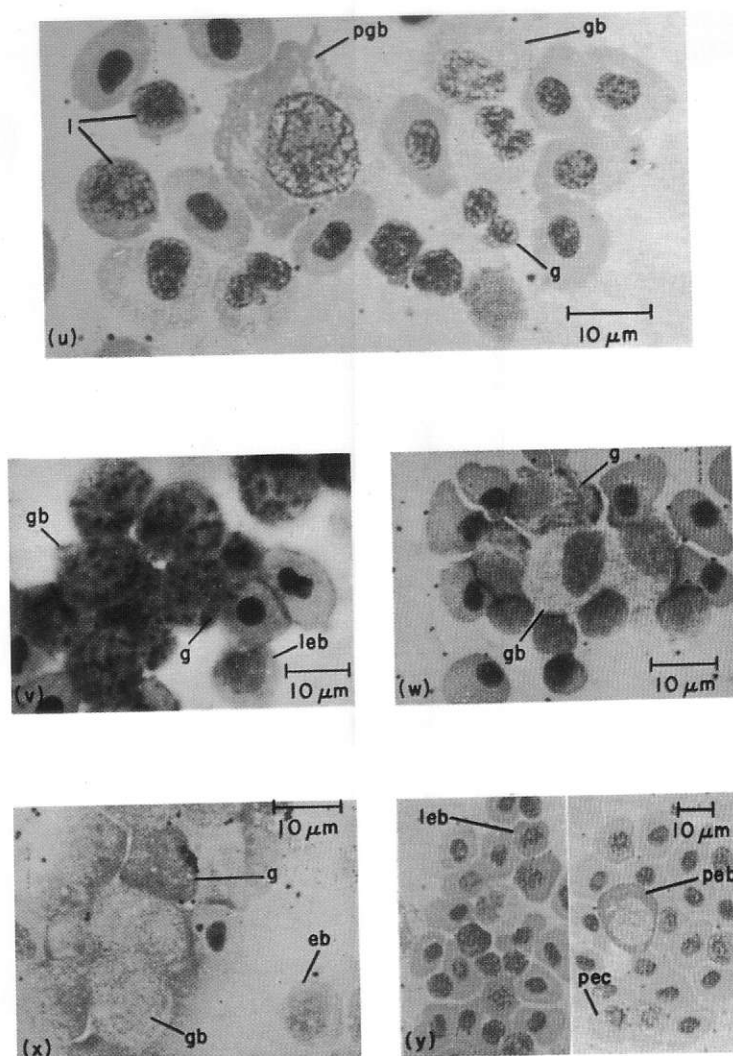


Fig. 2.23. Blood cells of the plaice (continued). (u) Kidney smear showing stages in the development of the neutrophil. Progranuloblast (pgb); granuloblast (gb); mature granulocyte (g); lymphocytes (l). Leishman $\times 1025$. (v) Kidney smear. Stages from the granuloblast to mature granulocyte contain acid-phosphatase granules. The erythrocytic series are negative (gb) granuloblast; (g) granulocyte; (leb) late erythroblast. Acid phosphatase test counterstained haematoxylin $\times 900$. (w) Kidney smear. Granuloblasts (gb) are negative, positively staining granules appear only in the mature granulocytes. Sudan black B counterstained Leishman $\times 900$. (x) Kidney smear. The granulocyte series from the early granuloblast (gb) stain positively whilst the erythrocytic series (eb, erythroblast) are negative. PAS and haematoxylin $\times 900$. (y) Kidney smear showing stages in the development of the erythrocytes. peb, pro-erythroblast; leb, late erythroblast; pec, pro-erythrocyte. Leishman $\times 450$. (From Ellis 1975.)

injury. Thrombocytes are found in all non-mammalian vertebrates. Typically they are elongated cells, often being termed 'spindle cells', though most commonly, one pole of the cell is drawn out into a point. They clot readily and if care is not taken in the preparation of a blood smear the thrombocytes may cast off most of their cytoplasm and appear as small, densely staining nuclei, surrounded by a minute amount of

cytoplasm. It is this 'spent' thrombocyte which has been frequently confused with the lymphocyte. When observed in the living state by phase-contrast microscopy, a refractile vacuole can be seen at the base of the pointed end of the thrombocyte, just anterior to the nucleus. Acid phosphatase and PAS-positive material is associated with that same region.

The ultrastructure of the cytoplasm of the teleost thrombocyte has a remarkable similarity to that of mammalian platelets (Ferguson 1975b). A labyrinth of interconnecting vacuoles ramifies through the cytoplasm and opens via fenestrae to the exterior. Foreign particles can enter this labyrinthine system as in mammalian platelets but active phagocytosis is probably not involved.

The difficulty in distinguishing 'spent' thrombocytes from lymphocytes has led to much confusion regarding counts of these cells. Unless the thrombocytes are preserved in their mature, intact, pointed or spindle forms, then differential counts of these cells will not be reliable.

In plaice blood, Ellis (1975) found, by a specific fluorescent antibody technique, that the ratio of thrombocytes to lymphocytes was 1.4 to 1, total numbers of thrombocytes ranging from 60 000 to 70 000/mm³.

Eosinophils

Eosinophils are putatively considered to play a role in defence mechanisms in mammals by phagocytosing antibody/antigen complexes. They may therefore have an important role in maintaining homeostasis during infection and are particularly numerous when antigens are continually being released, as in parasitic diseases. In mammals, eosinophils comprise only 1–3% of blood leucocytes, though their numbers are modified by certain factors such as hormone levels.

Eosinophils are characteristically packed with large refractile granules which have a high isoelectric point, i.e. they stain with acid dyes like eosin in alkaline medium. Ultrastructurally the granules of mammalian eosinophils possess an electron-dense axial crystalloid, though this does not seem to be a constant feature of the eosinophils of other vertebrates (Kelenyi & Nemeth 1969).

The literature concerning the presence and nature of eosinophils in fishes is notoriously confused, with many claims both of their presence and absence, often in the same species. They are normally reported to be rare in fish blood and most of the descriptions of eosinophils in teleosts refer to the eosinophilic granular cells found in the skin, haemopoietic and digestive tissues, which are almost certainly distinct from the true blood eosinophil. The only criterion for identifying the eosinophil of fishes has been the presence of fairly large eosinophilic cytoplasmic granules.

Fish eosinophils have been implicated in inflammation and some reports of phagocytic activity exist. For example, phagocytosis of bacteria by eosinophils in goldfish and guppies has been reported, while phagocytosis of thorotrast and carbon

particles has been both claimed and denied by different workers.

Basophils and mast cells

The basophils of vertebrates are uncommon granular leucocytes, characteristically containing large basophilic metachromatic granules similar to those of mast cells. The function of basophils is not clear and though they contain 5-hydroxytryptamine (5HT), in resemblance to mast cells, their relationship to tissue mast cells is not established. Like eosinophils they are affected by hormones from the adrenal gland and also seem to be involved, in an as yet undetermined way, in allergic and stress phenomena.

The presence of basophils in fishes, is, like that of eosinophils, claimed by some workers and disputed by others (see review by Ellis 1975). Affirmative reports of their presence liken them to the basophils of mammals in their morphology and staining reactions. This cell has not, as yet, been implicated in any recognized defence mechanism in the fish.

The cells designated as mast cells in fishes have been identified solely on the grounds that they have, in common with mammalian mast cells, a connective tissue habitat and cytoplasmic granules which are basophilic and metachromatic. Recent work has, however, also shown that the metachromatic granular cells present in the dermis of plaice skin, similarly contain 5HT.

A property of fish mast cells observed by many workers is the lability of the cytoplasmic granules. Roberts *et al.* (1971b) were able to stain the subepidermal mast cells of plaice only after the tissue was fixed in 4% paraformaldehyde and embedded in araldite, and Bullock *et al.* (1976) were only able to stain them in vital preparations (Fig. 2.24). All agreed with the original conclusion of Michel (1923) that the mast cell granules of fish were extremely soluble structures. Bucke (1972) found that the mast cell granules of the goldfish stained with the pinacyanol erythrocyanate method only after fixation in 10% buffered formalin.

In mammals the mast cells are mediators of anaphylaxis, causing the contraction of smooth muscle, dilation of blood vessels and increased vascular permeability. It is not at all clear that this phenomenon exists in fish, since attempts to induce anaphylactic-type reactions in fish have not generally been successful although occasional anaphylactoid reactions can be observed clinically.

In summary, although the presence of eosinophils and mast cells in fishes is disputed, they certainly appear to be present in some species and probably are present in all species. In

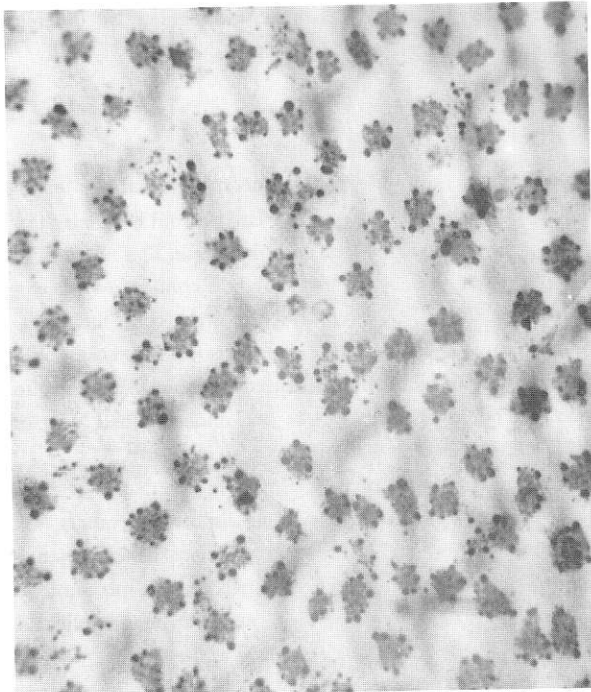


Fig. 2.24. Wet preparation of mast cells on under-surface of a whiting scale, stained with 0.1% neutral red in 0.85% saline. $\times 350$. (From Bullock *et al.* 1976.)

mammals the defensive role of these cells is coming to light only slowly. At the present time the functional role of so-called eosinophils and mast cells in fish can only be inferred and their relationship to the obvious eosinophilic granule cell of teleost mucosae and submucosae, the EGC, while probably close, is as yet not defined (Reite, 1998).

Lymphocytes

The lymphocyte is the cell responsible for the immune response. Its properties are discussed in detail in the chapter on the immune process, and the present description is confined to the haematological features of the cell. The morphology of the lymphocyte is remarkably similar throughout the phylum Vertebrata.

They are usually and arbitrarily separated into large and small categories, for reference, though they probably represent different functional states of cells within populations of cells rather than a difference in functional capacity. The average size of small lymphocytes may differ between species, e.g. their diameters average $4.5\ \mu\text{m}$ in plaice, $8.2\ \mu\text{m}$ in goldfish and about $6\ \mu\text{m}$ in man. The nucleus occupies virtually the whole

of the cell, leaving only a narrow rim of basophilic cytoplasm in which there are a few mitochondria and isolated ribosomes. In morphology the majority of circulating small lymphocytes appear as inactive undifferentiated cells. They circulate in this form until stimulated into action by their specific antigens. A lymphocyte is said to be mature when it is competent to respond to its antigen.

Lymphocytes circulate throughout the blood and lymph of the vertebrate body and congregate in organs which filter body fluids.

The number of lymphocytes in the blood is noticeably greater in fishes than in mammals. For instance, the density of lymphocytes in plaice is $48 \times 10^3/\text{mm}^3$ while in man it is only about $2 \times 10^3/\text{mm}^3$.

There is great variability in the reported counts of lymphocytes in fish blood, such as to give rise to grave doubts as to their diagnostic value, although Blaxhall and Daisley (1973) in their review suggest that useful results can nevertheless be obtained. Much confusion probably stems from inaccurate differentiation of lymphocytes and thrombocytes (see above).

HAEMOPOIETIC TISSUE

Since teleost fish have no lymph nodes and their bones usually have no medullary cavity, haemopoietic tissue is located in the stroma of the spleen and the interstitium of the kidney. To a lesser extent it is also found in the periportal areas of the liver, the intestinal submucosa and the specialized lymphoid organ, the thymus.

Renal haemopoietic tissue

In the kidney the haemopoietic tissue forms a support matrix for the nephrons of the posterior kidney but the anterior or head kidney is almost exclusively haemopoietic. The blast cells are situated within a stroma of reticuloendothelial tissue similar to that of the bone marrow of the mammal. The endothelial cells line numerous sinuses, through which blood from the renal portal vein is passed for filtration of effete cells and addition of new ones. The corpuscles of Stannius and the internal (adrenal) tissue (cortex and medulla) are embedded within the haemopoietic tissue. Another cellular structure, found throughout teleost haemopoietic tissue but not in higher vertebrates, is the melanomacrophage centre (Roberts 1975b).

Melanomacrophage centres vary in their degree of organization, depending on species. In the lower teleosts they are clusters of dark cells distributed throughout the haemopoietic

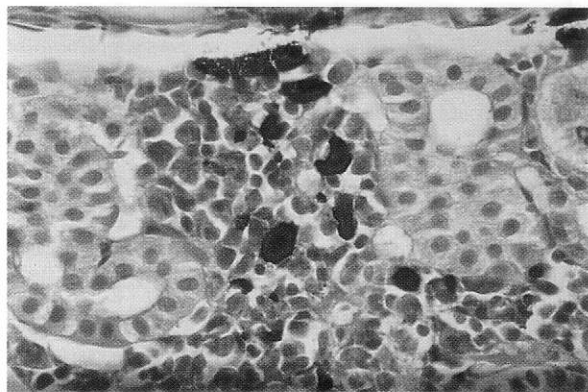


Fig. 2.25. Melanomacrophage aggregation in kidney of trout. In lower teleosts melanomacrophages are diffuse and black brown in colour. H + E $\times 400$.

tissue (Fig. 2.25). The degree of melanization varies with age but at all ages the pigment present is dark brown or black and has all the biochemical and chemical properties of melanin, although not necessarily laid down on the characteristic melanosomes of integumental melanin.

In higher teleosts the amount of dark pigment present in the melanomacrophage centres of spleen or kidney of normal fish is usually very small, the majority of their pigment being much lighter in colour (Fig. 2.26). Histochemically this is lipofuscin but Edelstein (1971) has produced evidence to suggest very close chemical affinities between lipofuscins or age pigments and melanin.

The morphology of the melanomacrophage centres of higher teleosts is also much more closely defined. They are usually nodular, with a delicate argyrophilic capsule. In many species they are closely applied to vascular channels and may



Fig. 2.26. Section through spleen of plaice showing characteristic pale coloured focal melanomacrophage centre. H + E $\times 120$.

have a collar of lymphocytes. Circulating macrophages, replete with particulate matter, possibly microbial in origin, or metabolic waste products such as ceroid or haemosiderin, home selectively on the melanomacrophage centres, which can therefore be considered as metabolic dumps.

The spleen

The spleen is the only lymph node-like organ to be found in teleost fish. It is dark red or black in colour and in health usually has sharply defined edges. It is situated near the greater curvature of the stomach or the flexure of the intestine. Although usually single, it may in some species be divided into two or more smaller spleens. The splenic capsule is fibrous and devoid of muscle and does not have the dense trabeculae extending into the tissue which are found in the mammalian spleen. In some species the pancreas is located as a subcapsular layer to the spleen but in most fish the main elements of the spleen are the ellipsoids, the pulp and the melanomacrophage centres. Ellipsoids are the thick-walled filter capillaries, which result from the division of the splenic arterioles. Each comprises a thick basement membrane-bound tube within which the vessel runs, usually eccentrically, separated from the membrane by a layer of sheathed compartments. These contain erythrocytes and phagocytic cells and are capable of trapping large quantities of particulate matter from the circulation. Replete macrophages then migrate from ellipsoids to the melanomacrophage centres (Fig. 2.27) (Ferguson 1976).

The splenic pulp consists of sinusoidal phagocytic tissue similar to that of the kidney, in which large numbers of red blood cells may be held, and haemopoietic tissue, which is supported by argyrophilic fibres. This is mainly lymphopoietic but not exclusively so. Melanomacrophage centres, similar to those of the kidney, occur and are usually located close to a vessel; they may even be invested by strands of its externa and usually have a fine reticulin limiting membrane.

The thymus

The thymus is a paired organ, an ovoid pad of primary lymphoid tissue situated subcutaneously in the dorsal commissure of the operculum. It arises from primordia associated with the epithelium of the pharyngeal pouches. Lele (1932) made a comparative study of thymic morphology in teleosts and found that its lifespan was very different in different species, involuting in lower teleosts before sexual maturity but surviving, and even growing, for several years after maturity in

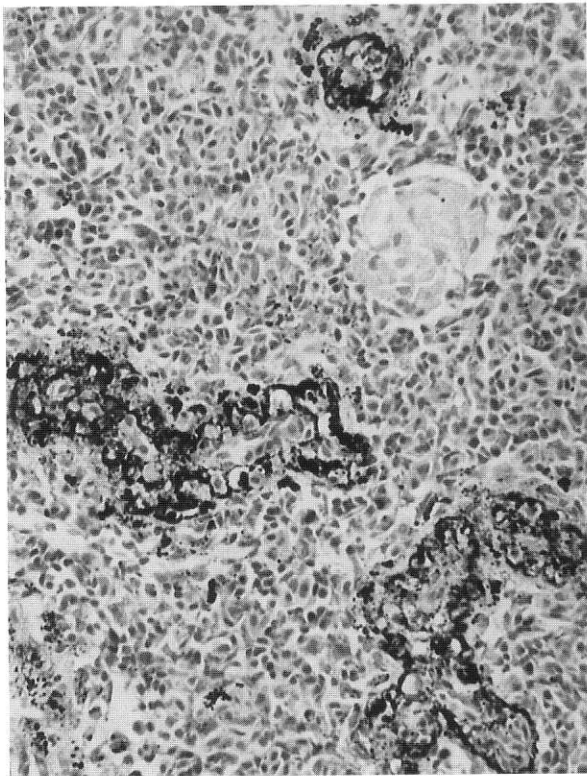


Fig. 2.27. Section of spleen from plaice inoculated intravenously 6 hours previously with Indian ink. The ink particles have been trapped within the walls of the ellipsoids. H. + E $\times 300$.

higher teleosts. In section the thymus is an aggregate of small lymphocytes with a fibrous capsule and fine argyrophilic supporting cells. Macrophages are very numerous in the thymus of some fish (e.g. *Lophius*) and are found in close association with lymphocytes. The thymus is thought to be the site of immunocompetent T cell maturation. Occasional epithelial cords are seen and, rarely, focal epithelial nests which may correspond to the Hassl's corpuscles.

THE RETICULOENDOTHELIAL SYSTEM

The reticuloendothelial system (RES) is the system of phagocytic cells, widely dispersed throughout the body, which is responsible for the removal of effete cells and particulate matter from the circulation.

The criteria by which cells are included in the RES are high phagocytic activity and capacity to concentrate and segregate such phagocytosed material. The weight of evidence suggests that in mammals and probably teleost fish, tissue

macrophages are derived from monocytes circulating in the blood and their precursors; thus there are two populations of macrophages, one fixed and the other free to move about.

The cells of teleost fish which are considered to comprise the RES, are the promonocytes of the haemopoietic 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. The most important organs are the kidney because of its avidity and the atrium because of its peculiarly vulnerable site.

There are a number of differences between the mammal and the teleost with regard to the RES. The lining of the cardiac atrium is a highly significant site in many species of fish whereas it has no such activity in the higher animals (Fig. 2.28). Lymph nodes do not occur in fish, and the liver, whose Kupffer cells provide the largest area of phagocytic tissue in mammals, is virtually inert with regard to phagocytosis in the teleost (Ferguson 1975a).

Giant cells and epithelioid cells, which are found in teleost chronic inflammatory lesions, are also considered part of the RES, or more properly the 'mononuclear macrophage system' (Van Furth 1970), since they are formed from fusion of individual macrophages in the presence of certain undegradable irritants (Timur 1975). The anatomy of the various tissues, which possess a phagocytic component, is described under their respective organ systems.

An interesting feature of the macrophages of the teleost RES is their capacity, whether fixed or circulating, to form aggregates once they are replete. Usually these aggregates are in the areas of the melanomacrophages of the haemopoietic tissues (Roberts 1975b) but such aggregates are also found,

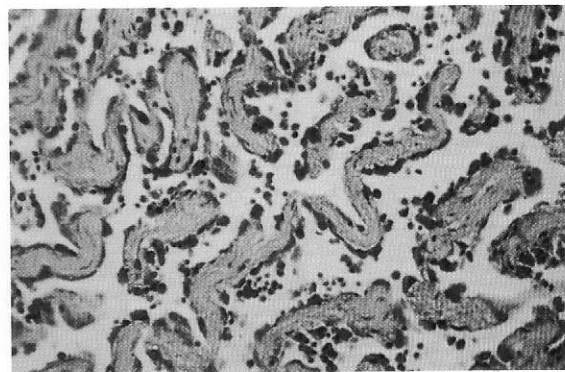


Fig. 2.28. Section through the atrium of a plaice injected with yeast cells 6 hours previously. The fixed phagocytes of the atrium have taken up the yeast and stain bright red. PAS $\times 760$.

frequently pigmented, within or around chronic inflammatory lesions. It is not known whether the pigments found in such aggregates are of purely exogenous origin, nor is it completely clear why there is so frequently a collar of lymphocytes in association with such aggregates. However, melanin and related pigments are considered to play a defensive role in many organisms, in their capacity for H_2O_2 generation (Edelstein 1971).

THE RENAL AND EXCRETORY SYSTEMS

The regulation of the internal body fluid composition of fish is a complex process. The skin is essentially impervious in adults but, as pointed out previously, fluxes of water and ions readily take place across the gills. Other surfaces at which transfers take place are the gut wall and the kidneys. Thus it is the control of fluxes in all three organs—gills, kidney and alimentary canal—that constitutes the osmoregulatory and excretory requirement of fishes.

The excretory kidney

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

The kidney of teleosts is usually located in a retroperitoneal position up against the ventral aspect of the vertebral column. It is a light or dark brown or black organ normally extending the length of the body cavity. It is usually divided into anterior or head kidney, which is largely composed of haemopoietic elements, and posterior or excretory kidney. Although embryologically arising as a paired structure, its adult form varies with species, from two separate parallel organs in species such as the angler fish, through varying degrees of attachment, to the complete fusion found in salmonids. The ureters or archinephric ducts, which conduct urine from the collecting ducts to the urinary papilla, may fuse at any level and may be dilated, after fusion, to form a bladder (Fig. 2.29). The urinary ducts open to the outside posterior to the anus.

Arterial blood is supplied to the kidney by renal arteries arising directly from the aorta or from segmental vessels. Except in the small number of aglomerular species, these serve the glomerular capillary bed and then drain into efferent arterioles. In marine and euryhaline species the peritubular capillaries also receive blood from caudal or segmented vessels,

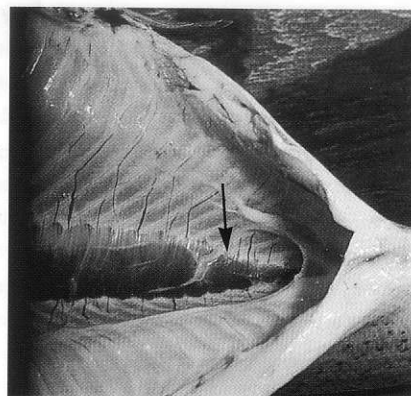


Fig. 2.29. Kidney of rainbow trout showing white archinephric ducts joining to form urinary bladder (arrowed).

draining the tail region and constituting a renal portal system. This portal blood is thus venous (Hickman & Trump 1969).

The structure of the teleost nephron varies considerably between marine, euryhaline and fresh-water forms, mirroring the significant differences between their respective functions.

The nephron of the typical fresh-water teleost comprises a well vascularized glomerulus (Fig. 2.30), a ciliated neck, two distinctive proximal segments (one with a prominent brush

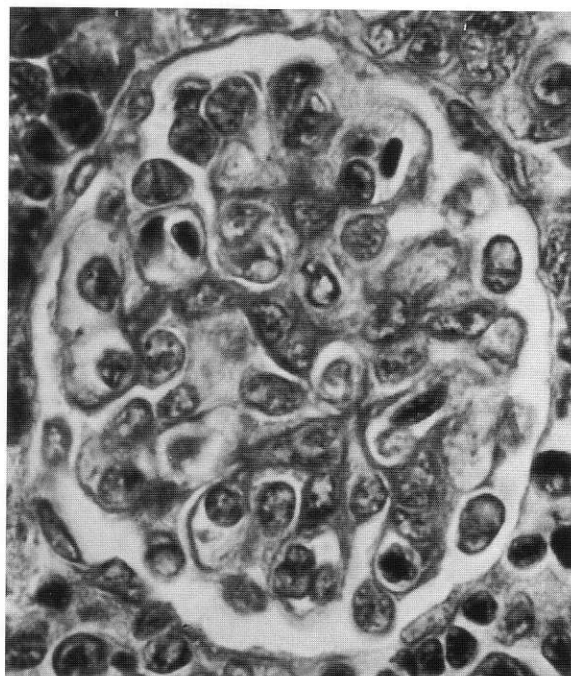


Fig. 2.30. Glomerulus from the mid-kidney of common carp. PAS $\times 600$. (By courtesy of Dr M. Yokote.)

border and the second containing numerous mitochondria but a less developed brush border), a narrow ciliated intermediate segment, a distal segment and a collecting duct system.

The marine nephron is typically smaller in size and consists of glomerulus, neck segment, two or three proximal segments, which constitute the major component, an intermediate segment occasionally found between the first and second proximal segments, and the collecting duct system.

The euryhaline teleost usually has a nephron combining the structure of both types, being similar to that of the marine teleost with, in addition, a distal segment similar to that of the fresh-water teleost. Certain marine and fresh-water species are anomalous in that they do not possess glomeruli in the nephron. There are many individual species variations in nephron structure and the above descriptions provide only a broad summary. The major work on this field is that of Hickman and Trump (1969) and this should be consulted for more detailed comparative information.

Osmotic and ionic regulation

In fresh-water the environment is hypo-osmotic and water tends to pass into the body fluids through the gills and permeable surfaces of the pharynx. This is compensated by the kidney producing large volumes of dilute urine, so that a glomerular filtration is of great importance.

Ions are also passively lost through the gills and significant amounts are lost in the urine. The latter is minimized, by the urine having very low Na^+ and Cl^- concentrations. Equilibrium is maintained by active uptake of Na^+ and Cl^- by the gills and absorption from the food through the wall of the gut.

In the marine environment the blood has a relatively low osmotic pressure so that, by passive diffusion, water is lost through the gills and ions are gained. Urine is produced in small quantities with low or negligible glomerular filtration rates. The urine is approximately isosmotic or slightly hypo-osmotic and contains mainly the divalent ions Mg^{2+} and SO_4^{2-} . Large volumes of sea-water are taken up by drinking to compensate for the passive water loss. The divalent ions are generally not taken up by the gut so they appear in the faeces. Excess monovalent ions in the body are excreted through the gills.

Key structures in ionic exchange across the gills in teleost fish are the chloride cells, which are implicated in pumping salt inwards in fresh-water and outwards in sea-water. They are located on both primary and secondary lamellae, principally at the junction between the two, and are found in greater densities in marine-adapted fish. It has been suggested that salt pumping from the gills of marine fish results from diffusion

from a high concentration of salt in a specialized space between adjacent chloride cells, which are open to the sea. The salt gradient is produced by a very high density of sodium pumps, on the membranes, facing these intercellular spaces (Fig. 2.31). The mechanism of inward pumping of salt in fresh-water is thought to be essentially the same as in amphibian skin.

Euryhaline species can alternate between the mechanisms of fresh-water and marine species according to requirements, although the glomerular apparatus of some undergoes degeneration in the marine environment. There are also basophilic primordia, adjacent to glomeruli, in teleost nephrons, which may be the protoglomeruli which will form new glomeruli, if required.

Osmoregulation is mainly under endocrine control via the posterior pituitary and interrenal corticoid hormones and possibly the caudal neurosecretory system and the juxta-glomerular apparatus of the kidney (Lagler *et al.* 1962). Prolactin-like hormones apparently control Na^+ efflux from the gills but they also affect the kidney and bladder wall. Corticoids also modify the ionic transport and permeability characteristics of different tissues. Thus many endocrine changes associated with sexual maturation can secondarily affect ionic and osmoregulatory mechanisms. In anadromous fish such as salmon the young fish has to reach a certain stage

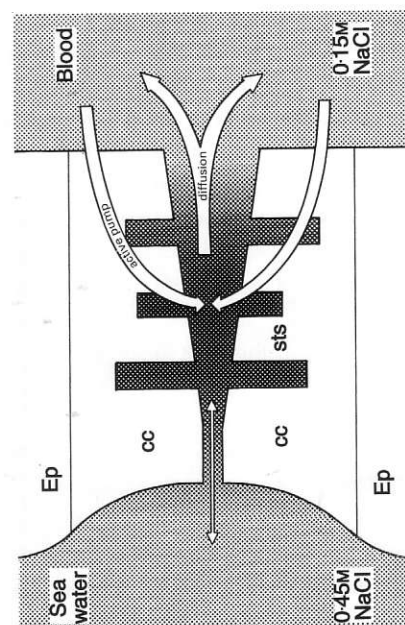


Fig. 2.31. Model for NaCl pumping across the gills in sea-water. cc = chloride cells, sts = smooth tubular system, Ep = epithelial cell.

of development involving drastic endocrine changes to become a smolt before being capable of living in sea-water (Langdon *et al.* 1984).

The mechanisms of ionic regulation and osmoregulation and their control are particularly complex and the specialist text of Maetz (1974) should be consulted for further details. In pathological investigations it should be noted that the structures involved in water and ion exchange can undergo highly significant changes according to the animal's state of development, the composition of its aquatic environment and endocrine factors. All of these must be taken into account when examining clinical material from the gills, gut epithelium and renal tissues.

The main nitrogenous end-products of metabolism are ammonia and urea; both are highly soluble and are readily expelled into the surrounding water, mainly by diffusion through the gills. Trimethylamine oxide occurs generally in fish and can be an important nitrogenous excretory component of the urine under marine conditions.

THE DIGESTIVE SYSTEM

The digestive tract

Teleost fish feed on a very wide variety of substrates so that differences in oral structures and digestive tract, adaptations

and specializations to suit specific diets, are often very distinctive. Overall length of the digestive tube is a major difference, that of herbivorous fishes being very much longer than that of carnivorous species. Other specializations, related to diet, including dentition, presence and numbers of diverticula and even in some species complete absence of stomach, are also of considerable taxonomic significance (Fig. 2.32).

The swim-bladder, which is derived embryologically from the upper digestive tract, but has no digestive function, is considered as a separate system.

Mouth

The mouth and buccal cavity are shared by the respiratory and digestive systems. Their digestive function is confined to selection, seizure and orientation of food for transfer to the stomach. Details of the range of feeding mechanisms used by fish are reviewed by Alexander (1981). Chewing and predigestion, found in mammals, are not usually a function of the mouth of the teleost except in a few highly developed herbivorous species. The mouth and perioral regions are well endowed with sensory nerve endings and teeth, which vary greatly in location, morphology and number. The lining of the buccal cavity consists of a stratified mucoid epithelium on a thick basement membrane with a very condensed dermis binding it to bone or muscle.

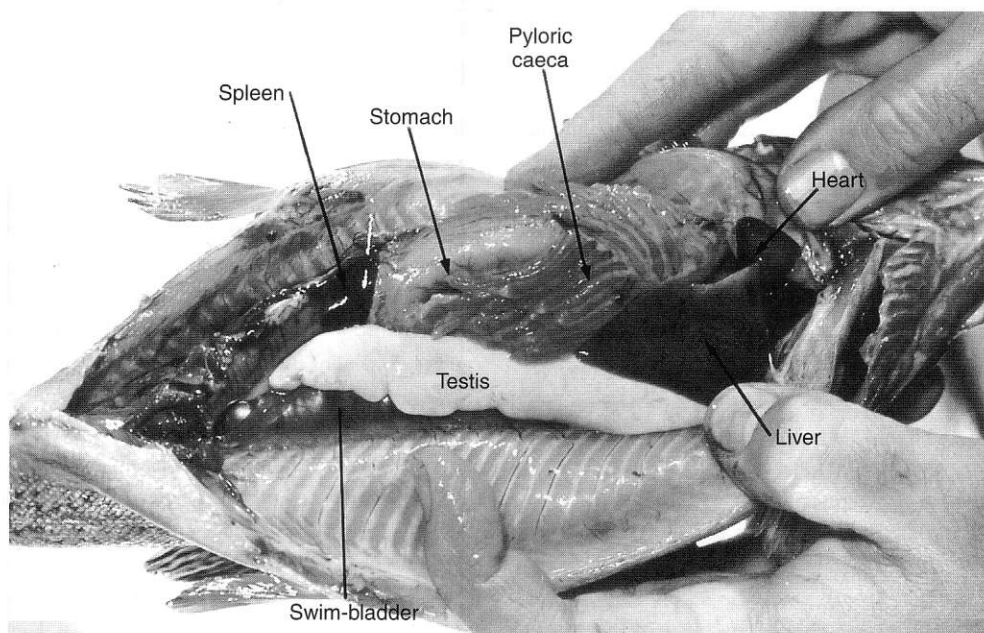


Fig. 2.32. The abdominal contents of a rainbow trout, a top predatory carnivore with a very short intestine.

Oesophagus

The oesophagus is usually a short, straight and very muscular tube passing from the mouth to the cardia of the stomach. Its epithelial lining is usually well endowed with mucous cells and this, and the extensive longitudinal folds into which its inner surface is thrown, allows for easy swallowing of awkward food particles. In some tropical species there are blind diverticula (oesophageal sacs) or oesophageal teeth.

Stomach

The stomach varies in size. It is usually a sigmoid, highly distensible sac with numerous folds in its lining. It is highly muscular and the cardia demarcates the change from the striated muscle of the anterior digestive tract to the smooth muscle occurring distally. There are a number of layers of muscle, with a muscularis mucosa, and the layers of connective tissue adjacent to this are often endowed with large numbers of eosinophilic granule cells. The gastric mucosa itself is very mucoid, with numerous glands at the bases of the folds. Some species are agastric; no particularly convincing reason for this anomalous situation is available but Kapoor *et al.* (1975) have suggested that it is a modification to allow for consumption of large amounts of indigestible ballast which must be passed quickly through the digestive tract.

Pyloric caeca, blind-ending diverticula from the distal, pyloric valve region of the stomach and from the anterior intestine, are found in many species but notably in the salmonids, where they may number 70 or more. Their histological and histochemical features resemble those of the intestine rather than the stomach.

Intestine

Although its relative length may vary according to diet, the intestine of most fish is a simple tube, which does not increase in diameter to form a colon posteriorly. It may be straight, sigmoid or coiled, depending on the shape of the abdominal cavity. It has a simple, mucoid, columnar epithelium, overlaying a submucosa often richly endowed with eosinophilic granule cells and limited by a dense muscularis mucosa and fibroelastic layer (Fig. 2.33). Rodlet cells are frequently seen in the lining of the intestine.

Rectum

The rectum has a thicker muscle wall than that of the intestine and its lining is highly mucigenic. It is capable of considerable distension.

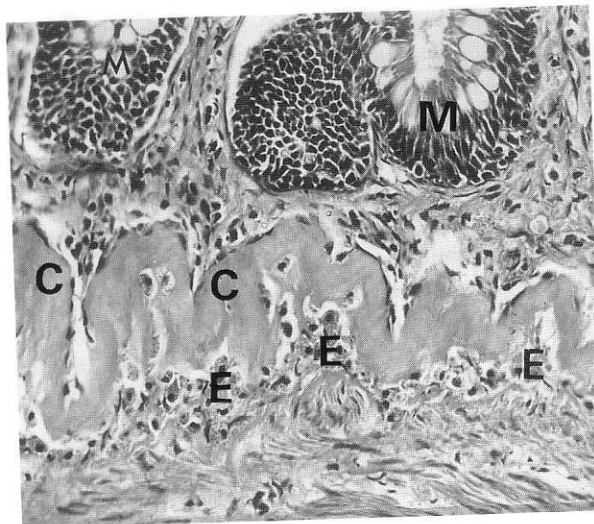


Fig. 2.33. Section of the pyloric region of the intestine of a rainbow trout. E = eosinophilic granule cells; C = circular muscle; M = mucous cells of mucosal lining. H + E $\times 240$.

Physiological changes take place in the digestive tract of many species of teleost during cyclical periods of starvation, migration or spawning. These changes are particularly well marked in catadromous and anadromous species such as the European eel and the Atlantic and Pacific salmon, where intestinal folds flatten, cells become shrunken and dark-staining and there is often extensive epithelial necrosis into the tenacious mucoid intestinal content.

The liver

The teleost liver is a relatively large organ. In wild fish it is usually reddish brown in carnivores and lighter brown in herbivores but at certain times of year it may be yellow or even off-white. In farmed fishes, where diets generally contain higher levels of lipid, it is usually lighter in colour than in the equivalent wild specimen. The liver may be a localized organ in the anterior abdomen or may, in some species, have processes which extend the length of the abdomen or are closely applied to the other viscera. In some species it is a compound organ in the form of a hepatopancreas, but in others the pancreas is a separate organ.

The histology of fish liver differs from the mammalian in that there is far less tendency for disposition of the hepatocytes in cords or lobules and the typical portal triads of the mammalian liver are not apparent. Sinusoids, which are irregularly distributed between the polygonal hepatocytes, are fewer in number and are lined by endothelial cells with very prominent nuclei. Functional Kupffer cells are not found in

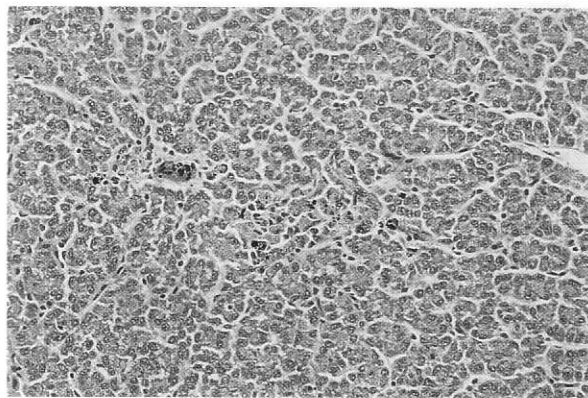


Fig. 2.34. Section through liver of normal trout showing sinusoids between muralia of polygonal hepatocytes. H+E $\times 110$.

the lining of the sinusoids. This was first demonstrated by Varichak in 1938 and has since been adequately confirmed (Ellis *et al.* 1976) but descriptions of so-called Kupffer cells based solely on morphological criteria continue to appear (Hinton & Pool 1976). The sinusoidal lining cells are fenestrated and overlie the space of Disse which is the zone between sinusoid cells and hepatocytes and contains microvilli from both, as well as numbers of fat storage cells, the cells of Ito. Hepatocytes are polygonal and have a distinctive central nucleus with densely staining chromatin margins and a prominent nucleolus (Fig. 2.34). The hepatocytes are often swollen with glycogen or neutral fat when nutrition is even marginally less than ideal and during cyclical starvation phases the cells may be shrunken and the entire liver loaded with yellow ceroid pigments.

The biliary system also differs from that of mammals in that intracellular bile canaliculi occur which eventually anastomose to form typical bile ducts. The bile ducts fuse and ultimately form the gall bladder, which contains the greenish-yellow bile which is conducted to the intestine via the common bile duct. The gall bladder lining is transitional epithelium which often contains rodlet cells. Haemopoietic tissue, complete with melanomacrophage centres, is found in varying amounts around the larger vessels of the liver, and where a hepatopancreas is present this invests the larger branches of the hepatic portal vein as a glandular externa.

The pancreas

The pancreatic tissue is more variable in location, even within a single species, than the other abdominal viscera. The most common sites for it are as scattered islands of secretory tissue interspersed among the fat cells in the mesentery of the

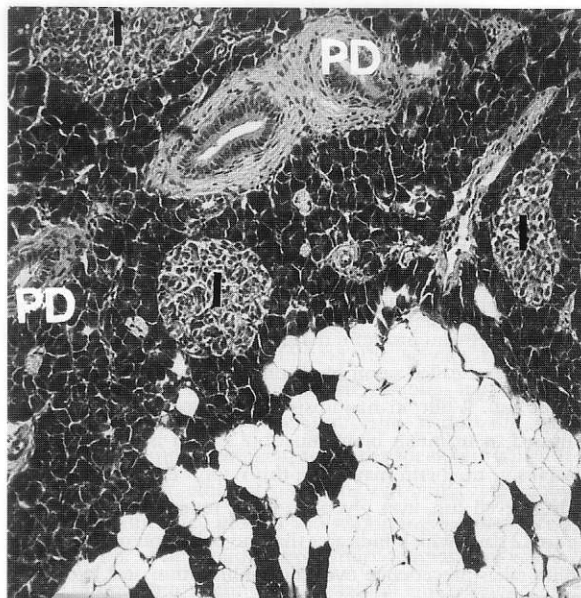


Fig. 2.35. Section of normal rainbow trout pancreas. The darkly staining exocrine pancreatic acini surround the pancreatic ducts (PD). The characteristic pancreatic fat cells are at bottom of picture. I = islets of Langerhans. H + E $\times 70$.

pyloric caeca, as a subcapsular investment of the spleen and as an external layer around the hepatic portal vein.

The acinar structure of the exocrine pancreatic tissue is very similar to that of the mammal and is comprised of cells with a very dark basophilic cytoplasm. In actively feeding fish these contain large numbers of bright, eosinophilic, secretory granules. The pancreatic duct usually joins the common bile duct somewhere along its length. The endocrine components of the pancreas, the islets of Langerhans, consist of a number of lightly capsulated, poorly staining structures comprised of the small fusiform α , β and δ cells. The size of islet cells may vary with season and, in some species, there is one major islet, known as the Brockman body (Fig. 2.35).

NUTRITION, METABOLISM AND GROWTH

Most knowledge of food requirements for fish is based on work on salmon and trout in artificial culture. More recently, however, information concerning the dietary requirements of omnivorous and herbivorous cultured species such as carp and tilapia has become available for comparison (Jauncey 1982; Jauncey 1999). The main energy foods are carbohydrates, proteins and fats, as in all other vertebrates.

Carbohydrates

Although many species of fish can utilize carbohydrate as well as lipid as an energy source, at levels up to 25% of the diet, it is generally not an important dietary component. Essentially, fish energy metabolism resembles that of a diabetic mammal. Thus the ingestion of glucose will produce persistent hyperglycaemia over many hours. This slow glucose metabolism is partly due to low catabolic activity in tissues such as liver, muscle and kidney. Insulin, of fish origin, will lower blood glucose levels and promote transfer to tissues, but it seems that insulin is not called upon to modulate blood glucose levels in teleost fish. While in mammals rapid glucose homeostasis is required to maintain brain function, in fish higher glycogen reserves in the brain may make this response unnecessary.

In starved fish, liver glycogen levels remain unchanged for up to 22 days, which indicates that the oxidation of other substrates takes precedence over the mobilization and hydrolysis of glycogen and the oxidation of glucose. Thus the capacity of fish to metabolize glucose aerobically is low relative to mammals. It also appears that the nervous tissue, which uses glucose as a primary fuel, may rely on gluconeogenesis rather than glycogenolysis. The former process is highest when the diet is high in protein and lowest when protein is low and carbohydrate is high.

It would seem that fish, which are predominantly carnivores, use non-essential amino acids and lipids as preferred sources of energy. When dietary carbohydrate levels are low, blood glucose and glycogen reserves can be maintained by gluconeogenesis.

Protein

Protein requirements for fish are linked to growth and gonad development. In fact there is a clear linear relationship between daily protein requirement and specific growth for a wide range of fish. This implies that the utilization of protein is relatively constant and is independent of the feeding category, i.e. carnivores, omnivores and herbivores.

Dietary protein requirement for fish ranges between 45 and 70% of the gross energy content of the diet. Compared with farmed homeotherms, this seems high. On the other hand, for a homeotherm to maintain a constant elevated body temperature, the total energy requirement for maintenance is higher than that of an ectotherm of similar size. This difference will lead, consequently, to a higher proportion of non-protein energy components in the diet. If daily protein requirements are related to flesh production then all categories of fish have

Table 2.1 Mean essential amino acid requirements, based on data from various species of teleost fish, mainly belonging to the Salmonidae

EAA	Daily weight-related requirement (mg/kg body weight/day)
Arginine	4.3±1.0
Histidine	1.7±0.2
Isoleucine	2.6±0.5
Leucine	4.0±0.6
Lysine	4.9±0.7
Methionine	1.6±0.4
Phenylalanine	3.1±0.7
Threonine	3.1±0.7
Tryptophan	0.6±0.2
Valine	3.1±0.3

a range from 420 to 766 g/kg live weight gain, which is not dissimilar to terrestrial farmed animals.

Although many abiotic factors influence metabolism and thus growth (see later), as yet there is no evidence that factors such as temperature or salinity affect the relative size of the protein component of the diet.

Those species which have been studied so far require the same essential amino acids (EAA) as terrestrial farm animals (Table 2.1).

Lipids

Fish require lipids as a source of energy and to maintain the structure and function of cellular membranes. With lower body temperatures than homeotherms, fish utilize lipids with low melting points. Many of the commercially important marine species feed on crustacean zooplankton, which contain high levels of polyunsaturated fatty acids in the form of wax esters. As a consequence of this diet, fish such as herring and capelin lay down large lipid depots in the liver and muscle. The constituent lipids are oils consisting of triglycerides containing polyunsaturated fatty acids of the $\omega 3$ series. Possession of such large amounts of oils, which make up 10–20% of the body weight, means that lipids rather than carbohydrates are the main energy reserve. As a rule, body lipid content is inversely proportional to body water content; this is a useful index of fish condition in some circumstances.

In general an increase in the non-protein components of the diet leads to improved protein utilization. Diets containing between 10 and 20% lipid by weight, optimize protein utilization, although commercial salmonid diets now frequently contain up to 30%. Also the protein-sparing action of lipid is more effective than that of carbohydrate. Excess lipid,

however, can cause mortality, associated with fatty livers, particularly in salmonids.

Polyunsaturated fatty acids of the $\omega 3$ series have been shown to be essential components of the diet of fish. A deficiency can lead to cessation of growth, caudal fin erosion, fatty liver, cardiac myopathy and the development of the shock syndrome. When linolenic acid (24:6 $\omega 3$) is added to the diet, at levels of around 1% by weight or 2.7% of the total energy content, growth and improved food conversion is stimulated. A comprehensive review of the lipid nutrition of fishes is given by Bell (1998).

Fresh-water species achieve highest weight gains with diets containing both $\omega 3$ and $\omega 6$ series fatty acids. The latter series is more typical of terrestrial organisms and may reflect the origin of food in fresh-water fish. It is interesting to note that dietary requirements of a given fish species closely match the body composition of that species caught from the wild, and presumably feeding on a totally natural diet.

There are, as might be anticipated, non-energy food requirements for minerals and vitamins. These requirements are broadly similar to those of higher animals, and there is a gradually accumulating body of information on the effects of their deficiency in the diet.

Metabolic rate

The measurement of metabolic rate, primarily as oxygen consumption, provides estimates of the energy requirements of fish, for both husbandry and water management. Also, as aquatic ectotherms, fish are very responsive to changes in their environment, which is reflected in changes in metabolic rate. Thus the measurement of oxygen consumption is a sensitive method of establishing the relative importance of various environmental factors. As a result of the variability of metabolic measurements the standard metabolic rate is taken as an arbitrary non-active level which is extrapolated from the relationship between swimming speed and oxygen consumption, measured after at least 24 hours fasting. This standard rate can then be used to estimate minimal maintenance rations and the influence of environmental factors (for reviews see Brett 1962; 1970; Brett & Groves 1979).

Abiotic factors influencing metabolic rate

Temperature. The ectotherm body temperature follows ambient temperature very closely, except in the large game fish such as tuna and marlin. The metabolic responses to temperature are governed not only by the extent of temperature

change but also by the rate and direction of the change. Abrupt and substantial increase even within the normal temperature range can be lethal due to a metabolic overshoot and a failure in the transport of respiratory gases. A smaller decrease in temperature is less traumatic and has been found to be useful for handling and transporting fish, since activity is suppressed and the metabolic overshoot reduces oxygen consumption disproportionately.

If the temperature is increased gradually (1°C per day) fish can be acclimatized to a wide range of temperatures, often spanning 20–30°C, depending on the species (Fig. 2.36). Within this range the metabolic rate doubles, approximately, for each 10°C rise in temperature. Also, within this range, there is an optimum temperature for the species, at which it is performing most efficiently. At this optimum temperature, conversion efficiency, growth and swimming performance are maximal. For each species the optimum temperature is related to the temperature regimen of its natural environment.

As temperature increases, the level of spontaneous activity also increases, and this is reflected in swimming performance and active metabolic rate, both of which increase up to the optimum temperature, after which they flatten out and eventually crash at the upper lethal temperature. In most species the curtailment of the increases in maximal active metabolic

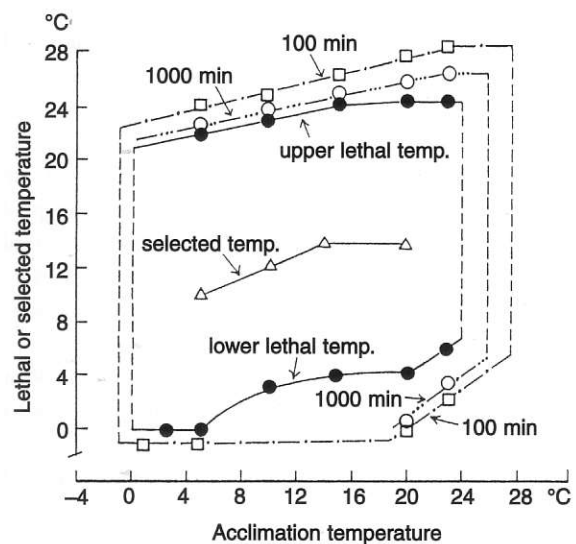


Fig. 2.36. The effect of thermal acclimation on upper and lower lethal temperatures of the sockeye salmon is shown as a series of tolerance polygons. The inner polygon indicates the temperature tolerance at which 50% of the sample survives on unlimited time of exposure. The two outer polygons refer to 1000 and 100 minutes of exposure. Temperatures selected by fish acclimated between 4 and 20°C are also shown.

rate is due to the reduction in the concentration of dissolved oxygen as the temperature rises. Swimming performance and active metabolic rate will often continue to increase above optimum conditions if the PO_2 of the water is increased above air saturation level.

Fish have been shown to adjust their metabolic rates to compensate, to a limited extent, for long-term changes in body temperature. Thus the differences in metabolic rates of individuals kept for long periods at either end of the thermal range are less than that predicted by the temperature coefficient (Q_{10}). The cellular bases for this thermal acclimatization are thought to involve switching between isozymes with differing thermal properties such as substrate affinity. There is also evidence for the opening of alternative metabolic pathways to supplement temperature-sensitive energy-releasing reactions. Low-temperature acclimatization produces reduction in the melting points of membrane lipids, through the incorporation of polyunsaturated fatty acids to maintain inter- and intracellular exchange.

Oxygen. Fish are fundamentally aerobes, although there is evidence of cyprinids surviving for several weeks in hypoxic conditions, by making use of anaerobic metabolic pathways. In general it can be said that oxygen is a limiting factor in that it governs the metabolic rate by virtue of its operation within the metabolic chain. The availability of oxygen in the aquatic environment is low, due to its relatively low diffusibility and low solubility, which is further reduced by increase in temperature and salinity. In consequence oxygen is more commonly a limiting factor in water than in air.

It has been shown for several species that the standard rate of oxygen consumption is constant over a wide range of oxygen tensions from atmospheric PO_2 down to a critical tension. This critical tension, which varies with temperature and species, marks the point where the standard rate increases rapidly as the lethal level is reached, after which there is a crash. The independent zone represents the range of ambient PO_2 in which the fish are able to extract sufficient oxygen without increasing the ventilation of the gills. The rise in standard rate below the critical level indicates the increased cost of ventilating the gills when oxygen becomes limiting.

Although the metabolic responses of fish to low oxygen tensions under standard conditions indicate the maximum extent to which they can tolerate low oxygen tensions, it does not show the extent to which the normal activities are constrained. In actively moving fish active metabolic rate and maximum swimming speeds are reduced at oxygen tensions considerably higher than the critical level. The oxygen tension

at which these restrictions occur is called the *incipient limiting level*, above which the fish is totally independent of oxygen tension. Below the incipient limiting level swimming activity and active metabolic rate decrease with decrease in oxygen tension. When active metabolic rate and standard rate are identical the fish cannot move without incurring an oxygen debt and has reached the *level of no excess activity*, which corresponds to the critical level. Between the level of no excess activity and the incipient limiting level is the zone of tolerance in which a fish can survive indefinitely but at a reduced pace. The short range of oxygen tensions between the level of no excess activity and the lethal level where the standard rate is elevated and no activity is possible is referred to as the *zone of resistance*. In this zone of resistance metabolism begins to show anaerobiosis with production of lactic acid and free shortchain fatty acids in muscle and liver tissue. In the zone of resistance most fish are unable to survive indefinitely.

Acclimatization to low oxygen tensions does not appear to alter the incipient limiting or no excess activity levels but does in some species extend the zone of resistance. From Table 2.2 it is clear that few species are independent of oxygen tensions much below air saturation levels. In the Salmonidae the level of no excess activity at optimum temperatures is when the water is about 30% air saturation (for review see Hughes 1973).

Carbon dioxide. Dissolved carbon dioxide, on its own, has little effect on the standard metabolic rate. However, high PCO_2 values do depress the maximum active rate of oxygen consumption (Fig. 2.37). The relationship between PCO_2 and active metabolic rate is species-specific and in general the sensitivity is reduced at higher acclimatization temperatures. Also there appears to be an interaction between increasing PCO_2 and decreasing PO_2 to augment the reduction of the maximum active rate of oxygen consumption.

Ammonia. Fish are very intolerant of dissolved ammonia (NH_3). Concentrations as low as 0.1 mg/litre are harmful. However, in water below neutral pH ammonia exists mainly

Table 2.2 The relationship between oxygen consumption and body weight for several species of fish

Species	b value	Equation of line	Temperature (°C)
Cod	0.73	$\log y = 0.73 \log x + 0.364$	13
Coalfish	0.73	$\log y = 0.73 \log x + 0.364$	13
Atlantic salmon	0.78	$\log y = 0.78 \log x - 0.182$	20
Brown trout	0.88	$\log y = 0.88 \log x - 0.847$	10
Brook trout	1.04	$\log y = 1.04 \log x - 0.91$	20
Sockeye salmon	0.85	$\log y = 0.85 \log x - 0.52$	15

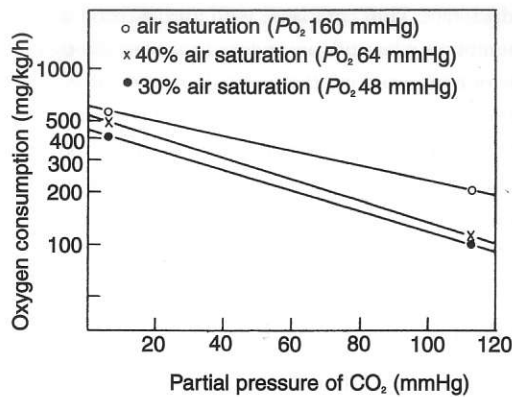


Fig. 2.37. The influence of increase of PCO_2 on the active respiratory metabolism of the common carp at 25°C at three partial pressures of oxygen. (From Beamish 1964.)

in the non-toxic ionized form, a process enhanced, in the presence of carbon dioxide, by the formation of ammonium carbonate. Unless pH is significantly alkaline, therefore, ammonia will not be a major influence on metabolic rate. However, in recirculation systems biodegradation of ammonia can produce harmful nitrites.

Salinity. The influence of salinity on the metabolism of fish is only relevant to those euryhaline species which are able to tolerate a certain range of environmental salinities. Many of the commercially important species such as the Salmonidae and Anguillidae fall into this category. The interest in the effects of salinity on the physiology of these species comes from reports that juveniles transferred into higher salinities show increased growth. However, there is little evidence that within the salinity range 0–28 g/litre the metabolic rate or conversion efficiencies are significantly changed. There are reports that the metabolic cost of locomotion at maximal cruising speeds is minimal at the iso-osmotic point of 11.6 g/litre when the osmotic gradient is zero. However, no such effect has been demonstrated under standard conditions and the only explanation for improved growth at higher salinities is that there is an increase in feeding rate (Fig. 2.38).

Biotic factors influencing metabolic rate

Body size. Small fish have a relatively higher metabolic rate than large fish and this general rule applies both intra- and interspecifically. The relationship between body weight and oxygen consumption is an exponential function which is normally represented by the following equation:

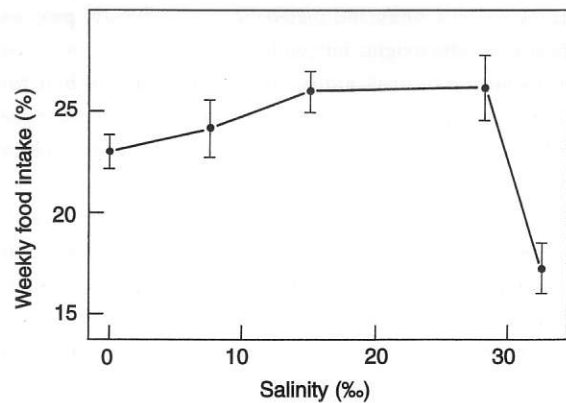


Fig. 2.38. Influence of increase in salinity on the weekly food intake, expressed as a percentage of the fresh weight of rainbow trout at 10°C.

$$\log Y = \log a + b \log X$$

where Y is the oxygen consumption in mg/hour; X is the fresh weight of the fish in grams; b is the exponent of weight; and a is a constant equivalent to the oxygen consumption of a fish weighing 1 g. This equation is shown in Fig. 2.39 as a linear relationship between the logarithm of weight and the logarithm of oxygen consumption, with b the slope of the

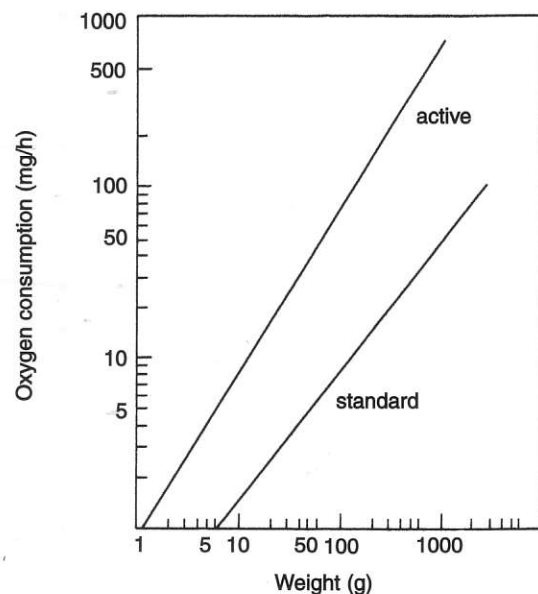


Fig. 2.39. Relationship between standard and active rates of oxygen consumption and weight of juvenile *Oncorhynchus nerka* at 15°C. The 'b' values for standard and active rates are 0.78 and 0.97 respectively.

line. When b is unity the metabolic rate is directly proportional to body weight, but with values less than unity the metabolic rate of small fish is higher than in large fish. It has been shown for a number of species of fish that the b value under standard conditions at 20°C is within the range 0.73–1.04 with a mean value of 0.83.

The weight exponent appears to increase in value with decrease in temperature even within the same species. This phenomenon appears to be intraspecific since there are no significant differences in b values even between tropical and polar species. Swimming activity also tends to push the value of b upwards. In sockeye salmon the weight exponent increases from 0.78 under standard conditions to 0.97 when maximally active.

The a values under differing environmental conditions provide an interesting intraspecific comparison. At the optimum temperatures for many species the a values are remarkably similar, suggesting that there is an optimum level of energy expenditure which, through thermal acclimatization, individual species have evolved to exploit their temperature regimen efficiently. Thus tropical, temperate and polar species have similar metabolic rates for their particular thermal range. (Otherwise tropical fish would tend to burn themselves up while polar species would take too long to develop and grow.)

Muscular activity. Spontaneous swimming activity of aquarium fish has been shown to have a pronounced effect on oxygen consumption. The maximum routine rate (the oxygen consumption at normal levels of spontaneous movement in moderate confinement) can be as high as ten times the

standard rate. This correlates with the increase in oxygen consumption when fish are induced to swim throughout the range of cruising speeds (Fig. 2.40). However, these periods of intense activity are of short duration and swimming activity, when spread over the 24-hour period, increases oxygen consumption less than twice the standard rate at optimum temperatures. In the lower end of the temperature range the routine level of metabolism is even less.

Specific dynamic action and starvation

Specific dynamic action (SDA) is the term used for the increased metabolic rate following the ingestion of a meal. It is the energy used in digestion, assimilation, growth, deamination of amino acids and the synthesis of nitrogenous excretory products. The energy released as SDA is related to the components of the diet. SDA is highest for protein at 30% of the total energy content of the diet. Lipid and carbohydrate have SDA costs of 13 and 5% respectively. The proportions of these components in the diet will affect the contribution of SDA to the total metabolic rate. Since most commercially important species are carnivores, with a high requirement for protein in the diet, SDA will be high. The traditional view is that SDA represents the energy cost of processing food and is therefore competitive with growth. In other words best diets are those which have the lowest SDA per energy intake. However, Jobling (1983, 1985) has proposed the opposite view that SDA is the energy expenditure associated with growth and is therefore interactive. His alternative view is that diets promoting

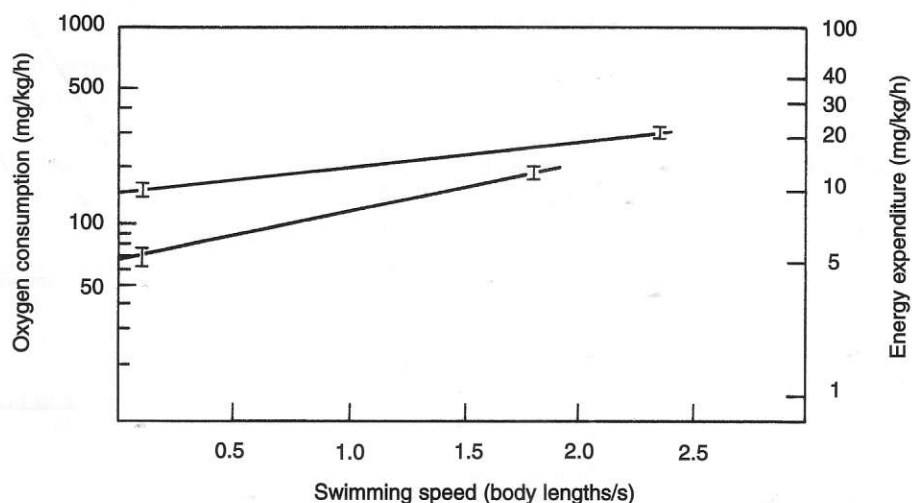


Fig. 2.40. Relationship between metabolic rate and swimming speed for 1 + year group haddock at 5°C and 15°C. Note that the ordinate has a logarithmic scale.

good growth will induce high SDA. Thus diets rich in protein promote growth in fish.

SDA is of particular importance in farmed carnivores, with a high oxygen demand, since feeding at the upper end of their temperature range can result in lethal overdemand for oxygen. Gill pathology and reduced water supply will exacerbate the situation.

Starvation has been shown to decrease standard metabolic rate by 25–50% of the non-starved rate in 7–300 days. Rapid decrease characterizes the early stages of starvation, followed by an exponential decay. Even for short periods of starvation (1–2 days) the standard rate can fall by 10%.

Finally, changing day length and the onset of sexual maturity and reproductive behaviour can change both the standard and the maximal routine metabolic levels. Differences in the order of 30% have been found between the resident and migratory phases of some species of white fish. Unfortunately it is extremely difficult to separate out the combined effects of photoperiod and temperature upon the physiology of fish and the role that endocrines have on metabolism.

THE REPRODUCTIVE SYSTEM

The teleost fish show greater diversity in their reproductive patterns than does any other group in the animal kingdom. Although most species have male and female sexes, hermaphroditism and bi-sexuality occur. Parthenogenesis (development from an unfertilized ovum) and gynogenesis (development from an ovum stimulated to divide by penetration from a sperm which does not contribute genes) are also recorded, either in the wild or in the laboratory (Purdom 1972). Eggs and sperms may be discharged into the water for external fertilization or copulation may take place, resulting in either discharge of fertilized eggs or viviparous release of young fish (Hoar 1969). Young fish may be hatched in nests and protected by male or female, hatched from eggs deposited in redds, released into the plankton from floating eggs or even held in the mouth of a parent.

An understanding of teleost reproductive anatomy and physiology and its pathophysiology is particularly important in cultured species, where the egg production and larval stages are normally the most critical in terms of economic as well as biological efficiency of the system (Bromage & Roberts 1995).

The testes

The testes are paired organs, suspended by mesenteries from the dorsal abdominal wall, alongside or below the swim-

bladder. They vary in size from small strands of tissue in the juvenile to large white flabby organs approaching 12% of the total body weight. There is a main collecting duct for genital secretions from the testis—the vas deferens—which conducts mature spermatozoa to an excretory meatus at the urinary papilla.

The testis itself comprises a series of tubules or blind sacs, the seminiferous tubules, which are lined with spermatogenic (or seminiferous) epithelium. The process of maturation of the male gamete involves the multiplication of spermatogonia, or sperm mother cells, which develop from the spermatogenic epithelium to form spermatocytes. Many of these eventually undergo a meiotic division to become the haploid spermatozoa. Spermatozoa attach to the surface of the pyriform, nourishing cells of the seminiferous epithelium known as Sertoli cells, until ready for release. Secretion of testosterone, the male secondary sex hormone, is by the interstitial cells of the testis, located in the fibrous supporting tissue or else in the basement membrane of the seminiferous tubules.

The ovary

The female genital tract of the teleost varies in structure from the simple cluster of ovarian follicles found in the lower teleost to the very complex organ found in viviparous species. This not only produces eggs but also acts as a spermatozoa store, a vagina and a uterus where the young embryos can be nourished.

The mature ovaries can represent as much as 70% of the total body weight. They are suspended from the abdominal wall by a mesentery and usually appear as a small cluster of minute orange-white spheres in the immature fish. The primary ovarian cells are the ovarian follicles. These line a hollow cavity or potential cavity which has a very complex series of folds in its lining. Ova are passed into this cavity as they mature. In the higher teleosts the ova are passed directly to the outside via an oviduct, but the more primitive species such as the salmonids pass the eggs into a fold of mesentery which ultimately ruptures and releases the eggs directly into the abdominal cavity, for evacuation via the genital opening.

Oogonia, the cells which are beginning to mature, are surrounded by a single layer of small epithelial cells and it is this aggregate of ova and epithelial cells which is known as the ovarian follicle. The epithelial cells grow as the ovum grows and are separated from it by a gradually thickening hyaline capsule, the zona pellucida. These granulosa cells, as they are known, are responsible for nourishing the ovum and secreting its yolk. Should an ovum degenerate before ovulation they

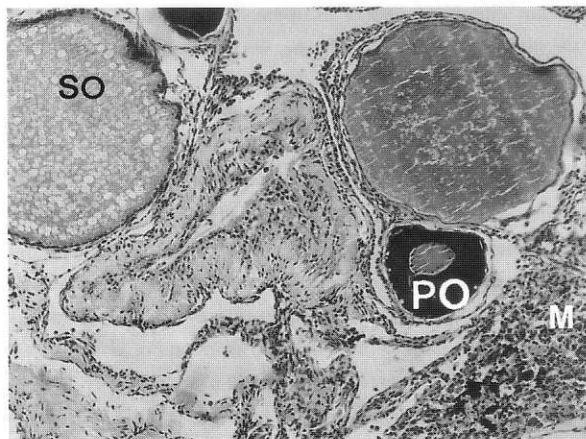


Fig. 2.41. Section through ovary of rainbow trout showing primary and secondary oocytes. PO = primary oocyte; SO = secondary oocyte; M = macrophages at site of oocyte degeneration. H + E $\times 60$.

invade the degenerating cell before themselves in turn being invaded by macrophages and melano-macrophages. In many species several generations of ova may be found in different stages of development (Fig. 2.41).

The fecundity of teleost fish varies. Those fish which merely liberate their eggs into the water, usually produce considerable numbers, to compensate for the lack of care. The mature common cod, for instance, can produce some nine million eggs per season, whereas the Nile tilapia, which cares for its offspring by mouth brooding, produces only a few thousand, in several broods, over an annual cycle (MacIntosh & Little 1995).

THE NERVOUS SYSTEM

The nervous system of the teleost extends throughout the body as an interconnecting system of integration centres and communication pathways: the neurones and their axonal and dendritic processes. The largest concentrations of nervous tissue are in the brain and its posterior extension, the spinal cord, and these together comprise the central nervous system (CNS). The peripheral nervous system (PNS) comprises the nerves passing out from the CNS and their nerve endings or organs of special sense.

Only a part of the nervous function is under conscious control—the nerves serving the striated myotomal muscles and voluntary cranial muscle. Regulation of heart beat, chromatophores, gill respiratory movements, peristalsis and the other functions of smooth muscles are controlled by autonomic components of the system, as in higher species.

The neurones of fish resemble those of other species except that there are some, such as the Mauthnerian groups, which are very large compared to those of mammals. Supporting cells, the neuroglia (astrocytes, oligodendrocytes and microglia), are also present. CNS tissue is divided into the classical grey and white matter consisting of nuclei of neurones and neuroglia, and myelinated axonal processes, respectively (Ariëns Kappers *et al.* 1960).

The brain and spinal cord are protected by a single primitive meningeal layer, the meninx primitiva, enclosing cerebrospinal fluid (CSF) produced by the choroid plexuses. These ependymal, glomerulus-like, invaginations of the ventricles are often in very different sites within the brain, compared with the mammal because of the differences in infolding of the teleost brain.

The roots of the spinal nerves, especially in the region of the dorsal root ganglia, are usually overlaid by clusters of eosinophilic granular cells, which are morphologically similar to those frequently observed in the teleost intestinal submucosa and other loose connective tissues.

The brain

The teleost brain is similar in its basic components to the brain of higher animals, but with many differences in form and complexity. For ease of description it is usually divided into five divisions comprising, from the anterior: the telencephalon, the diencephalon, the mesencephalon, the metencephalon or cerebellum and the medulla oblongata (Fig. 2.42).

Telencephalon

The telencephalon or forebrain is responsible for olfaction and for aspects of colour vision, memory and reproductive and feeding behaviour. The olfactory bulbs, situated in the nostrils, are directly connected to the telencephalon via axons from the telencephalon proper, known as the olfactory tract.

The telencephalon itself has no obvious lateral ventricle but is usually divided into pars ventralis and pars dorsalis. The olfactory component of the dorsalis, dominant in lower teleosts, decreases with evolutionary level so that in higher teleosts it is a highly differentiated centre processing much more than simply olfactory information. In fact ablation of telencephalon in actinopterygians causes disruption of a wide range of behaviour, including startle reflexes, learned behaviour and reproductive behaviour (Fig. 2.43).

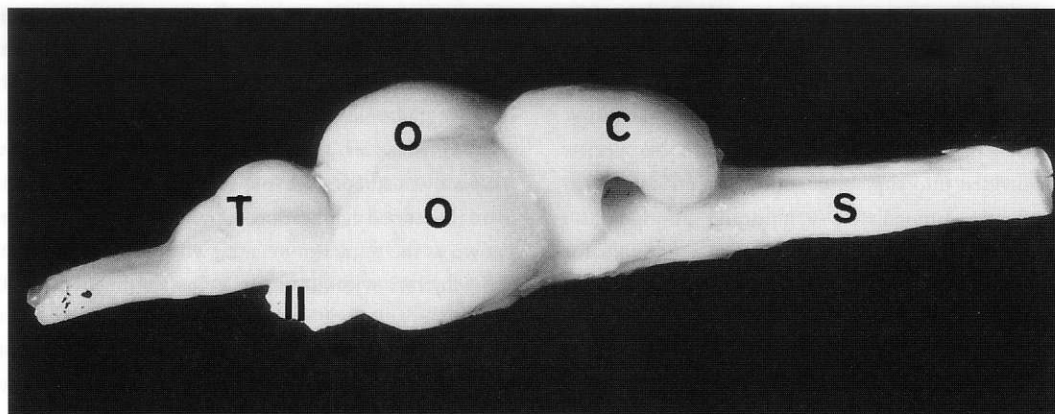


Fig. 2.42. The brain of an Atlantic salmon ($\times 2$). S = spinal cord; O = optic lobes; C = cerebellum; II = optic nerve; T = telencephalon.

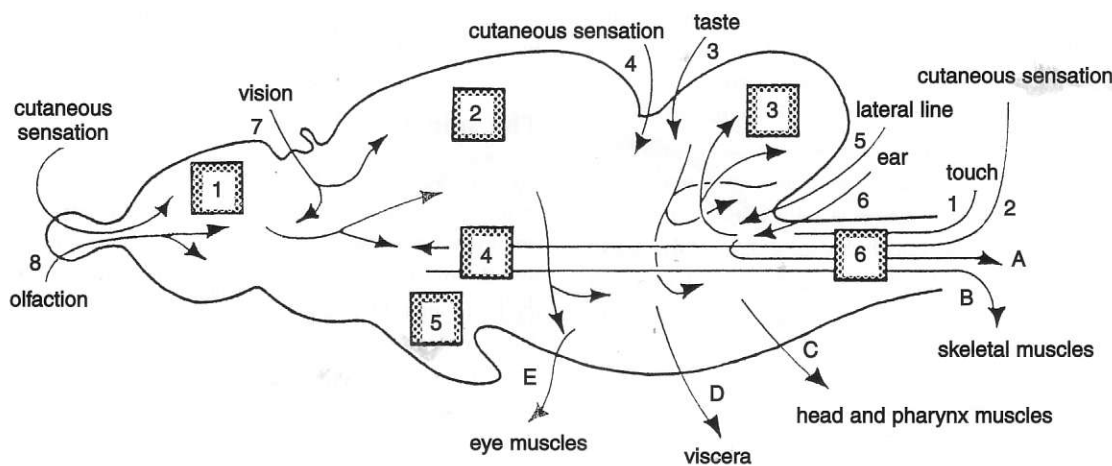


Fig. 2.43. Generalized fish brain showing functions of different regions. Stippled rectangles: regions of general integration. 1, learning, appetitive behaviour, attention; 2, sensory coordination, motor integration; 3, postural control and autonomic regulation; 4, alerting mechanisms; 5, homeostatic and appetitive coordination; 6, motor coordination. Efferents: A, reticulospinal tract; C and D, cranial nerves 5, 7, 9, 10, 11; E, cranial nerves 3, 4, 6. Afferents: 1, spinocerebellar tract; 2, spinoreticular tract; 3, cranial nerves 7, 9, 10; 4, cranial nerves 0, 5, 7, 9, 10; 5, cranial nerves 7, 9, 10; 6, nerve 8; 7, nerve 2; 8, nerve 1. (Redrawn from Laming 1981.)

Diencephalon

The diencephalon is very variable in form but is usually small and subdivides into three distinct components, the epithalamus, the thalamus and the hypothalamus.

The epithalamus consists of the pineal body, which is a light receptor with possible endocrine functions, and the habenular nuclei, which serve to coordinate outputs from pineal and telencephalon to the thalamus. This dorsal region of the thalamus also has neural links with the retina and the optic lobes.

The thalamus is very complex in structure and homologies with higher species or even between teleost species are often

exceedingly difficult to infer. It has a number of nuclei whose sizes vary considerably with species. In general terms it can be stated that the ventral parts of the diencephalon function mainly as correlation centres for sensory inputs such as gustation and olfaction.

The hypothalamus is more readily defined and usually relatively large in fishes. It appears to comprise mainly nuclei responsible for coordination of forebrain stimuli and lateral line impulses. An area controlling feeding behaviour has been located in the inferior lobe of the hypothalamus which receives both olfactory and gustatory information and seems

to have motor control over the jaw muscles involved in feeding. The neurohypophysis or pars nervosa is a downward pouching of the floor of the hypothalamus, the infundibulum, carrying axonal tracts of the preoptic ganglionic neurones. These tracts may be massive at spawning time in some species, but are not divided into supraoptic and paraventricular as in higher vertebrates. Immediately behind the infundibulum lies the saccus vasculosus, a choroid plexus responsible for cerebrospinal fluid secretion.

Mesencephalon

The mesencephalon is relatively large and anatomically subdivides into the optic tectum, which provides the roof of the third ventricle, and the tegmentum, which is its floor. Usually the optic tectum is a relatively large component and is divided by a longitudinal furrow into two globular structures, the corpora bigemina. It is particularly concerned with reception and coordination of optic nerve inputs, which reach the tectum after complete crossing over at the optic chiasma. In fish with well formed eyes the optic tectum has a cortex containing cells with various visual functions. For example, cells with dendritic processes arranged at right angles to the tectal surface are thought to be responsible for positional fixing and detail in the visual field. Another group of cells with processes orientated parallel to the tectal surface seem to be involved in the perception of movement.

The cerebellum or metencephalon

The cerebellum of teleost fish varies considerably in size and morphology between species. It is generally associated with reception and coordination of proprioceptive and balance stimuli. In most teleosts it has two components, a vestibulolateralis (basal) lobe, which receives stimuli from the vestibular apparatus and lateral line inputs, and the corpus cerebelli, more dorsally situated, which receives sensory stimuli via the spinal cord from extremities and proprioceptors. The size of the basal lobe is related to the degree of development of the lateral line.

Medulla oblongata

The medulla merges with the spinal cord without any distinct demarcation. It comprises mainly four columns of nerve fibres, the visceral sensory and motor and the somatic sensory and motor tracts. These dorsal and ventral tracts also form the roots of cranial nerves V to X. In cyprinids the taste fibre component of the medulla is everted to encompass the very considerably

enlarged gustatory elements derived from their very well developed taste receptors. Autonomic supply from the medulla is restricted to the oculomotor and vagus nerves, with the latter supplying, *inter alia*, the branchial vascular bed, heart, stomach and swim-bladder. The intestine is not usually innervated by the parasympathetic. Nervous regulation of respiration is vested in an ill-defined aggregation of motor neurones of the medulla known as the respiratory centre, which coordinates the ventilation rhythms without higher influence (Shelton 1970).

The nerve supply to taste receptors—extensively studied because of ecological, nutritional and fishery significance—is located within the medulla, as are the acoustic fibres, with sound perception fibres in the pars superior and vestibular function in the pars inferior. The medulla also acts as a centre for control of chromatophores. The Mauthner cells, two very large neurones, lie in the medulla at the level of the VIIIth nerve root, but their axons pass ventromedially right down the spinal cord and help to coordinate swimming movements.

The spinal cord

The spinal cord of teleosts extends the length of the body and terminates, in higher teleosts, in an endocrine structure, the urophysis. Grey and white matter in the teleost spinal cord is well demarcated, increasing in complexity with evolutionary level, although the two dorsal horns of grey matter are fused. They contain numerous large motor neurones in both the dorsal and the ventral horn tissue. The dorsal and ventral roots of the spinal cord do not have a demarcation of motor and sensory nerve fibres as in higher vertebrates: both tracts contain a mixture of nerve fibres. Major features of the cord tracts are the very large ventromedial axons, the Mauthnerian axons from the medulla.

Peripheral nerves

There are ten cranial nerves serving both sensory and motor, voluntary and involuntary functions of the head and, in the case of the vagus, para-sympathetic supply to the main visceral organs also.

Special sense organs

The eye

The teleost eye is remarkably similar to the eye of all other vertebrates (Fig. 2.42). The sclera, the outer fibrous coat, has insertions for three pairs of oculomotor muscles innervated by the third cranial nerve and has cartilaginous or osseous sup-

ports. The cornea, the external window of the eye, is similar in its refractive index to water. Its layers comprise an epidermal conjunctiva, a basement membrane (Bowman's membrane), a dermally derived substantia propria and an internal basement membrane and endothelial layer (Descemet's membrane and endothelium). The teleost lens is not lenticular in shape but usually completely spherical. It protrudes partially through the iris to provide a very wide angle of view (a 'fish eye' lens). The short focal length of the eye means that the fish has a large field of view and depth of focus. A small degree of accommodation is achieved by moving the lens towards the retina, by the action of the *retractor lentis* muscle (Fig. 2.44). In many species the lens, or the cornea, is tinted.

The choroid vessels of the eye form a subscleral network of capillaries for nourishment of the retina. They are associated

with the large choroid gland, a network of capillaries which is active in oxygen secretion and whose function is considered to be related to ensuring a high level of oxygen for the retina, although it also has blood-monitoring functions. The oxygen secretion mechanism is the same as in the gas gland of the swim-bladder, so certain syndromes may become apparent simultaneously in both organs.

The ocular humours have not been investigated in any detail but, although there are considerable differences in viscosity between species, they can usually be compared with the aqueous and vitreous of higher animals.

The iris is virtually fixed, having very poorly developed sphincter and dilator muscles even in advanced species. The teleost retina, the light-sensitive tissue, is organized as in other vertebrates, with transparent nervous elements innermost,

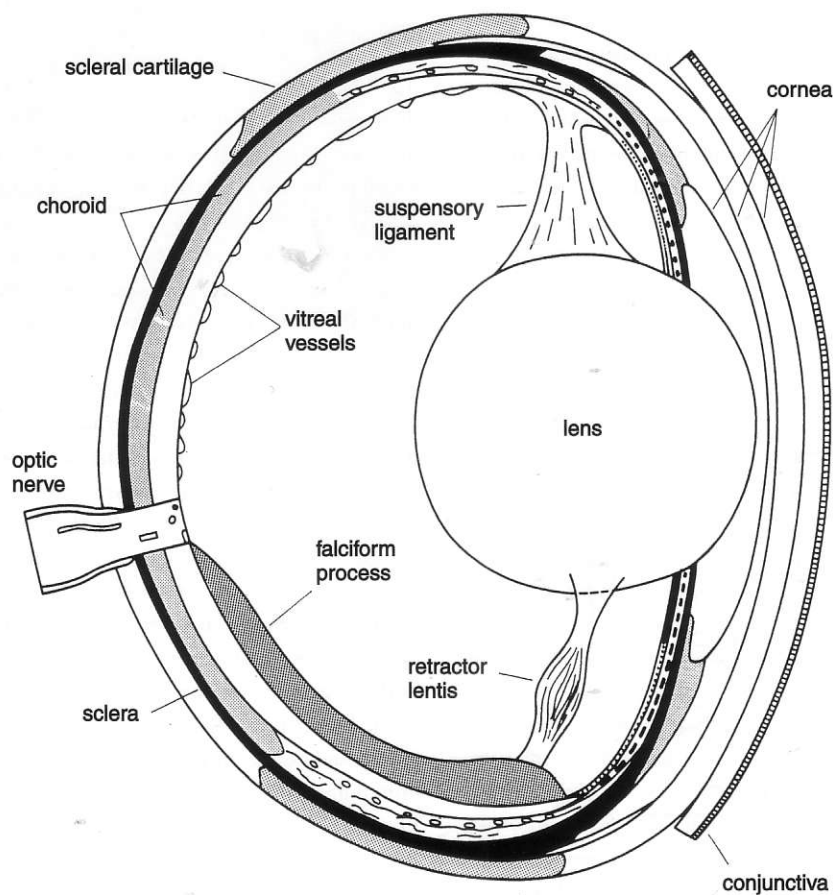


Fig. 2.44. Diagrammatic representation of the teleost eye. (After Walls 1942.)

overlying the rod and cone receptor cells with the black pigmented layer peripherally. Most shallow water fish have colour vision but cones and colour vision are absent in fish living at depths where absorption of long-wavelength light by the water column produces low intensity blue-green monochrome illumination (Munz 1971). The visual pigments, rhodopsins, vary in spectral sensitivity according to the visual environment of the fish. The optimum wavelength of light tends to match that which is best transmitted by the water in which the fish lives. Thus oceanic fish are most sensitive to light with a blue bias, while the eyes of fresh-water fish respond best to light with reddish tint. Some fish have two types of visual pigment, the proportions of which can vary seasonally or during migrations between sea- and fresh-water.

The labyrinth

The labyrinth is an evolutionary development of the anterior lateral line, forming a complex sensory organ associated with maintenance of equilibrium and 'hearing'. It consists of two connected parts: the semicircular canals and the otolith organs (Fig. 2.45).

The semicircular canals comprise three fibrous semilunar tubes embedded in the skull. The ends of each canal insert into the labyrinthine cavity which is expanded at one end to form the spherical ampulla which contains the cristae, small ridges

with a tapetum of sensory hairs similar to the mechanoreceptors of the lateral line. The sensors are stimulated by movements of the fluid in the canals, the endolymph, which indicate angular acceleration, and the stimuli are passed to the cerebellum via the acoustico-lateralis tract.

The otolith organ usually consists of three inter-connecting chambers, known as the membranous labyrinth (the utricle, saccule and lagena). The semicircular canals insert in the utricle. In each chamber are located otoliths, distinctively shaped white calcified 'stones' overlying the sensory epithelium, which consists of sensory cells which are again very similar to the lateral line neuromasts. The membranous labyrinth is also filled with endolymph, and movement of the otoliths, which are denser than the endolymph, over the sensory tissue, provides stimulation by the force of gravity and low-frequency sound vibrations. The otolith organs are important in the perception of sound, acceleration and change in equilibrium.

The lateral line system

Although it has origins and nervous components in common with the labyrinth, the lateral line is a distinct organ, found only in lower vertebrates. The main components are the paired lateral line canals but in some species there are also well developed contiguous head canals.

The canal is a groove in the trunk of the fish, on each side, with a bony support and an integumental cover which is punctuated by sequential pores along its length. The mechanoreceptors are located basally in the canal, alternating with the position of pores. These 'neuromasts' are stimulated by transfer of motion in the external *milieu* to the water inside the canal, which mechanically displaces the receptors of the neuromasts. These consist of pyriform receptor cells with a bundle of sensory hair-like structures which extends up into the gelatinous cupula (Fig. 2.46) (Flock 1971). Lateral line neuromast organs are very sensitive to water particle displacement such as that induced by nearfield sound of frequencies up to 200 Hz. It has been suggested that some fish are capable of detecting the vibrations of active prey over distances up to 32 m. The nerve supply to the lateral line nerve, which is parallel and slightly medial to the canal itself, is mainly derived from the vagus (Xth cranial nerve).

Olfactory and gustatory senses

The olfactory organs are paired pits on the snout with a single opening traversed by a cusp of skin dividing it into an anterior

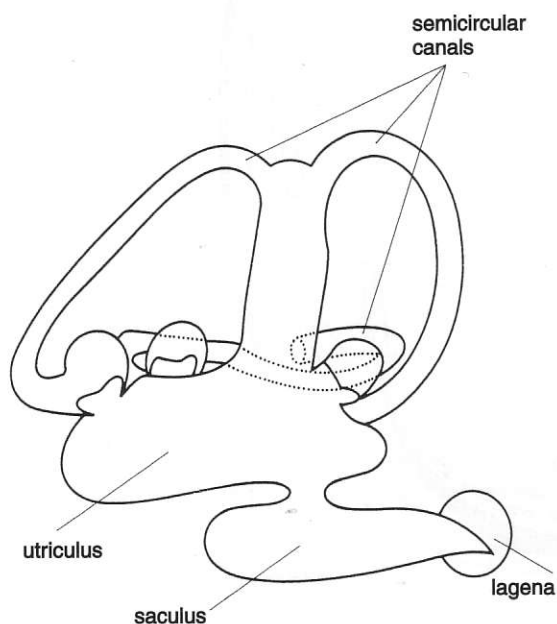


Fig. 2.45. The labyrinth organ of a typical teleost.

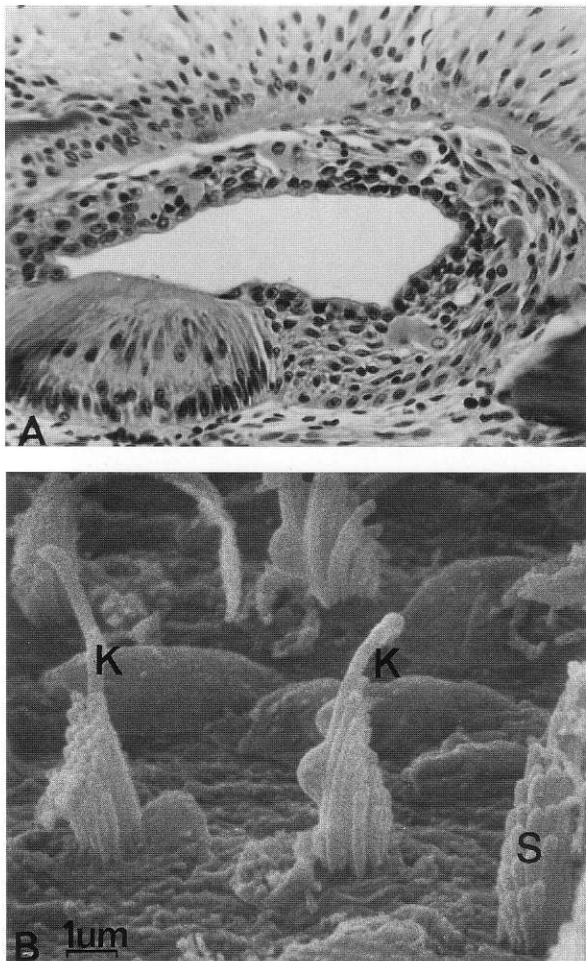


Fig. 2.46. The lateral line organ. A: The mandibular canal and neuromast of *Fundulus heteroclitus* H + E $\times 250$. B: Scanning electron micrograph of surface of sensory epithelium of a canal organ of the burbot. In centre are two sensory hair bundles with kinocilia (k) facing in opposite directions. Steriocilia (s) are bundles of increasing length. (A by courtesy of Dr G.R. Gardner; B by courtesy of Dr A. Flock.)

inlet and posterior exhaust. Swimming and breathing allow passage of water through the sacs, passing over the olfactory epithelium, which is raised in a series of very vascular folds or fingers which increase the surface area of sensory tissue.

The actual olfactory tissue consists of focal groups of receptor cells surrounded by mucoid and ciliated columnar epithelium. Axons from the olfactory bulb collect from the bases of receptor cells to form the olfactory tracts to the telencephalon. In many species the loose connective tissue of the subepidermal tissue of the nasal mucosa is heavily endowed with eosinophilic granule cells.

The gustatory organs—the taste buds—are found both on the outer surface of the lips, head, barbels and fins and on the gill rakers, gill arches and mouth. Indeed in some species they may be found spread over the entire body surface. Normally innervated by branches of the VIIth, IXth and Xth cranial nerves, the buds are composed of clusters of elongated segment-shaped cells forming a sphere. They consist of receptor, supporting and basal cells. There are also many free neurones in the teleost epidermis.

SWIM-BLADDER

Structure

The gas-filled swim-bladder is a conspicuous (up to 7% of body volume) and characteristic feature of the viscera of teleost fish (Fig. 2.47). Its primary function is as a buoyancy mechanism since the teleost body has a specific gravity 107% and 105% of that of fresh- and sea-water respectively. It is also used for sound and pressure reception and in some species is equipped with drumming muscles for sound production. The swim-bladder is absent in many bottom-living species, where neutral buoyancy is not necessary, and in some fast-swimming pelagic species, where it would increase drag by increasing the surface area.

In the fish larva the swim-bladder develops as a dorsal diverticulum of the foregut, so that in the adult many structural features of the digestive tract are retained. Histologically it consists of two main layers: a tunica interna, which lines the gas space, and a tunica externa. The tunica interna has a transitional epithelial layer overlying a muscularis mucosa and a submucosa of loose, vascular connective tissue. The tunica externa consists of an outer serosa beneath which lies a tough fibrous layer in which muscle and elastic connective tissue are found.

The embryonic connection between the gut and the swim-bladder is retained as a pneumatic duct in many of the more primitive teleosts (Isospondyli). Fish with this type of swim-bladder are referred to as 'physostomes'. In most of the spiny-rayed fish the functional pneumatic duct is lost: the closed or 'physoclistous' swim-bladder. In both physostomes and physoclists the swim-bladder has a wide range of morphological variation related to habitat and behaviour (Fig. 2.48). Many physostomatous swim-bladders have two chambers separated by a diaphragm. The anterior chamber is then associated with gas reception and retention and consequently has a thicker wall. The posterior chamber, being involved in gas reabsorption, has a thin tunica interna.

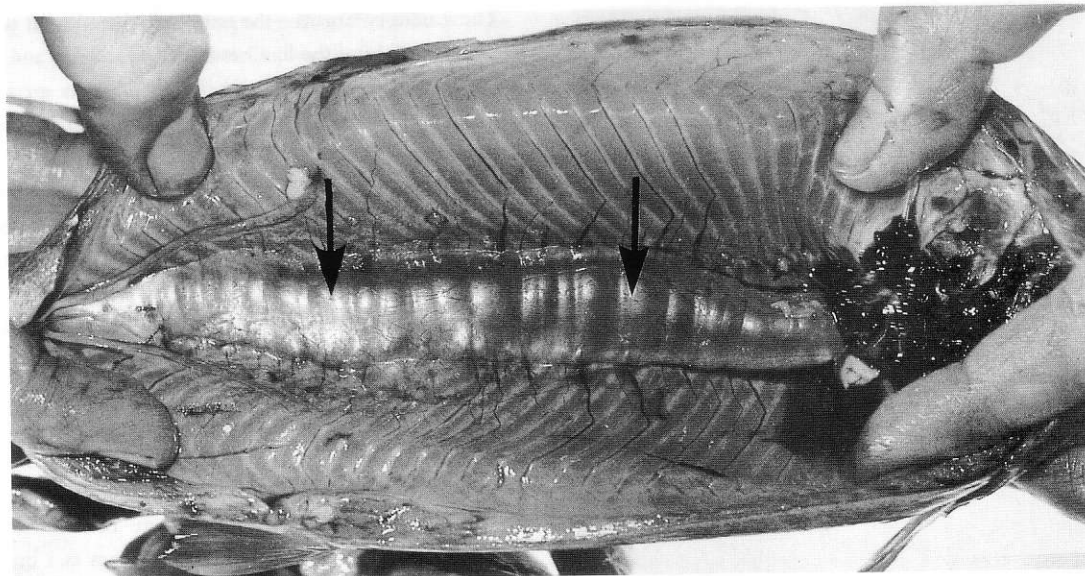


Fig. 2.47. The swim-bladder of the rainbow trout (arrowed).

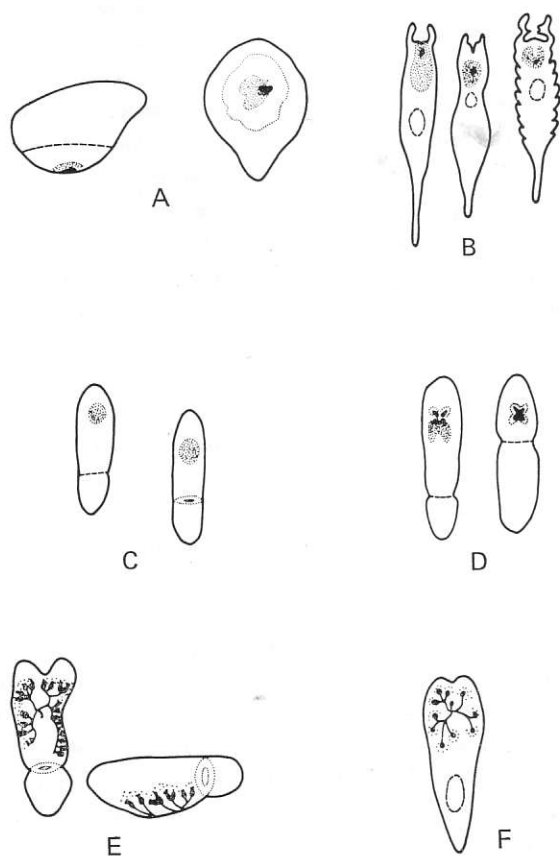


Fig. 2.48. Diagrammatic representation of swim-bladders from a variety of shallow water fishes. They are drawn as though transparent, with the stippled area representing the extent of the gas-secreting complex and the broken lines the sphincter separating secretory and absorbent chambers or areas of the swim-bladder. A, The goby: an example of a single gas gland with a large oval area. B, The gadoid pattern: a single gas gland and oval sphincter. C, The wrasse pattern: double-chambered swim-bladder with a four-part gas gland. D, A variant of C in which the surface areas of the chambers can be altered by muscular displacement of the sphincter. E, The gurnard: a multilobulate gas gland in a double-chambered bladder. F, The perch: a multilobulate gas gland with an oval sphincter.

Buoyancy adjustment

Neutral buoyancy depends on maintaining a constant volume in a flexible, gas-filled, buoyancy chamber, irrespective of the depth of the fish. In physostomes with access to the water/air interface, inflation is produced by swallowing air which is then forced via the pneumatic duct to the swimbladder. In physoclists, and those physostomes with no access to the water/air interface, inflation is by the release of gas from arterial blood passing through a gland situated in the tunica interna of the anterior ventral area of the swim-bladder. Gas reabsorption occurs when a capillary plexus (the oval) arising from the dorsal aorta is exposed to swim-bladder gas. The oval is an impervious muscular diaphragm which controls the area of exposed plexus and hence absorption. The components of the gas are mainly oxygen, nitrogen and carbon dioxide but the proportions are, in many cases, different from those in air. The partial pressure of oxygen can be as high as 176 atmospheres in some species of deep-sea macrurid. In physostomes such as the cyprinids the swim-bladder contains pure nitrogen, while carbon dioxide is a more variable component occurring mainly in physoclists (for further information see Steen 1970 and Tytler 1976).

Sound and pressure reception

Sound and pressure reception are similar, in that sound propagation through water involves pressure oscillations as well as water particle velocity and displacement. Thus the flexible gas-filled swim-bladder which responds to pressure changes by changing volume is an obvious potential pressure receptor. The swim-bladders of many species are found to have direct or indirect linkage with the perilymphatic system of the inner ear.

Although near sound sources can be perceived by fish without swim-bladders, by bone conduction, otolith vibration or lateral line response, they are insensitive to distant sound sources above 400 Hz. Fish with swim-bladders but without connections with the inner ear (e.g. gadoids) can produce good conditioned responses at frequencies below 520 Hz, whereas the clupeids, ictalurids or cyprinids, with their direct connection, may have frequency ranges of perception between 13 and 4000 Hz. The presence of Weberian ossicles pushes the upper frequency response to as high as 5000 Hz. As with buoyancy, any uncompensated change in depth will affect the sensitivity of sound perception by changing the volume of the swim-bladder (Chapman & Hawkins 1973).

Fish with swim-bladders can perceive relative pressure changes equivalent to less than 0.5% of the ambient hydrostatic pressures, while those without can only detect pressure changes between 2.5% and 10% (Tytler & Blaxter 1973).

Sound production

Although they do not possess a larynx, some fish can produce sounds by rubbing the serrated surfaces of special skeletal components. Many species of the families Sparidae, Tetrodontidae and Holocentridae produce high-pitched sound by grinding their teeth, but the vibration of the swim-bladder wall by special muscles provides the greatest repertoire of noises or calls produced in fish. The members of the Triglidae are well known for their sound production. In fact, the crepuscular choruses of the sea robins caused great confusion amongst operators of anti-submarine acoustic echolocation devices on board warships of the US navy during World War II, until the target was eventually recognized (Moulton 1956).

THE ENDOCRINE SYSTEM

The endocrine system of fishes has the same basic components as that of higher vertebrates but, because of the considerable differences in environmental constraints and evolutionary development experienced by the teleosts, it has many distinctive features (Matty 1985). The most striking difference is the number of endocrine structures which have no apparent analogue in the mammals. These include the urophysis, the corpuscles of Stannius and possibly the pseudobranch.

The pituitary

The teleost pituitary, as in all vertebrates, comprises two embryologically distinct components. These are the neurohypophysis, which grows down from the diencephalon of the brain, and the adenohypophysis, which originates as an upward budding of the ectoderm of the embryonic buccal cavity. The two fuse together with their respective mesenchymal vascular supply to form a composite endocrine gland enclosed above by the diencephalon and laterally and below by a bony cupula, the cella turcica. Extracts of hypophyseal tissue are frequently used to stimulate ovulation in cultured fishes and the protection afforded by these structures makes its removal from cadavers somewhat difficult.

The teleost neurohypophysis is much simpler than that of mammals and comprises a stalk of nervous tissue with an

enlargement at its tip, which forms the core of the complete gland. The stalk is composed of the axons of neurosecretory neurones, whose cell bodies are located in the hypothalamic nuclei.

The adenohypophysis secretes a variety of protein or peptide hormones and is usually divided anatomically into a pars intermedia and pars distalis. It is composed of a number of different cell types with a variety of tinctorial properties. The precise correlation of the different cell types with their specific hormones has not yet been completely worked out and may differ between species (Ball & Baker 1969).

The pituitary hormones of teleosts can be divided into two groups: those which stimulate activity of other endocrine organs, e.g. the thyroid, gonads, and adrenal, and those influencing physiological processes such as the movements of dermal melanophores, osmoregulation, metabolism and growth. A more detailed description of the teleost pituitary and its hormones is presented in the excellent review by Matty (1985).

The thyroid gland

The teleost thyroid is similar in basic structure to that of the mammal, and the thyroid hormone, which has a stimulatory effect on many metabolic processes, is a similar iodinated thyroxine to that of higher animals (Gorbman 1969). The thyroid follicles are, as in the mammal, usually round to oval with low cuboidal epithelium and PAS-positive colloid secretion, but a very important point of differentiation is the anatomically diffuse distribution of the follicles, which varies considerably between species or even between individuals. Instead of being located within a discrete capsule they are distributed throughout the connective tissue of the pharyngeal area or even, in some species, around the eye, ventral aorta, hepatic veins and renal haemopoietic tissue (Fig. 2.49). This diversity of distribution has led to a number of reports of such follicles as being neoplastic but although thyroid tumours do occur, most such reports are probably merely of normally ectopic follicles.

Control of thyroxine and tri-iodothyronine is through pituitary thyroid stimulating hormone (TSH). Both are transported in the blood, bound to plasma proteins. Unlike the case in mammals, thyroid hormones do not produce a calorogenic response in fish, but seem to influence carbohydrate metabolism and the mobilization of lipid reserves. These responses of fish to thyroid hormones are very dependent on the nutritional state, ambient temperature, photoperiod, and salinity. Recent work has shown that thyroxine promotes growth in juvenile fish by stimulating appetite.

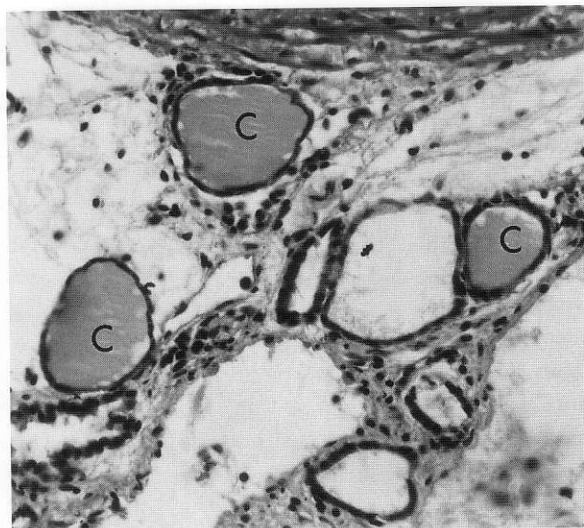


Fig. 2.49. Follicles of thyroid tissue in the connective tissue around the ventral aorta. Colloid (C), the thyroid hormone secretion, is present in the lumen of three follicles. H + E $\times 70$.

Adrenals (interrenal and suprarenals)

The compact adrenal-type endocrine gland comprising both cortex and medulla is only found in a few teleost groups such as the sculpins. Normally, in teleosts an adrenal cortical equivalent, the interrenal, a series of strands of lightly staining cuboidal eosinophilic cells, is situated in the anterior kidney, often in association with major blood vessels passing through the area (Fig. 2.50). The steroid hormones of the adrenal

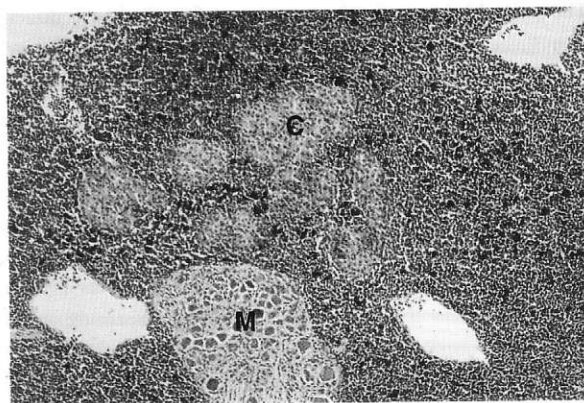


Fig. 2.50. Section of anterior kidney of rainbow trout showing strands of pale-staining adrenal cortex cells and the single nodule of neurosecretory neurones forming the adrenal medulla. The stroma of haemopoietic tissue is also well endowed with individual dark brown melanomacrophage cells. H + E $\times 110$.

cortex include gluco- and mineralocorticoids, and these have very similar functions to those in the higher animals.

The adrenal medulla, the chromaffin tissue (so called because of its staining reaction to chromic salts), is variable in location. It may be found accompanying the sympathetic ganglia, in clumps between anterior kidney and spine or, as indicated above, in close contact with the interrenal tissue, within the head kidney. It secretes sympathomimetic substances such as adrenaline, associated with immediate stress responses. In particular, increase in blood levels of catecholamines causes hyperglycaemia and increases the functional area of the gills for gaseous and ionic exchange.

Ultimobranchial glands

All vertebrates have the capacity to regulate their serum calcium and in fish this is achieved by the activity of the ultimobranchial glands, cords of polygonal cells lying just ventral to the oesophagus within the septum separating the sinus venosus from the abdomen (Copp 1969). The organ is an embryonic fifth gill arch and corresponds in function to the parathyroid in mammals.

The corpuscles of Stannius

The corpuscles of Stannius are usually paired whitish clusters of endocrine tissue normally located retroperitoneally on the surface of the kidney. The large clear endocrine cells appear to

secrete into the centre of the cluster (Fig. 2.51). They secrete a glycoprotein hormone called teleocalcin, blocking absorption of calcium by the gills, so they are in some ways akin to the parathyroids of higher animals. They also have other properties such as secretion of pressor substances and possible involvement in osmoregulation, but they do not seem to secrete steroid compounds.

The endocrine pancreas

The distribution of the pancreatic tissue varies considerably with species. The endocrine component is also varied, with small islets of Langerhans scattered throughout the tissue, in the salmonids or anguilliforms. In the higher teleosts the endocrine tissue consists of a small number of scattered islets and a large compact islet which varies in size with the stage in the life cycle and is known as the Brockman body (Epple 1969). They have a delicate fibrous capsule within which are the three types of islet cells, cells which produce glucagon, cells producing insulin and cells of unknown function (Fig. 2.52). There is considerable change in islet size at spawning and senility and with dietary changes but there are also reported seasonal differences in the proportions of the different cell types.

Insulin causes hypoglycaemia but fish do not exhibit the rapid blood glucose clearance response typical of mammals. Insulin release is linked to blood glucose levels but only to enable relatively low levels to leave the extracellular space to

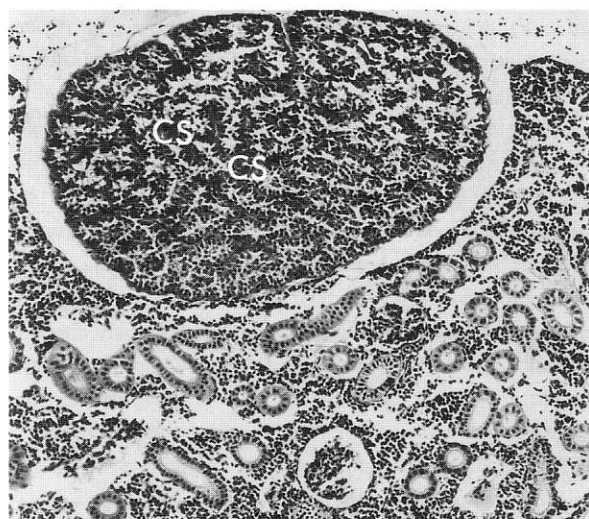


Fig. 2.51. Section through the mid-kidney of rainbow trout passing through a corpuscle of Stannius (CS). H + E $\times 35$.

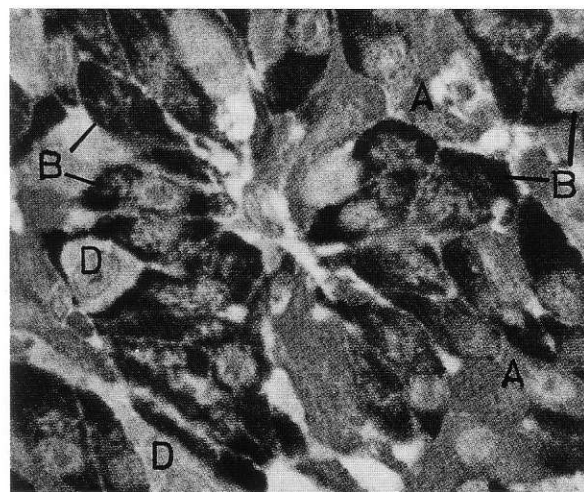


Fig. 2.52. Islet of Langerhans cells in the pancreas of the common carp. A = alpha cells; B = beta cells; D = delta cells. AT-azocarmine G $\times 800$. (By courtesy of Dr M. Yokote.)

function as cellular fuel. It may be directed to oxidative clearance of glucose but not to glycogen deposition. Insulin has been found to inhibit gluconeogenesis from amino acids and to reduce the turnover of liver protein. The prime role of insulin may be to conserve protein and amino acids and promote tissue deposition.

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.

The urophysis

The caudal endocrine secretory structure is only found in sharks and bony fish. Its function is still somewhat obscure but its anatomy has been well described by Bern (1969). It is a small whitish ventral expansion of the spinal cord, at its posterior end. It is invested with a meninx, like the rest of the cord, and has a very extensive vascular supply draining to the renal portal system. It is comprised largely of large neurosecretory cells with polymorphic nuclei, whose non-myelinated axons extend from the cord, in a urophyseal stalk analogous to that of the hypophysis. They terminate in pallsades adjacent to capillary walls of the neuro-haemal complex, akin to those of the hypothalamus.

The urophyseal hormones are peptides which are concerned principally with osmoregulation, although one acts specifically on the smooth muscle of the reproductive tract (Bern & Nishioka 1993).

The pseudobranch and choroid body

The pseudobranch is not present in all teleosts but where present it is a red, gill-like structure derived from the first gill arch and attached to the internal surface of the operculum. It consists of parallel blood capillaries supported by cartilage rods. The pseudobranch has a direct vascular connection with the choroid of the eye, which is composed of similar arrays of capillaries alternating with rows of slender fibroblast-like cells. Those fish which do not possess a pseudobranch (e.g. 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.

The gonads

As well as their obvious gametogenic function, the teleost gonads also secrete hormones, which have a generalized effect on a wide range of tissues. Their output is controlled by the output of gonadotropins from the pituitary, the 'conductor of the endocrine orchestra'. The oestrogens and androgens produced in the gonads cause thickening of skin, colour changes, development of breeding tubercles, kypes and swelling of the urogenital area.

Androgens and oestrogens also have far-reaching effects on carbohydrate, lipid and protein metabolism. Androgens stimulate protein anabolic processes, promoting growth by increasing the protein and RNA content of liver, kidney and muscle. Oestrogens tend to increase body reserves of lipids and initiate the synthesis of vitellogenic proteins.