Invited Review

The phylogenetic odyssey of the erythrocyte.
III. Fish, the lower vertebrate experience

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Summary. The piscine erythrocyte can be considered the prototype of the red cells that are distributed among inframmalian vertebrates. It is a permanently nucleated, hemoglobin-ladened, oval, flattened, biconvex disc. Ultrastructurally it demonstrates a cytoskeleton comprised of a marginal band and a membrane skeleton which are responsible for the erythrocyte's conversion to an ellipsoid during morphogenesis and endow it with resilience to physical trauma. Erythropoiesis initiates in the yolk sac, followed in many fishes, by the intermediate cell mass. These sites are the sources of the transitory, primitive generation red cells which apparently make their first phylogenetic appearance in fishes and which are subsequently represented in all classes of vertebrates including mammals. Production of definitive generation erythrocytes is centered in evolutionary «pre-splenic» tissue of the gastrointestinal tract or in the spleen in cyclostomes, dipnoi, and chondrichthyes while in teleosts it is typically located in the kidneys with or without splenic participation. The blood is a major site of erythrocyte maturation in the lower fishes and exhibits significant numbers of immature erythroid cells plus occasional mitotic figures. Some teleosts also circulate developing erythroid cells. Certain fishes have occasional circulating erythroplastids, conceptually a portent of phylogenetic changes in higher vertebrates. Remarkably, some bristlemouths have denucleated erythrocytes exclusively in the circulation. The largest piscine erythrocytes are found in the dipnoi, myxines, and chondrichthyes. Primitive fish with the exception of the endothermic sharks tend to have lower hemoglobin concentrations than the modern teleosteans. The very highest hemoglobin concentrations are attained by the endothermic scombrids. Erythrocyte-based data have a broad extent and are variably affected by age, sex, season and environment. This report includes a substantial selection of illustrations (fish species and rbc micrographs).

Key words: Erythrocyte, Hemoglobin, Fish erythrocyte, Erythropoiesis, Hemopoiesis

Introduction

Erythrocytes have a sporadic, scattered representation among invertebrates and it is in the fishes that the red cell first becomes uniformly expressed throughout a taxonomic class (Glomski and Tamburlin, 1989, 1990). The piscine red cell can be considered a prototype of the circulating nucleated, hemoglobin-bearing cell that is phylogenetically retained by all other submammalian vertebrates. The purpose of this discussion is to present a configurational, ultrastructural, ontogenetic, phylogenetic and quantitative profile of the erythrocyte in primitive and modern fishes. Phenomena such as yolk sac erythropoiesis, primitive (larval) and definitive generation red cells, evolutionary progression in erythropoietic loci, erythromorphogenesis, and the «early» circulation of erythroplastids are addressed. The red cells are also studied and compared in the perspective of their indices (MCV, MCH, MCHC). The varied cytology and functional expression of the ichthyic erythrocyte present an intriguing discipline. Its appreciation also serves as a basis for an enhanced understanding of red cells in other species.

Discussion

The phylum Chordata embraces those animal species having an anatomical structure advanced beyond that observed in the invertebrates. Its members manifest, either at some stage in their existence or throughout life, an axial rodlike notochord for support of the body, a single dorsal tubular nerve cord and paired gill slits that

establish a passageway between the pharynx and the exterior. Aggregates of highly vascularized tissue termed gills are located at the internal aspect of the gill slits and are designed for the extraction of oxygen from the aqueous environment. The latter activity, in all but extremely rare species, is facilitated by erythrocytes that circulate through the gill capillaries. The chordates embrace the subphyla Urochordata, Cephalochordata (also called protochordates), and Vertebrata which because of its importance has also been viewed as a phylum in its own right. The subphylum Cephalochordata is not a major one but notable in that although its members are small, marine, fishlike creatures that manifest blood vessels and many gill slits they nevertheless have neither erythrocytes nor hemoglobin. They, like the vast majority of marine invertebrates are capable of obtaining oxygen sufficient for their requirements from the ambient sea water without the need of erythrocytes to enhance oxygen
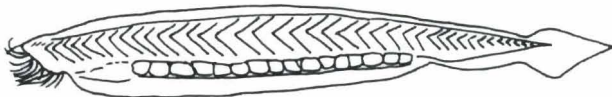

Fig. 1. *Branchiostoma lanceolatum,* amphioxus, a lancelet. The mouth is surrounded by tentacle-like processes (cirri). This fishlike creature is semitransparent thereby permitting its myotomes (muscle segments) to be externally visible.

transport or storage. The classic example of a cephalochordate is the semitransparent lancelet or amphioxus *Branchiostoma lanceolatum* (Fig. 1).

The vertebrates demonstrate certain structural features as a cranium-enclosed brain, cartilaginous or osseus vertebrae, visceral (gill) arches and a closed circulatory system containing hemoglobin-bearing erythrocytes. The most primitive extant vertebrates are the jawless fish (class Agnatha or the cyclostomes). They have a cylindrical eel-like body with median fins only, a smooth skin without scales, no true jaws, and as would be

Fig. 2. *Petromyzon marinus,* a lamprey. This primitive vertebrate's low evolutionary status is reflected in its erythrocytes which manifest monomeric hemoglobin.

anticipated, multiple pairs of gill pouches. The sea lamprey *Petromyzon marinus* (Fig. 2) and the Atlantic hagfish *Myxine glutinosa* are representative members of this group. The adult lamprey as well as its larval form, the ammocoete, have modest sized, circular, nucleated, disc-shaped erythrocytes. Their dimensions (12 - 14 μm diameter and 4 μm thickness) as well as their uncommon (for submamalian vertebrates) circular form were
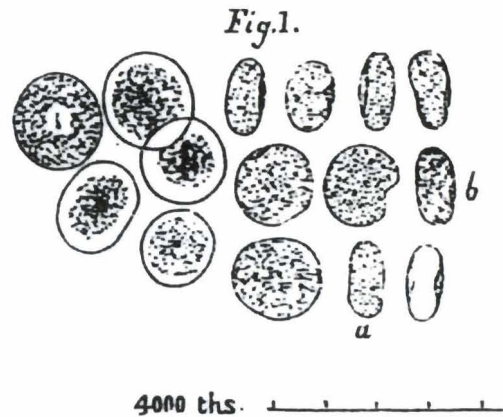

Fig. 3. Erythrocytes of the lamprey *Petromyzon* suspended in plasma. Their round shape and thick, flat or slightly biconvex profile was recognized early in hematologic studies. Erythrocytes viewed edgewise are indicated by a. and b. From a woodcut published by George Gulliver in 1870. Each division in the scale represents one four thousandth of an inch. Proc. Zool. Soc. (London).

established more than a century ago by Gulliver in 1870 (Fig. 3). Hagfish *Myxine glutinosa* red cells are oval, about 30 x 22 μm in long and short diameter, somewhat flattened, permanently nucleated, and have a volume of approximately 1500 femtoliters (fl, μm^3) as opposed to the human erythrocyte with a volume of 100 μm^3 (Jordan and Speidel, 1930; Wintrobe, 1933). This erythroid disc is slightly biconcave along its lateral margins; the
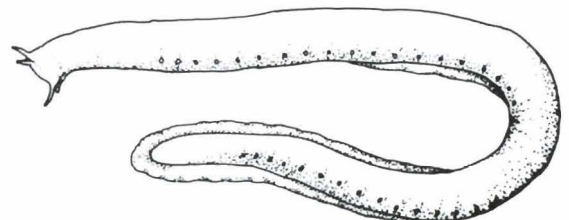

Fig. 4. *Myxine glutinosa,* Atlantic hagfish. Sensory tentacles are identifiable around the mouth and slime-producing glands are distributed linearly along the sides of the body. The hagfish's eyes are small, covered by pigmented skin and consequently are not apparent.
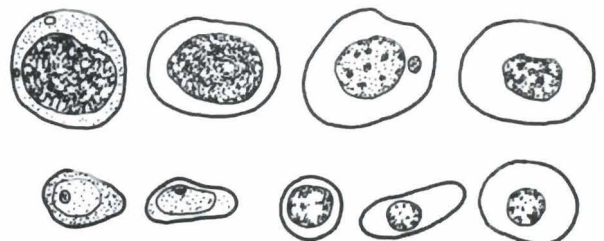

Fig. 5. Development of erythrocytes in the hagfish *Myxine glutinosa.* Top row: (first cell, left) a hemoblast followed by an early erythroblast, late erythroblast, and a mature erythrocyte respectively. Blood smear, Wright's-stain; x 650. Bottom row: same sequence terminating with two mature erythrocytes, profile, and en-face views respectively, from sections of liver, eosin-azure stain, x 800. After Jordan and Speidel, 1930.
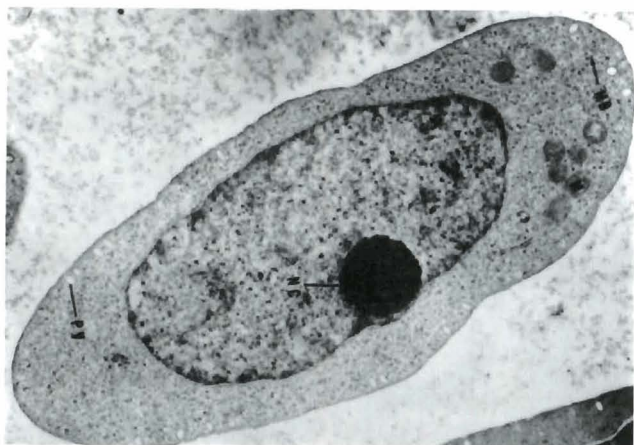
Fig. 6. A young erythrocyte from the Pacific hagfish *Eptatretus stoutii*. The chromatin is delicate and the nucleolus (NC) is very prominent. Ribosomes, pinocytotic vesicles (PV) and mitochondria are identifiable. Microtubules of the marginal band (MB) are present but not well resolved at this magnification, TEM x 9200. Sekhon S.S. and Maxwell D.S., Fine structure of developing hagfish erythrocytes with particular reference to the cytoplasmic organelles. J. Morph., The Wistar Institue Press, 1970. Reprinted by permission of Wiley-Liss, division of John Wiley & Sons, Inc. This acknowledgement also applies to Fig. 7.
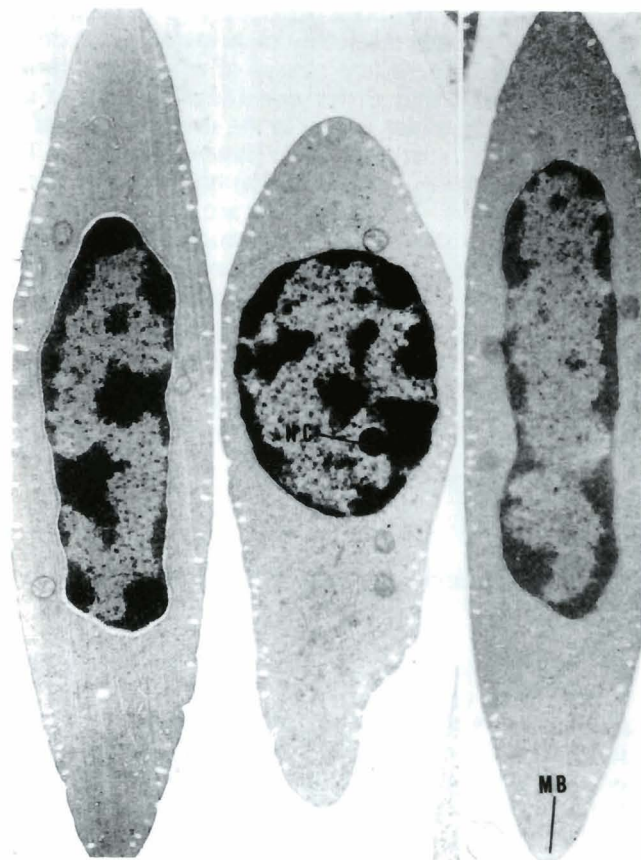

Fig. 7. Mature erythrocytes from the Pacific hagfish *Eptatretus stoutii*. The ellipsoidal shape of the cells is apparent. The numerous pinocytotic vesicles are particularly well illustrated. The grey electron-dense cytoplasm reflects the predominant quantity of hemoglobin. A few mitochondria and ribosomes persist; a small nucleolus (NC) is present in the center cell. MB indicates site of the marginal band, TEM x 10,000. Sekhon and Maxwell; Wiley-Liss.

centrally located elliptical nucleus however, bulges outwardly and imparts to the cell an element of biconvexity (Figs 4 - 7). This nuclear to cytoplasmic spatial relationship is typical among lower vertebrate red blood cells.

The erythrocytes in agnathic fish can be considered, from a conceptual viewpoint, as red cells appropriate for the lowest vertebrates. They manifest characteristics common to erythrocytes of all vertebrates and to some rbc in occasional invertebrates that also maintain erythroid cells (e.g. a free independent existence in a fluid milieu, a high level of the respiratory pigment hemoglobin, and a minimal or reduced complement of cytoplasmic organelles in the mature cell). In addition, they bear the cytologic hallmarks of submammalian vertebrate red cells, some of which are subsequently modified in higher evolved species. Thus erythrocytes in lower vertebrates can be expected to be oval like those of the hagfish; the nucleus is retained throughout the life of the cell and is usually correspondingly ellipsoidal in the mature specimen. The substantial diameters of the hagfish's red cells in particular are also representative of this species' level of development. The consideration that cyclostomes have erythrocytes that are consistent with their low taxonomic status is further supported by the fact that their hemoglobin is monomeric (one heme group per molecule), a primitive characteristic observed in some invertebrates (e.g. annelids). Indeed, the cyclostomes are the only vertebrates that have monomeric instead of tetrameric hemoglobin (i.e. composed of four heme subunits and two pairs of α and β globin chains) in their red cells. Unlike the red cells in some invertebrates which have

both nutritive as well as hemoglobin-dependent functions and exhibit cytoplasmic inclusions such as lipid granules and large amounts of glycogen which subserve the former activities (e.g. *Urechis caupo*, the inkeeper worm), the lamprey's and hagfish's erythroid cells have hemoglobin as their single, major cytoplasmic product. Finally, an interesting phenomenon regarding the circulating erythrocytes of both the lamprey and the hagfish is the occurrence of occasional denucleated red cells (lamprey 1 - 3%).

The further advanced fishes are the jawed fishes (superclass Pisces or Gnathostomata). They have a scaled skin, usually median and bilateral fins, and a set of jaws (derived from the modification of one set of visceral/gill arches). They are cold blooded and respiration is accomplished by means of gills. Aristotle (circa 350 B.C.) organized fishes phylogenetically by dividing them into the cartilaginous (chondrichthyes) and bony (osteichthyes) fishes, a classification that stills

stands today. This classification has proved to be convenient for the discipline of hematology since the erythrocytes in these two classes of aquatic vertebrates have red cells that differ considerably in size and number but otherwise adhere to the structural format for erythroid cells of inframmalian vertebrates. The cartilaginous fish (elasmobranchs) embrace the sharks, skates and rays. The latter two are believed to have evolved from the former and hence erythrocellular agreement observed among these marine creatures is not surprising. The hemoglobiniferous cells observed in
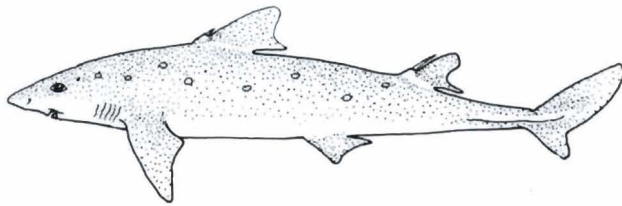
Fig. 8. *Squalus acanthias*, spiny dogfish, piked dogfish, dogfish shark.

these species are oval, nucleated discoids and according to the data of Gulliver (1875), Wintrobe (1933), Saunders (1966), and others are in the order of 17 to 30 μm in length and 14 to 20 μm in width. An interesting note apropos to the evolution of the erythrocyte is that tetrameric hemoglobin is first observed in the shark.

The piked or spiny dogfish *Squalus acanthias* (Fig. 8) is the most common shark in the coastal North American and European Atlantic ocean, the best known, and possibly the world's most abundant shark (Compagno, 1984). It has red cells with long and short cellular diameters of~23 μm and 16 μm, and a volume of 900 femtoliters (fl) (Kisch, 1951; Sherburne, 1974). This elasmobranch, also known as the dogfish shark, has a firmly established presence among contemporary
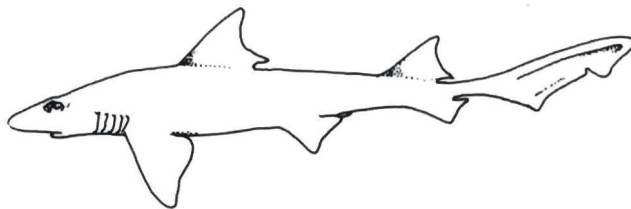
Fig. 9. *Mustelus canis*, dusky smooth hound, smooth dogfish.

biologists since it is commonly employed as a dissecting specimen in college comparative biology courses. *Mustelus canis* (dusky smooth hound), the second most abundant shark found in the continental shelf waters of southern New England and middle Atlantic states (USA) has red cells that have been subjected to extensive ultramicroscopic analysis in the study of the cytoskeleton of lower vertebrate erythrocytes (Fig. 9). Its cells are flattened, elliptical, biconvex due to a nuclear bulge and have major and minor axes of 17 - 22
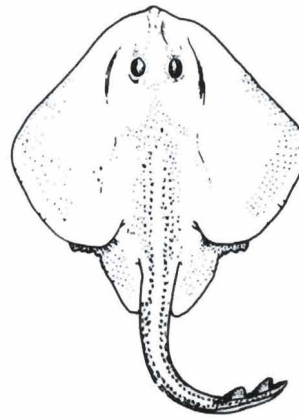
Fig. 10. *Raja erinacea*, little skate, common skate.

μm and 13 - 15 μm (Cohen et al., 1982). The skate's erythrocytes are similarly configured and representative of the chondrichthyes, (skates and rays are fishes whose pectoral fins are greatly developed giving them a flattened, wing-like shape particularly at the cephalic end). *Raja erinacea* (Fig. 10) the most common skate along the Atlantic coast of New England as well as the most familiar one because of its habit of coming into shoal water, presents erythrocytes with long and short
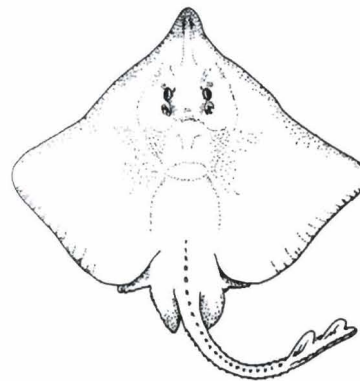
Fig. 11. *Raja laevis*, barndoor skate. The eyes and an immediately caudal pair of spiracles on the dorsal surface of the fish are readily identified.

diameters of 24 μm and 14 μm. The western Atlantic *Raja laevis*, the barndoor skate (Fig. 11), has red cells with dimensions in the range of 25 x 16 μm (Wintrobe, 1933; Kisch, 1951). In a recent study the electric rays *Torpedo marmorata* (marbled ray) and *T. ocellata* (eyed ray) were found to have adult erythrocytes 33 x 24 μm with a volume >1000 fl (Figs. 12, 13) (Pica et al., 1983). Although sharks, skates, and rays exhibit similar erythrocytes, according to most investigators' reports there is a tendency for skates and rays to have slightly larger rbc than the sharks (Table 1).

The circulating erythrocytes in a given cartilaginous fish have a greater morphologic diversity than those exhibited by a typical modern fish or mammal because the maturational process is conducted, in major part, in the peripheral blood. Mitotic figures have also been noted (Dawson,
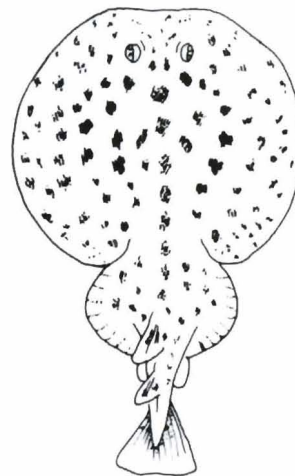
Fig. 12. *Torpedo marmorata*, marbled electric ray or skate.

Erythrocyte Odyssey

Table 1. Erythrocyte counts and Related Values in Representative Primitive and Modern Fishes.

Family and Species	RBC	Hct	Hb	L x W	MCV	MCH	MCHC
Myxinidae (hagfishes)							
Myxine glutinosa	0.15	22.2	4.6	26.2 x 18.0	1530	318	21
Petromyzonidae (lampreys)							
Petromyzon marinus	0.33	23.5	5.8	14.3 x 14.3	710	176	25
Squalidae (spiny dogfishes, dogfish sharks)							
Squalus acanthias (MW)	0.07	6.8	1.4	23.7 x 17.0	946	201	25
Squalus acanthias (BK)	0.24	18.9	3.8	22.7 x 15.2	820	158	20
Triakidae (smoothhounds)							
Mustelus canis	0.46	23.5	4.6	19.1 x 13.8	541	100	20
Torpedinidae (electric rays)							
Torpedo marmorata							
Torpedo ocellata (T. torpedo) }	0.20	24.5	3.2	33.0 x 24	1039	149	13
Rajidae (skates)							
Raja eglanteria	0.30	23.4	4.5	23.7 x 14.4	823	150	19
Raja laevis	0.12	15.2	2.9	28.4 x 17.5	1274	246	19
Acipenseridae (sturgeons)							
Acipenser sturio	0.43	21.5	5.0	14.0 x 9.7	506	116	23
Salmonidae (salmons)							
Salmo gairdneri	1.07	39.5	7.5	15.7 x 10.4	380	73	19
Salmo trutta	1.0	32	6.8	15.2 x 9	320	68	21
Esocidae (pikes)							
Esox lucius	1.89	32	8.8	11.0 x 8.6	169	47	28
Cyprinidae (carps)							
Carassius auratus	1.8	32	8.3	13.3 x 8.4	178	46	26
Cyprinus carpio	0.84	31.3	10.5	13.4 x 10.2	373	125	34
Catastomidae (suckers)							
Ictiobus bubalus	1.28	32.9	8.5	15.3 x 9.0	257	64	26
Ictiobus cyprinellus	1.08	34.6	7.8	–	320	67	23
Ictaluridae (catfishes)							
Ictalurus nebulosus	1.22	27.9	6.9	13.8 x 9.4	228	57	25
Ictalurus punctatus B et al.	1.61	22.7	4.0	–	139	22	17
Ictalurus punctatus H et al.	2.16	29.4	6.6	11.0 x 7.6	136	31	22
Anguillidae (freshwater eels)							
Anguilla rostrata	2.48	37.9	9	13.0 x 8.0	156	37	24
Gadidae (codfishes)							
Gadus morhua	1.55	29.1	5.9	12.2 x 9.0	186	38	20
Syngnathidae (pipefishes & seahorses)							
Syngnathus (Siphostoma) fuscus	2.05	–	5.6	10.6 x 9.1	–	27	–
Centrarchidae (sunfishes)							
Micropterus salmoides	1.57	26.0	5.8	10 x 7.6	166	37	22

Family and Species	RBC	Hct	Hb	L x W	MCV	MCH	MCHC
Notothenidae (Antarctic blennies)							
Trematomus borchgrevinki	1.19	38	5.3	11 x 11	319	45	14
Scombridae (mackerels)							
Scomber scombrus	3.91	57.5	14.5	12.3 x 8.3	147	38	26
Thunnus alalunga	2.90	54.5	15.8	9.0 x 6.6	188	54	29
Katsuwonus pelamis	4.14	59.3	18.0	8.0 x 6.4	143	43	30
Triglidae (searobins)							
Prionotus carolinus	2.5	24	7.1	9.8 x 6.5	96	28	30
Prionotus evolans (strigatus)	1.93	22.2	6.2	10.4 x 7.3	130	32	28
Cottidae (sculpins & bullheads)							
Myoxocephalus scorpius	0.95	20.2	4.4	12.4 x 9	214	46	22
Pleuronectidae (flounders)							
Limanda ferruginea	1.23	14.6	3.2	10.3 x 7.7	118	27	23
Batrachoididae (toadfishes)							
Opsanus tau	0.68	27.5	6.2	14.9 x 12.9	404	91	23
Lophidae (goosefishes)							
Lophius piscatorius	1.12	17.9	4.4	13.6 x 9.5	204	39	25
Helostomatidae (kissing gouramis)							
Helostoma temmincki	2.51	18.7	5.6	9.5 x 5	75	22	30
Channidae (snakeheads)							
Channa punctatus	3.1	48	12.4	12.5 x 8.0	154	39	26

RBC = erythrocytes millions/mm³ or µl, Hct = hematocrit, Hb = g hemoglobin/deciliter, L x W = length x width (µm), MCV = mean cellular volume (µm³ or femtoliters, fl), MCH = mean cellular hemoglobin (pg), MCHC = mean cellular hemoglobin concentration (wt/vol%, gHb/100 ml rbc). Derived from Agrawal and Mahajan, '83; Albritton, '51; Alexander et al., '80; Altman and Dittmer, '71; Breazile et al., '82; Chlebeck and Phillips, '69; Coburn and Fischer, '73; Engel and Davis, '64; Haws and Goodnight, '62; Kisch, '51; Kooyman, '63; Mahajan and Dheer, '80; McCarthy et al., '73; Mulcahy, '70; Pica et al., '83; Smith et al., '52; Wintrobe, '33; Some values are averages of different studies and some erythrocyte indices have been calculated from reported data per Wintrobe formulas.
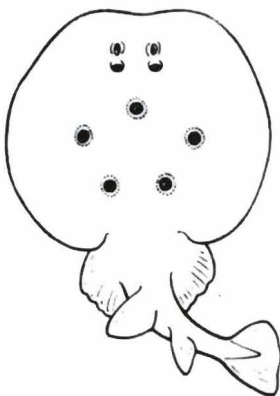

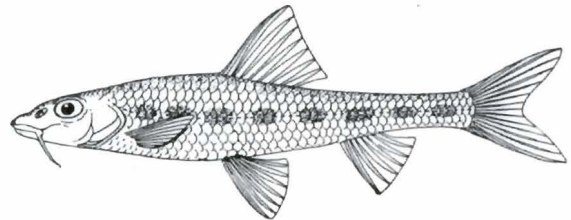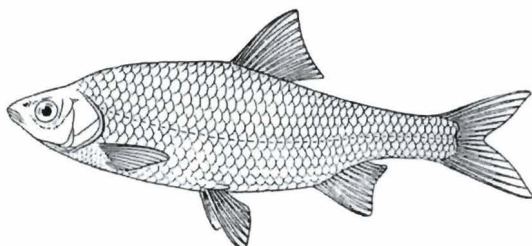**Fig. 13.** *Torpedo ocellata (T. torpedo)*, eyed electric ray.

Fig. 15. *Gobio gobio*, common goby.

Fig. 14. *Rutilus rutilus*, European or common roach.

1933). These bright field microscopic observations are further documented by the ultrastructural studies of Zapata and Carrato (1981) who found immature rbc in the circulating blood of two elasmobranchs, the skate *Raja clavata* (thornback ray) and the marbled electric ray *Torpedo marmorata*, a fish that stuns its prey with an electric shock (Fig. 12). Comparable immature erythrocytes were not identified in their concurrent examination of two bony fish, both members of the carp suborder (Cypriniformes) *Rutilus rutilus*, the European roach and Gobio gobio, the common goby (Figs. 14, 15). *Squalus acanthias* is described as having 4 - 35% (mean 9%) immature erythroid cells in its blood

(Sherburne, 1974).

In contrast to the cartilaginous fishes, the bony fishes (osteichthyes) have a skeleton that is partially or completely ossified. They have an external series of bones that form bilateral gill covers (opercula) that shield and protect the gills and gill arches. The erythrocytes that circulate within the gill and other capillaries in such fish have recognizable, and in some cases, striking dimensional differences from the red cells in their cartilaginous counterparts.

The osteichthyes can be divided into two categories (classes), the Sarcopterygii, the fleshy-finned fishes, and the Actinopterygii, the ray-finned fishes. The members of the former group are anatomically characterized, among other features, by paired fins which are freely moveable, have a narrow base, and a prominent, fleshy, muscular lobe. The members of this class were most prevalent in the Devonian period some 350 million years ago. The surviving genera are extremely limited in number, and indeed, are living fossils. They are hematologically of interest and also of specific biologic relevance because this assemblage is postulated to have given rise to the terrestrial vertebrates. That is, the amphibians and all higher
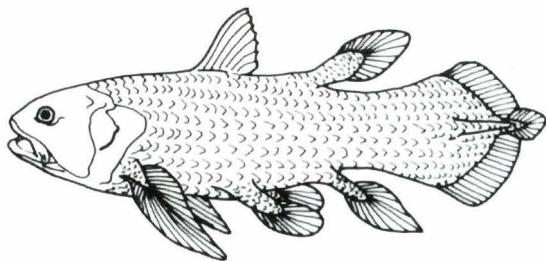

Fig. 16. *Latimeria chalumnae*, the coelacanth. The characteristic basal fleshy (muscular) segment of the fins is well illustrated.

vertebrates may have evolved from this group. Most aspects of this evolutionary processs, of course, remain unresolved, but some insight has been offered by hematologic investigation. Recently Gorr and others (1991) analyzed the primary structure of the hemoglobin of the sole enduring coelacanth *Latimeria chalumnae*, a sarcopterygian, (Fig. 16). They compared it with all other known hemoglobins of cartilaginous and bony fish as well as those of tadpoles, adult amphibians and the hemoglobin of this fish's competitively ancient cohort, the lungfish *Lepidosiren paradoxa*. They concluded that the coelacanth is the closest living aquatic relative of the terrestrial tetrapods, and by implication, that it is close to the human evolutionary tree (Fricke, 1988; Browne, 1991). Of parallel interest is the deep sea visual observation that four of this fish's fins move synchronously in a way common to four-legged animals (e.g. a trotting horse) but not to fish. Conversely the lungfish (dipnoi), also sarcopterygians, have similarly been proposed as the aquatic ancestors of the terrestrial tetrapods. Lungfish have ordinary gills but also primitive air-breathing lungs, thereby allowing some of

them, for example, to survive in a ball of mud in dry periods. Since the tetrapod line of land animals is likely to have evolved from fishes that adapted themselves to

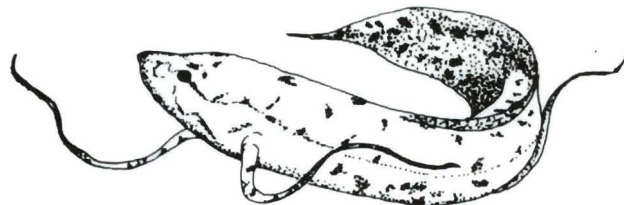**Fig. 17.** *Lepidosiren paradoxa*. South American lungfish.

Fig. 18. *Protopterus aethiopicus*, the African lungfish.

breathe air and move with the aid of limbs (e.g. modified fleshy, muscular sarcopterygian fins) the candidacy of the lungfish is well recognized.

Three genera of lungfishes have survived till modern times (Figs. 17, 18). They occur in South America *(Lepidosiren paradoxa),* Africa *(Protopterus aethiopicus)* and Australia (*Neoceratodus fosteri).* Their erythrocytes are extremely large, approximately 40 by 30 μm, a size exceeding those of both the cyclostomes and chondrichthyes. As might be predicted, they are elliptical and slightly biconcave while their nucleus is oval, centrally positioned and bulges slightly into the biconcavity. In accord with observations in other primitive fish all transitional stages between the erythroid stem cell and the fully hemoglobinized
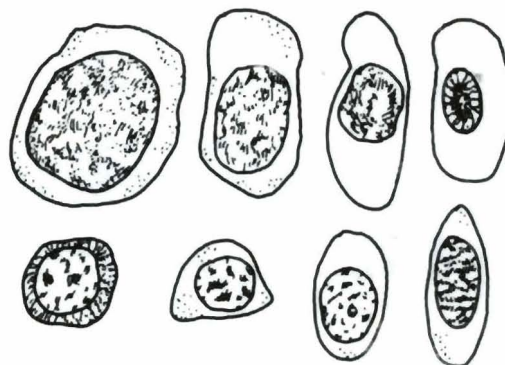

Fig. 19. Erythrocyte differentiation in the African lungfish *Protopterus aethiopicus*. Top row: the first cell (left) is a proerythroblast while the next is a transtion stage between it and the erythroblast. The third cell, an erythroblast, demonstrates some nuclear condensation and cytoplasmic accumulation of hemoglobin (recognizable as eosinophilia in the orginal preparations). The last cell is a mature erythrocyte. From Wright-stained blood filim, x 700. Bottom row: the first cell is a hemoblast, it has an extremely delicate nuclear pattern and a very basophilic cytoplasm. The second cell is a proerythroblast while the next two are early and late erythroblasts respectively. From sectioned spleen, azure II-eosin stain, x 800. After Jordan and Speidel, 1931.

erythrocyte are identifiable in the blood of the African lungfish (Jordan and Speidel, 1931). Mitotic figures are fairly common in the circulation; erythroplastids, however, are rare. The development of erythrocytes as seen in blood films and sections of the spleen have been graphically illustrated by these investigators (Fig. 19).

The Actinopterygii (ray-finned fishes) have fins supported by rays of dermal bone. They are a diverse group but can be divided into two time-honored groups, the ganoids and the teleosts. The ganoids are more primitive, have a limited number of existing
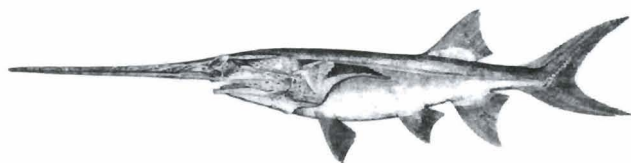
Fig. 20. *Polyodon spathula,* paddlefish, spoonbill surgeon.

representatives and are typified by their rhombic «ganoid» scales. These early ray-finned fishes can be further subdivided into the Chondrostei (paddlefishes

Fig. 21. *Acipenser oxyrhynchus,* Atlantic sturgeon. Five longitudinal rows of bony plates (scutes) are one of its recognizable features.

and sturgeons) and the Holostei (bowfins and gars). The paddlefish *Polyodon spathula* is a fish with an unusual spoon shaped snout that projects a great distance beyond its mouth (Fig. 20). The hematologic aspects of this fish have been documented by Downey (1909). The caviar-producing Atlantic sturgeon *Acipenser*
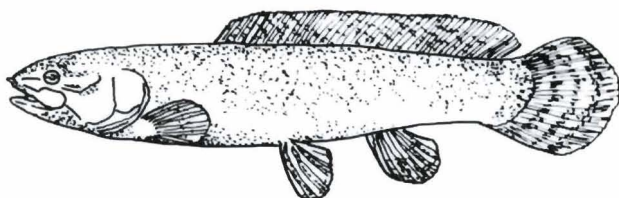

Fig. 22. *Amia calva,* bowfin, a ganoid fish.

oxyrhynchus (Fig. 21), a fish that can attain 6 meters in length and 200 kg in weight, has nucleated oval erythrocytes in the range of 15 x 10 µm. The bowfin *Amia calva* (mud fish, river dogfish) is an inhabitant of standing or slowly moving inland waters and is distributed throughout the eastern half of the United States (Fig. 22). Its hemopoiesis has been studied and illustrated by Robeson (1932). The red blood cell of the bowfin is a biconvex, elliptical disc about 19 x 10 µm.

Its nucleus is distinctive because in perhaps 50% of the cells it has an irregular contour. It is frequently bilobed and even binucleated. A few nuclei have an eccentric
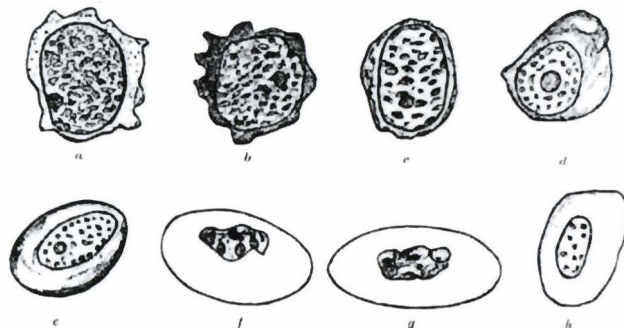

Fig. 23. Development of erythrocytes as observed in Wright-stained blood films of *Amia calva.* Top row. The first cell, a. the hemoblast which matures progressively into b. the proerythroblast, c. early erythroblast and d. late erythroblast respectively. The latter is the first stage to tinctorially demonstrate accumulation of hemoglobin. Botton row. The next stage in the series is the immature erythrocyte followed by the mature erythrocyte which has a deeply chromatic, irregular nucleus (two center cells, f. and g.). The last cell, h., is a senile erythrocyte. It has a decreased content of hemoglobin and a lighter staining nucleus. Robeson, 1932, x 750.

location apparently in relation to their extrusion which results in the presence of a small number of erythroplastids in the circulating blood. The erythrocyte arises from a lympho-myeloid hemoblast in the kidney or spleen, passes into the general circulation at the proerythroblast stage and completes its maturation in the blood (Fig. 23). The presence of significant numbers of immature red cells in the blood is, as a result, commonplace.

The teleosts or modern bony fishes are particularly numerous and represent 95% of all existing living fishes. They comprise some 20,000 species, a number about equal to all other vertebrate groups combined. They represent a diverse constellation of life cycles,
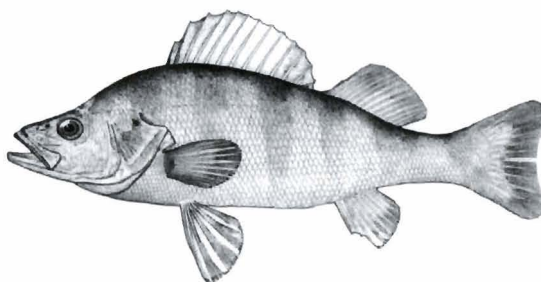

Fig. 24. *Perca flavescens,* yellow perch of North America. It is considered an ideal example of a teleost.

behaviors, habitats and adult sizes. In addition to their osseus skeleton, teleosts are also anatomically characterized by a homocercal tail (one in which the upper and lower halves are about equal). The yellow perch *Perca flavescens* is often cited as the most perfect
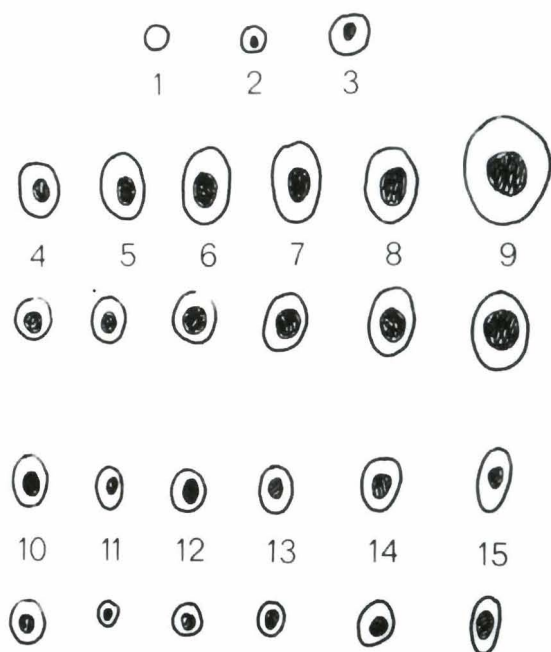
Fig. 25. Erythrocytes in fishes: size, shape and intra-individual variation. Erythrocytes illustrated: human, cyclostomic, selachian, ganoid and teleostean. Drawings 4 to 15 illustrate two erythrocytes from each species, the largest and smallest red cell found in a single blood film. 1. man, 2. pipefish, 3. lamprey, 4. hammerhead shark, 5. stingray, 6. smooth dogfish, 7. ray, 8. spiny dogfish, 9. electric ray, 10. sturgeon, 11. sharksucker, 12. searobin, 13. angler, 14. mackerel, 15. eel, x 400. After Kisch, 1951.
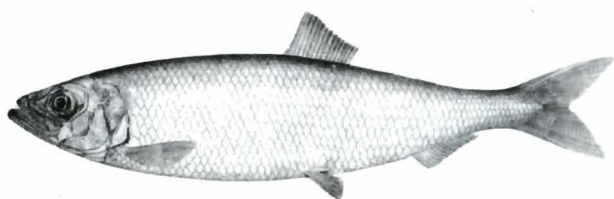

Fig. 26. *Clupea harengus,* Atlantic herring. A prized human food for centuries e.g. utilized fresh, frozen, pickled, smoked («kippers»), canned juvenile herrings («sardines»).

example of a fish, it serves equally well as a model of a modern ray-finned fish (Fig. 24). Many teleosts have a fusiform body shape (e.g. trout and the aforementioned perch) which aids in the reduction of the turbulence and drag of the water as the fish swims. A similar functional/morphologic relationship is probably also attributable to the ellipsoidal erythrocytes of fish and their flow in at least the large vessels. Support for this premise is obtained in the experimental observation that the normally biconcave, discoid erythrocytes of man assume a reversible ellipsoidal configuration when submitted to shear stress, a condition that presumably mimics intravascular, large vessel blood flow.

In general the erythrocytes of teleosts are nucleated ellipsoids, smaller than those of the chondrichthyes and the more primitive bony fishes such as the lungfish and
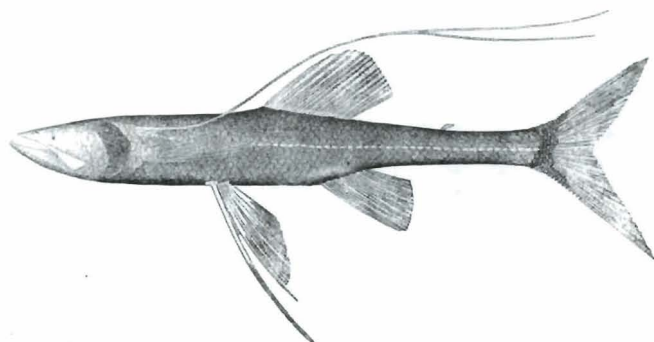

Fig. 27. *Bathypterois longipes,* a bottom fish of the deep sea. It is probably one of the more common species encountered at depths greater than 4,000 meters.

bowfin (Figs. 19, 23, 25). The typical erythrocyte in a teleostean is approximately 10 - 14 μm long and 8 - 9 μm wide (Table 1). The range, however, is broad as exemplified by the modest sized cells of the Atlantic

Fig. 28. *Ipnops murrayi.* It is hematologically noteworthy because of its large, oval erythrocytes. This deep-sea bottom inhabitant lacks the customary eyes. It has, instead, a bright yellow photoreceptive organ in the dorsal region of the head, covered by a pair of large transparent membranous skull bones.

herring *Clupea harengus* (9 x 7 μm) and the significantly larger rbc of the common roach *Rutilus rutilus* (16 x 10 μm), (Figs. 14, 26). This breadth is further extended by two closely related deep-sea bottom fishes whose life histories are still unknown, *Bathypterois longipes* and *Ipnops murrayi* (Figs. 27, 28). They have erythrocytes (in sectioned tissues) in the order of 19 x 14 x 6 μm, perhaps the largest among teleosts.

The readily apparent, oval shape of piscine red cells (Fig. 25) can be expressed mathematically as the ratio of the cell's length to its width; among modern bony fish the length: width (L:W) ratio is usually 1.4 : 1 or greater. Exceptions, however, are not rare and include

Fig. 29. *Siphostoma (Syngnathus) fuscus,* a pipefish. This fish has some erythrocytes that are circular and others that are elliptical. Either form can predominate (Kisch, 1951). This slender fish with a long tubular snout is developmentally notable in that the male nurses the eggs in a brood pouch into which the female deposits them.
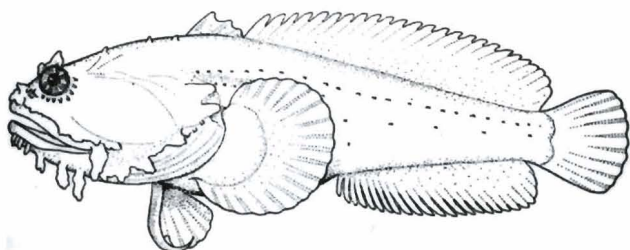
Fig. 30. *Opsanus tau*, toad fish, oyster toadfish. This fish is described as having a peculiar wary appearance due to the fleshy flaps it has over the eyes, on the cheeks, upper and lower jaws. It has only three gills and three gill arches. Its erythrocytes are relatively large, 404 fl, and rather circular, Illus. L. Cable.

Ictalurus natalis, a freshwater catfish (12.4 x 10.5 μm), *Siphostoma fuscus*, the pipe fish (10.6 x 9.1 μm) and *Opsanus tau*, the oyster toadfish (14.9 x 12.9 μm); their red cells are relatively wide and approach a quasi circular configuration (Figs. 25, 29, 30). The Antarctic-dwelling *Trematomus borchgrevinki* has round erythrocytes with a diameter of about 11 μm (Tyler, 1960).

Another erythrocytic structural extreme is offered by three deep-sea bristlemouths or lightfishes (Gonostomatidae), a family of teleosteans that are
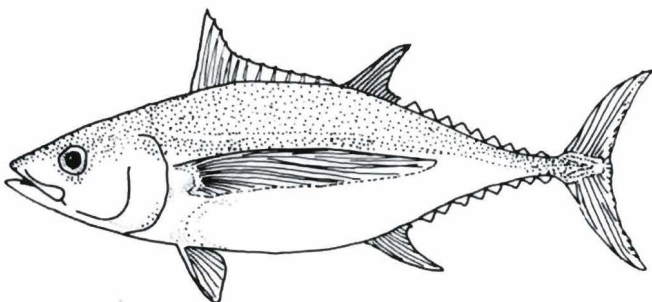

Fig. 31. *Thunnus alalunga*, albacore. This scombrid is characterized by its long sword-shaped pectoral fin which extends caudally to the level of the second dorsal fin.
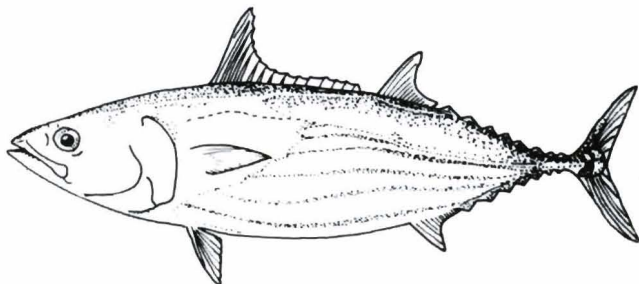

Fig. 32. *Katsuwonus pelamus*, skipjack tuna, bonito. This fish belongs to a tuna group which is characterized by longitudinal stripes on the sides of the body.

seldom seen by non-ichthyologists because of their deep habitat, small size and fragility. They are likely to be observed in plankton-net collections and in the stomach
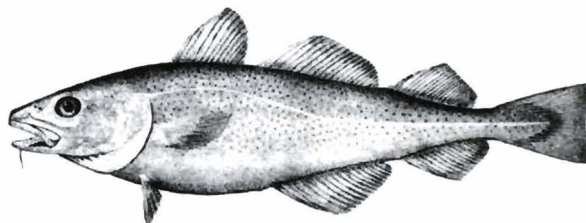

Fig. 33. *Gadus morhua (G. callarius)*, cod, Atlantic cod. Physical features that assist in recognition of the genus are a single, well-developed barbel, three dorsal and two anal fins, all separate from each other. This fish is among the most important of all commercial fishes.

contents of deep sea predators such as the albacore, skipjack, cod, herring and pollock (Ahlstrom and Counts, 1958) (Figs. 26, 31 - 33). This pelagic (inhabiting open oceans) family has an extensive distribution and though unimportant as human food is an
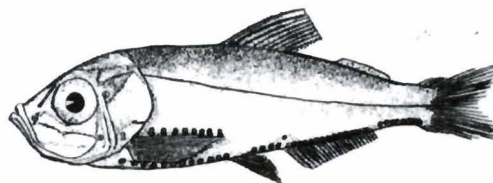

Fig. 34. *Maurolicus mülleri*, the first fish recognized to have solely denucleated erythrocytes in circulation. It is a small fish that resembles a miniaturized herring. This marine species has a rayless (adipose) dorsal fin anterior to its tail (a signature of the order Salmoniformes). A short and long row of photophores are identifiable on the lateral body surface. Atlantic fishes of Canada, Department of Fisheries and Oceans. Reproduced with permission of the Minister of Supply and Services. Canada. 1991.
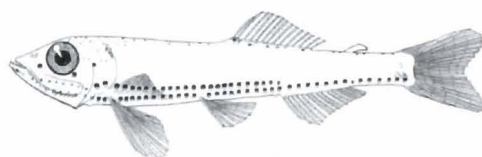

Fig. 35. *Vinciquerria lucetia*. Top: early juvenile, 20 mm long; bottom: adult, 44 mm long. One of the most abundant pelagic fishes in the eastern North Pacific. Their larvae are similar to, as numerous as, and found in the same regions as the larvae of the Pacific sardine. Like other gonostomatids, the number and distribution of the photophores distinguishes this genus from others. Ahlstrom and Counts, 1958.

important forage fish for larger oceanic species. Two members, *Maurolicus mülleri* and *Vinciguerria lucetia*, 5 - 6 cm long fish that are characterized by large silvery eyes and luminous surface organs, have erythrocytes that are small (7 x 2.5 - 3 μm), ellipsoidal, decidedly biconvex and (remarkably) denucleated (Figs. 34, 35) (Hansen and Wingstrand, 1960). *Maurolicus mülleri*, the best studied may have 1 - 2% erythroblasts in
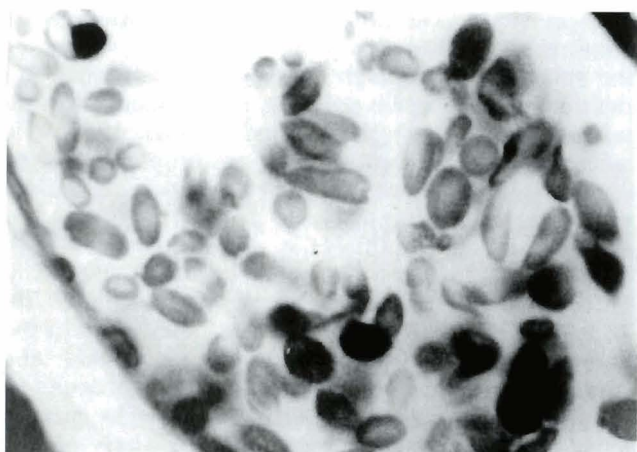
Fig. 36. Erythrocytes in a gill blood vessel in *Maurolicus mülleri*. Their ellipsoidal denucleated configuration is readily apparent. Dark cells are nucleated leukocytes. Paraffin section. x, 1,800. Wingstrand, Z. Zellforsch., 1956, with permission of Springer-Verlag.

circulation in immature specimens but none are found in the adult. Erythropoiesis is restricted to the spleen *(Maurolicus mülleri);* the erythroblast begins its maturation as a small round cell with a prominent nucleus and proceeds to evolve into an ellipsoid cell
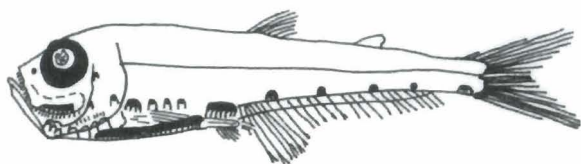

Fig. 37. *Valenciennellus tripunctulatus,* a widely dispersed, small (~25 mm) bathypelagic fish remarkable for its shperical, non-nucleated erythrocytes. The discrete dark structures on the side of the body represent its distinctive aggregates of photophores; after Jesperson, 1949.

(Fig. 36). Based on observations of sectioned material, it has been proposed that the nucleus is lost by karyolysis. Denucleated erythrocytes are also the usual form present in one other gonostomatid, *Valenciennellus tripunctulatus,* a fish found most frequently at 300 - 700
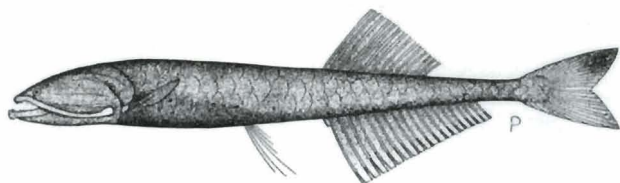

Fig. 38. *Cyclothone microdon* a member of the family Gonostomatidae that has oval nucleated erythrocytes typical of teleosts; illus. P. Parkin, The Sea Fishes of Southern Africa, 1961.

meter depths in the Pacific, Indian, tropical and subtropical Atlantic oceans (Fig. 37). These are spheroidal, approximately 7 x 7 μm in Bouin-fixed

sections predictably ~9 x 9 μm in dry film smears). The erythrocytes of five other Gonostomatidae including the ubiquitous *Cyclothone signata* and *Cyclothone microdon* (Fig. 38) have been surveyed; they all present the typical nucleated teleostean red cells. The three bristlemouths that have denucleated erythroid cells exclusively in their peripheral circulation are the only fishes to demonstrate this phylogenetically advanced form of red cell out of several hundred species examined to date. The cytology of their larval erythrocytes and the erythropoiesis of other gonostomatids await investigation.

Various biological determinants affect the size of an animal's red blood cells. Among these is the dimensional interrelationship of the various somatic cells (epithelial, muscle, etc.) in a given species (Szarski, 1968). That is, a subject with large epithelial or other cells would be expected to have large rbc's. This can be hematologically appreciated by comparing the relative magnitude of the erythrocytes and accompanying leukocytes in an elasmobranchian blood film. Given large erythrocytes, a species displays proportionately large granulocytes. The mean diameter of neutrophils in the spiny dogfish and the marbled electric ray (two fishes with «chondrichthian-size» rbc) are 20 and 25 μm respectively; in man these cells are half as large (Pica et al., 1983; Sherburne, 1974). A corresponding relationship exists with the lymphocytes. Pursuant to the consideration of red blood cell size in different species, it has also been hypothesized that in evolution the early vertebrates invaded brackish water first and later fresh waters (Szarski, 1968). As a result these animals' body cells (including erythrocytes) increased in size (partly in response to osmotic differences) and their metabolic level decreased (as a result of diminished cell surface to mass ratios). Among extant animals, it is the concensus that the dipnoans, tailed amphibians and elasmobranchs possess the largest cells (in all tissues, including hemopoietic). The large cell size is proposed to have evolutionarily persisted in those groups which retained a low metabolism (e.g. chondrichthyes) or continued to experience drastic osmotic challenges (e.g. dipnoans). Conversely, those species which evolved further (e.g. teleosts) decreased their cell sizes (hemic and other) and increased their rate of metabolism. Thus, the dimensions of a species' rbc mirror, at least in part, its phylogenetic history and its somatic cellular architecture. The size of erythrocytes (and other cells) in an individual are also correlated to the genome size, i.e. the quantity of nuclear DNA. The amount of DNA predictably increases with the complexity of a species but also with the degree of perseveration of its gene sequences. A large genomic constituency of repeated DNA sequences has apparently fostered large rbc among the elasmobranchs, dipnoans (and amphibians) but not in teleosts who seem to have economized in the amount of their «redundant» DNA (Fänge, 1985). Consequently, while the smaller teleostean red cells have in the range of 1 - 7 pg DNA

per nucleus, the jawless fish have 3 - 6, the elasmobranchs have 15 and dipnoans have a remarkable 225 pg per cell.
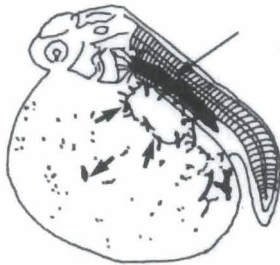

Fig. 39. *Salmo gairdneri* embryo, 8 days post-fertilization. Sites of developing primitive (larval) generation erythrocytes: blood islands and the intermediate cell mass. The blood islands are scattered over the surface of the yolk sac and are most numerous near the cell body. Representative islets are indicated by short arrows. The erythropoietically active intermediate cell mass is identified by one long arrow. O-dianisidine reaction. After Iuchi I. and Yamamoto M., 1983.

The sites of initiation and completion of erythropoiesis vary in fishes, reflect phylogenetic progression, and include to a greater or lesser degree, the peripheral circulation as one of the loci of activity. The mesenchymal layer of the yolk sac wall is the initial ontogenetic site of erythrocytic development, it occurs intravascularly (Fig. 39). It may be followed by the embryonic intermediate cell mass as in the rainbow trout *Salmo gairdneri*, the fundulus, a hardy, often experimentally used cyprinodont/killifish and the popular, easy to breed, aquarium-inhabitant *Brachydanio rerio* (zebra fish, a member of the carp family) but not in the angelfisch *Pterophylum scalare* (Iuchi and Yamamoto, 1983). These two regions generate the short-lived primitive (larval) generation erythrocytes (discussed subsequently). The next sequential erythropoietic site and source of definitive generation erythrocytes is splenic tissue and possibly other organs.

The cyclostomes constitute the earliest vertebrates with specialized hemopoietic tissue. It can be viewed as phylogenetically «pre-splenic», i.e. resembling this tissue but without an independent identity and incorporated into another organ, the intestinal tract. In higher species it has evolved into a specific organ, the spleen. Hagfish display diffusely scattered islands or cords of splenic-type hemopoietic tissue throughout the submucosa of the intestine. An identical location is noted in the lamprey *(Lampetra)*, also a cyclostome, except in this instance the tissue is aggregated in one region of the gut (spiral valve) (Jordan, 1938). Hemopoiesis (erythroid and other cell lineages) begins in this locus from a cell called the hemoblast or other term implying pluripotentiality. The developing erythroblasts enter the circulation and complete their maturation there (Fig. 5). The circulating blood consequently demonstrates great numbers of cells in transitional stages between ancestral hemoblasts and definitive erythrocytes as well as large numbers of hemoblasts. Occasional erythroblasts can be found undergoing mitosis, an activity biochemically confirmed by their incorporation of ^{3}H-labeled thymidine. The blood stream is thus the prime locus for erythrocytic maturation in fishes at this early evolutionary level

(Fänge, 1986). The lungfish *(Protopterus aethiopicus)* continues to follow this protocol (Fig. 19). However, the splenic tissue though histologically advanced to a definitive organ is nevertheless still embedded in the wall of the stomach (Jordan and Speidel, 1931). The spleen persists as the center of erythropoiesis in this species (granulopoiesis occurs in the splenic capsule and other organs). Hemoblasts, proerythroblasts and erythroblasts are identifiable in venous sinuses and pulp cords; these cells are also present in the circulating blood as are occasional erythroid mitotic figures. The selachians (sharks) and ganoids are noteworthy in that their spleens have separated from the alimentary tube; this organ subsequently maintains an independent existence at further evolutionary levels. The ganoids have been described as primitive fish likely closely related to the stock from which the teleosts have arisen. And once having attained this «ganoid» level, erythropoiesis in adult fishes is no longer restricted to the spleen. It can take place in other organs (most commonly the kidney), and the spleen, conversely, may even cease to be an erythropoietic center. The erythrocytes of the bowfin *(Amia calva*, a ganoid), as
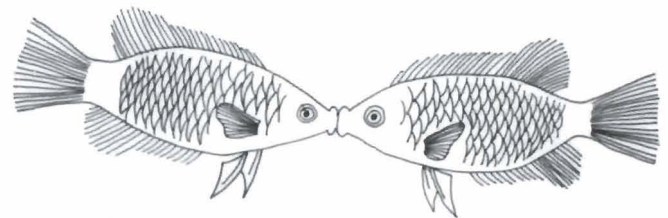

Fig. 40. *Helostoma temmincki*, kissing gourami, a tropical freshwater fish. The males display themselves by pursing their protrusible lips, pressing them against those of another male, and pushing each other about (hence «kissing»).

noted previously, differentiate from a hemoblast that originates in the spleen and also the kidney. The circulating blood, consistent with the cellular pattern of primitive fish, is the site of rbc development (Fig. 23). The paddlefish *(Polyodon spathula*, Fig. 20), also a ganoid, utilizes its kidney as the source of its erythrocytes (and granulocytes). At higher evolutionary levels, that is, among the modern fishes, e.g. the trout, and kissing gourami *Helostoma temmincki* (Fig. 40), the
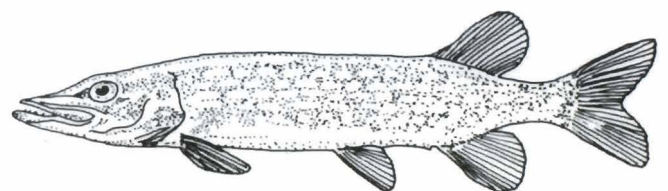

Fig. 41. *Esox lucius*, northern pike, pike.

developmental sites for erythropoiesis expand to include the spleen, kidney and possibly liver (Weinberg et al., 1976). In the freshwater teleost *Rutilus rutilus* (the
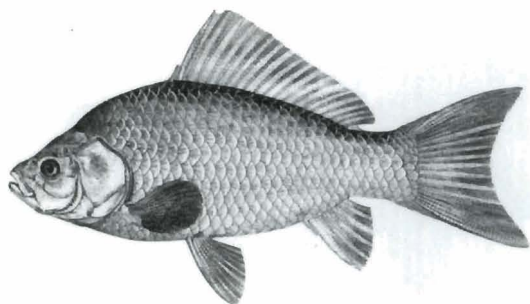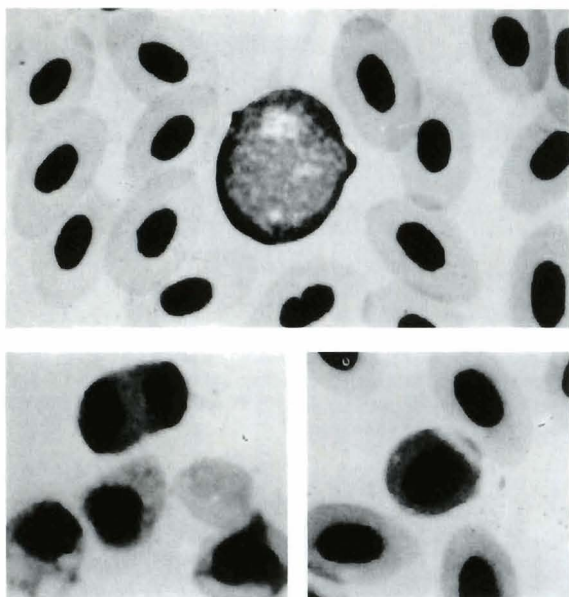
Fig. 42. *Carassius auratus.* goldfish.

Fig. 43. Developing and mature erythrocytes of *Carassius auratus*, the goldfish. Upper frame, peripheral blood: the prominent central cell with a large nucleus and delicate chromatin pattern is a proerythroblast (or hemoblast per Watson). The pale area most likely represents the negative staining Golgi apparatus. Left lower frame, spleen imprint: three early erythroblasts and a proerythrocyte (in telophase). Right lower frame, peripheral blood: central cell, a late erythroblast; x 1300. Watson et al., 1963.

common roach), and also the marine teleosts *Ctenolabrus rapestris* (goldsinny wrasse) and *Triglia cuculus* (a searobin called the red gurnard because of its ability to emit grunting sounds) only the kidneys are
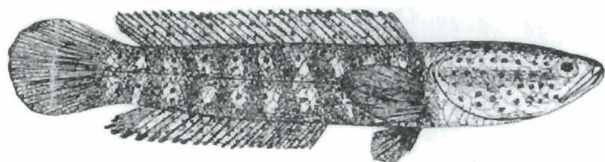

Fig. 44. *Channa punctatus*, snakehead, «live fish». A comment about this fish that helps to mentally segregate it from others is that this fish, among others, has lost its dependance on gill respiration because it also has a pair of richly vascularized pharyngeal sacs suitable for air breathing. It is common for this fish to be sold for food in its native India as «live fish» in earthenware pots containing a small amount of water, where it survives without difficulty.

hemopoietically active (Catton, 1951). The pike *Esox lucius* utilizes its kidney for erythropoiesis (Fig. 41). *Carassius auratus* (the goldfish) employs the kidney as its primary hemopoietic site while some erythrocytes are also produced in the spleen (Figs. 42, 43). A cell-specific combination of autoradiographic labelling of DNA and cytochemical staining of hemoglobin has localized erythropoiesis to the head kidney and the spleen in the air-breathing Indian fish *Channa punctatus* (Fig. 44). This study has identified a small DNA-synthesizing lymphoid hemoblast as the precursor of the erythroblast. It was observed in the kidney, spleen and the circulating blood (Mahajan and Dheer, 1980). In brief, the spleen is the principal erythropoietic locus in mature elasmobranchs as well as some other low species. While it continues to maintain its erythroid primacy in a few teleosts (e.g. the perch *Perca fluviatilis* and the bristlemouth *Maurolicus mülleri)* the predominant site for rbc production in mature modern bony fishes is the kidney.

Ichthyic erythropoiesis is stimulated by human erythropoietin, the major regulator of erythropoiesis in man, and by plasma from anemic fishes, potentially containing the same hormone (Weinberg et al., 1976). The occurrence of renal erythropoiesis in fishes and its absence in mammals, coupled nevertheless, with production of the erythropoietin in the mammalian kidney poses unresolved questions. That is, what renal-erythropoietic relationships expressed in fishes have been evolutionarily retained and are still in force in animals of the highest classes?

The undifferentiated precursor of the erythrocyte has been morphologically identified (as noted previously) in the circulating blood of the myxines, chondrichthians and other primitives. This lymphocyte-like cell, with a delicate, lightly staining, sieve-like nuclear chromatin pattern, has been variously termed the hemoblast, lymphoid hemoblast or hemocytoblast. It is considered capable of giving rise to cells of the erythroid as well as other lineages and can be viewed as probably equivalent in current terminology to the mammalian CFU-S (colony forming unit-stem) or possibly to a more committed erythro-myeloid progenitor. The hemoblast gives rise to the proerythroblast, the most immature, morphologically recognizable member of the erythroid series. It is identifiable with confidence in Wright/Giemsa-stained blood smears and is believed to be unequivocally committed to the erythroid line. The intermediate stages of development between it and the mature erythrocyte have been usually designated as the early (basophilic) erythroblast, late erythroblast, and immature erythrocyte (juvenile, proerythrocyte) (Figs. 19, 23, 43). The latter still retains a significant number of ribosomes and as a consequence is characterized by its polychromatophilic cytoplasm under Wright's staining. It is roughly comparable to the young mammalian reticulocyte. Mitotic capability is expressed at least through the late erythroblast level.

The proerythroblast is a round cell with a large

delicately-patterned nucleus and intensely basophilic cytoplasm. With maturation the nucleus decreases in size and accumulates coarse clumps of chromatin. The tinctorial quality of the cytoplasm concomitantly
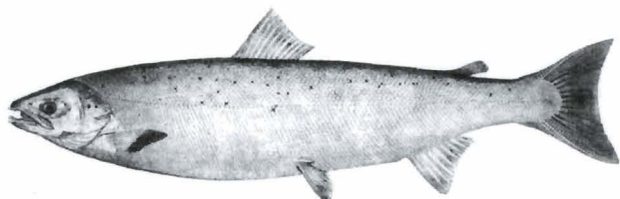

Fig. 45. *Salmo salar,* Atlantic and Baltic salmon.

evolves and reflects an increasing presence of acidophilic hemoglobin and a loss of basophilic-staining protein. In concert with these activities the configuration of the nucleus and cytosome gradually assume the typical oval form. As a general observation in teleosts there is a progressive increase in size of the erythrocyte with maturation (in contrast to the decrease normally observed in the mammalian rbc). In an autoradiographic study of ^{55}Fe⁻labeled circulating rbc in Baltic salmon *Salmo salar* (Fig. 45), the evolution of an immature
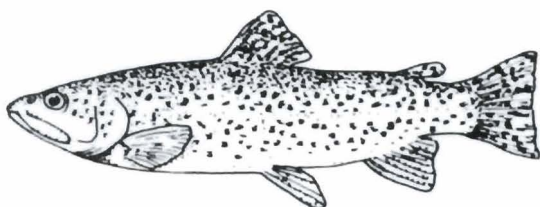

Fig. 46. *Salmo gairdneri,* rainbow trout. A fish frequently cited as demonstrating sex-linked, male dominant erythrocyte-based values.

circular erythrocyte to a mature ellipsoid resulted in increased dimensions (length and width) and a doubling of its surface area (Härdig, 1978). A longitudinal investigation of this species also revealed a confirming reciprocal correlation between the mean cell volume (MCV) and the percentage of circulating immature red cells (Härdig and Höglund, 1984). Separation of circulating rainbow trout *Salmo gairdneri* rbc by sedimentation at unit velocity reveals that these erythrocytes similarly increase in length, width and volume with maturation (Fig. 46). This is also accompanied by an increase of the L : W ratio (i.e. they become relatively narrower) (Lane et al. 1982). Immature erythrocytes in the herring *Clupea harengus* are smaller (5 x 4 μm) than the adult (9 x 7 μm) (Boyar, 1962). *Channa punctatus* demonstrates a gradual erythrocellular hypertrophy as the cell progresses through the small lymphoid hemoblast, erythroblast and reticulocyte stages (Mahajan and Dheer, 1980).

Most mature circulating erythrocytes in fish still retain some cytoplasmic ribonucleoprotein which is identifiable by supravital staining with brilliant cresyl
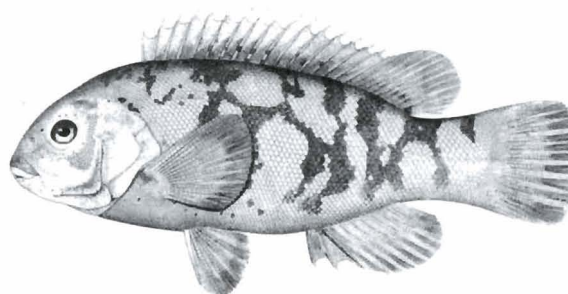

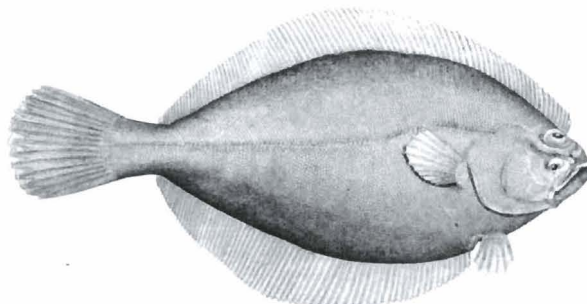**Fig. 47.** *Tautoga onitis.* tautog.

Fig. 48. *Hippoglossoides platessoides,* sand dab, American plaice. It has the flattened, broadened body characteristic of all flounders. Unlike other vertebrates, flounders are not dorsoventrally oriented (i.e. from back to belly) but laterally directed. The configuration has evolved as an adaptation to ocean bottom dwelling.

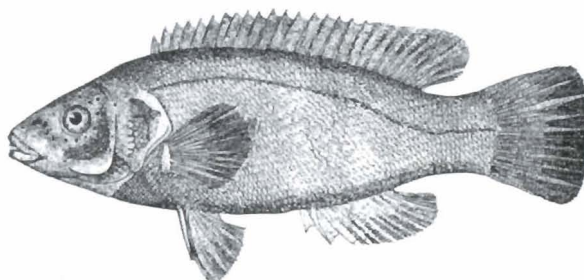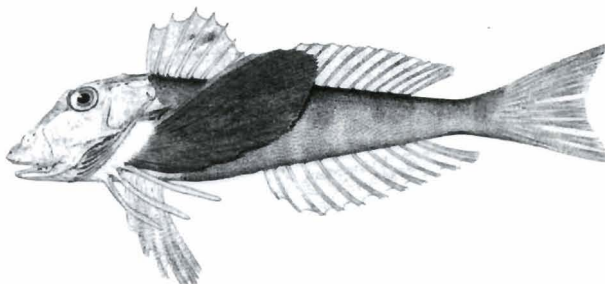**Fig. 49.** *Tautogolabrus (Ctenolabrus) adspersus,* cunner, sea perch.

Fig. 50. *Prionotus carolinus,* northern searobin. This marine marine fish's most distinctive physical feature is the modification of its elongated pectoral fins in which three lower rays are thickened, fingerlike and separated from the rest of the fin. They are used for support as well as feelers in the search for food on the sea bottom.
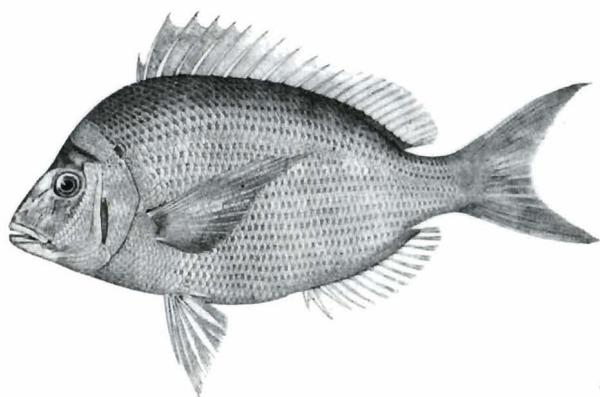
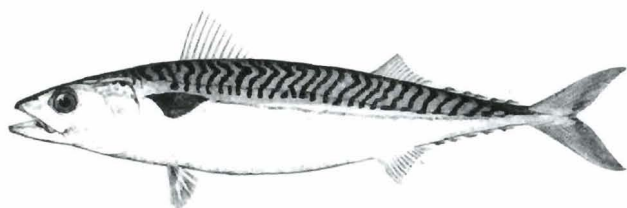**Fig. 51.** *Stenotomous chrysops.* scup. porgy.

Fig. 52. *Scomber scombrus,* mackerel, Atlantic mackerel. One of the most active, open sea migratory fishes. Members of its family (Scombridae) have a characteristic series of finlets located behind the dorsal and anal fins.
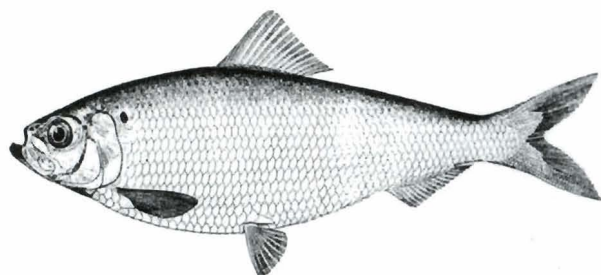

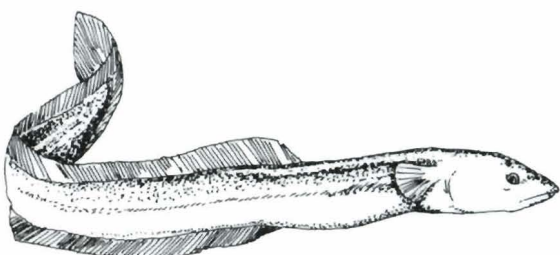**Fig. 53.** *Pomolobus (Alosa) pseudoharengus.* alewife.

Fig. 54. *Anguilla rostrata,* the common or American eel.

blue (Dawson, 1933). Some teleosts such as the tautog *Tautoga onitis* , toadfish *Opsanus tau,* sand dab *Hippoglossoides platessoides* and cunner *Tautogolabrus adspersus,* (Figs. 30, 47 - 49) have populations of erythrocytes that are comprised essentially of such mature cells and rarely any younger ones (recognized by marked stores of cytoplasmic ribosomal protein). However others (e.g. the searobin *Prionotus carolinus*

Fig. 55. *Anguilla rostrata* larva, the leptocephalus. This thin, transparent ribbon-like organism begins to develop erythrocytes only after its metamorphosis. Hence it exists at least several months without rbc. Approx. actual size.
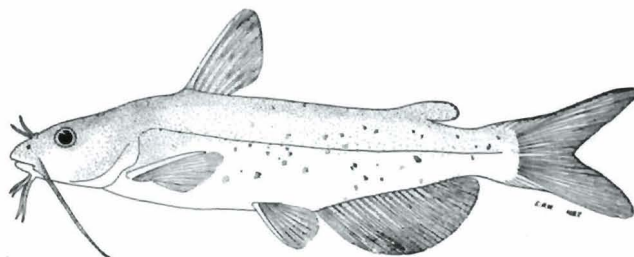

Fig. 56. *Ictalarus punctatus,* channel catfish. Fishes of Ohio, Ohio State University Press, 1981.

and scup *Stenotomus chrysops)* are likely to have 3 - 4% immature erythrocytes while the mackerel *Scomber scombrus,* alewife *Pomolobus pseudoharengus,* and a common eel *Anguilla rostrata* have approximately 20% immature forms (Figs. 50 - 54). Rainbow trout *Salmo gairdneri* have approximately 10% «juvenile» erythrocytes in their peripheral blood and the vast majority of all cells (juvenile and adult) synthesize some hemoglobin while in circulation (Keen et al., 1989). Polychromatophilic erythrocytes are commonly seen in the circulation of the extensively cultured species *Ictalurus punctatus,* the channel catfish; mitotic figures have also been occasionally noted (Fig. 56) (Williams and Warner, 1976).
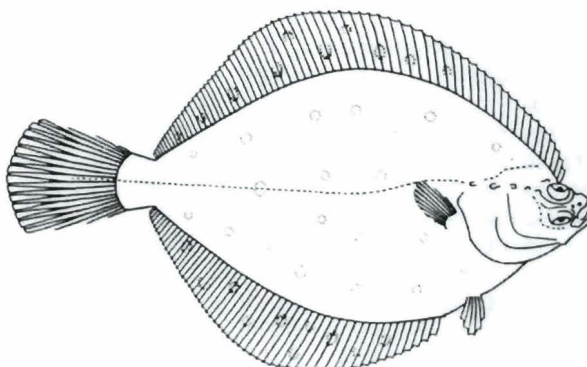
Fig. 57. *Pleuronectes platessa,* plaice. The best known and most marketed flounder species. The striking aspect of the flounder's larval metamorphosis is the migration of one of the eyes from one side of the body to the other. Ultimately both eyes lie on the same side.

Under undefined circumstances occasional erythrocytes in some fishes give rise to erythroplastids (denucleated rbc). The enucleation process has been interpreted as a pinching off of the nucleus and a surrounding rim of cytoplasm from the remainder of the

cell resulting in the generation of two circulating rbc's, one with a nucleus and one without (Watson et al., 1963). In addition to the priorly cited cyclostomes (lamprey), ganoids (bowfin), and dipnoi (lungfish) they are also seen in the goldfish *Carassius auratus*, European eel *Anguilla vulgaris*, plaice *Pleuronectes platessa* (Fig.57), perch *Perca fluviatilis*, roach *Rutilus rutilus*, channel catfish *Ictalurus punctatus,* and other random teleosts. The occurrence of incidental circulating erythroplastids throughout phylogeny (beginning with rare invertebrates, some fishes and extending on to higher inframammalian vertebrates) is interesting and in some ways a phylogenetically predictive phenomenon. The red cells of *Maurolicus mülleri* and the other bristlemouths that circulate only denucleated rbc are a marvel in this respect and are «far ahead of their time».

Amitotic red cell proliferation in the peripheral blood of primitive and teleostean fishes has been frequently described. Such derived cells are usually considered atypical and though recognized in various species e.g. the lamprey, lungfish, plaice, Atlantic salmon, white sucker, rainbow trout and goldfish, their numbers are low (Jordan and Speidel, 1931; Deutsch and Engelbert, 1970; Ellis, 1984; Benfey and Sutterlin, 1984; Houston, 1990; Murad et al., 1990). Their role and stimuli for presentation have yet to be definitively assessed.

Electron microscopy has added new dimensions to the cytologic analysis of erythropoiesis. Transmission electron microscopy (TEM) of circulating erythrocytes in the Pacific Hagfish *Eptatretus stoutii* verifies the tenet that profoundly immature erythrocytes are present in the blood of this primitive fish and that they undergo changes in shape, size, nucleocytoplasmic ratio and cytoplasmic content while in the circulation (Sekhon and Maxwell, 1970). Notable cytologic features of young and mature cells are the presence of an equatorial marginal band composed of 30 - 35 microtubules and the distribution of very numerous smooth and coated pinocytotic vesicles along the plasmalemmal border (a characteristic of vertebrate and some invertebrate erythroid cells). The major structural modifications associated with maturation are condensation of finely dispersed euchromatin into larger electron-dense masses of heterochromatin and loss of nucleoli, Golgi complex, ribosomes, lysosomes and associated lamellated figures (representing degenerated organelles) (Figs. 6, 7). Newly synthesized hemoglobin accumulates in the cytoplasm of developing cells and is seen as amorphous, moderately electron-opaque material. At maturity, the cytoplasm is devoid of organelles but nevertheless still retains occasional mitochondria and a few scattered ribosomes. The latter would be expected to yield positive brilliant cresyl blue staining under optical microscopy.

Ultrastructural studies of erythrocytes from the rays *Torpedo marmorata*, and *Raja clavata* (thornback ray) yield results in agreement with the hagfish including the recognition of putative hemoglobin in the nucleus (Zapata and Carrato, 1981). Hemoglobin has also been
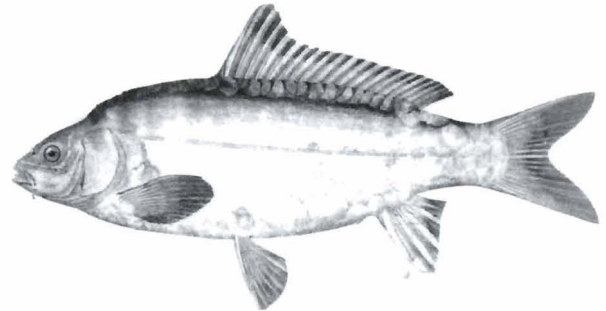

Fig. 58. *Cyprinus carpio*, carp, common carp. This fish can be totally or partially scaled or scaleless. This is an example of the latter, the so-called leather carp. The lateral line is very apparent.

cytochemically localized at this site in *T. marmorata* and *T. ocellata* (Figs. 12, 13) on the basis of a positive peroxidase-benzidine reaction (Pica et al., 1983). Intranuclear hemoglobin has been similarly reported for the hierarchal *Carassius auratus*, the goldfish, *Cyprinus carpio*, the carp (Fig. 58) and *Salmo gairdneri*, the rainbow trout.

The rainbow trout *Salmo gairdneri*, one of the world's most intensely investigated and commercially cultivated fish, has erythrocytes whose maturation follows the same ultrastructural sequence described for the jawless and cartilaginous fishes (Sekhon and Beams, 1969; Yamamoto and Iuchi, 1975). Its erythrocytes can be considered a morphologic paradigm for the teleosts. The trout (and presumably the vast majority of fishes) generate two sequential morphologically distinguishable populations of red cells. As indicated previously, the first is the transient larval or yolk sac series while the second is the definitive generation which populates the peripheral blood throughout the greatest part of the

Fig. 59. Definitive (adult) generation erythrocytes, *Salmo gairdneri*, rainbow trout. They are flattened ellipsoids with oval nuclei. The profile view reveals a biconcave depression that surrounds the nucleus whose bulge in turn imparts a biconvex configuration to the cell. From 23 day-old fry, SEM x 4,300. Yamamoto M. and Iuchi I. Electron microscopic study of erythrocytes in developing rainbow trout, *Salmo gairdneri irideus*, with particular reference to changes in the cell line. J. Exp. Zool. The Wistar Institute Press, 1975. Reprinted by permission of Wiley-Liss, division of John Wiley & Sons, Inc. This acknowledgement also applies to Figs. 65 - 68.

fish's life span. Definitive generation erythrocytes first appear in 3 day old fry (immature trout that have recently lost their yolk sac) as biconvex, relatively small, spheroidal cells (±10 μm in diameter); by day 6 they evolve into larger, flat, elliptical discs. In 12 day fry they are the predominant red cell in circulation.

Fig. 60. Definitive (adult) generation erythrocyte, *Salmo gairdneri*. An immature red cell. It has a round shape and the nucleus contains a moderate amount of heterochromatin. The Golgi complex (GC) and paired centrioles (C) are present in the lower left field. A mitochondrion (M) is indicated; some degenerating mitochondria (DM) are adjacent to or within vesicular structures. Longitudinally sectioned microtubules of the marginal band (MT) are faintly observable in the upper and lower cell margins, TEM x 19,000. Sekhon S.S. and Beams H.W., Fine structure of the developing trout erythrocytes and thrombocytes with special reference to the marginal band and the cytoplasmic organelles. Am. J. Anat. The Wistar Institute Press, 1969, Reprinted by permission of Wiley-Liss, division of John Wiley & Sons, Inc. This acknowledgement is also applicable to figures 61 - 64.

These erythroid cells continue to mature and increase their dimensions so that by day 23 they are uniformly larger, ~10 x 16 μm. Scanning electron microscopy (SEM) ideally verifies their three dimensional configuration, i.e. oval with a hemispherical central nuclear convexity surrounded by a ring-like depression on both surfaces (Fig. 59). They are 1 μm thick at the margin. Under TEM the immature, initially round cells are seen to have a large nucleus with a delicate

Fig. 61. Definitive (adult) generation erythhrocyte, *Salmo gairdneri*. The cell is intermediate in development. The cytoplasm and nucleus have attained an oval form. Polyribosomes are numerous, a degenerating mitochondrion is present in the right center (DM), and vesicular structues of the Golgi apparatus (GC) are identifiable in the central infra-nuclear region. The marginal band (MB) though present is poorly resolved, TEM x, 15,000. Sekhon and Beams; Wiley-Liss.
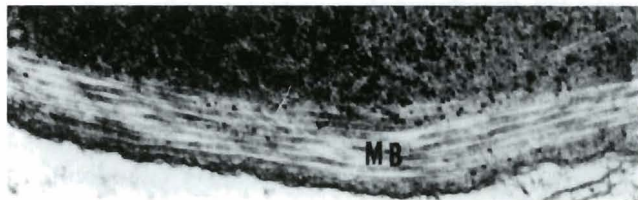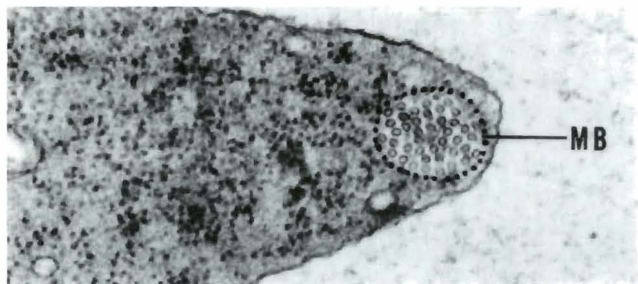
Fig. 62. Definitive (adult) generation erythrocytes at magnifications greater than the previous figure, *Salmo gairdneri*. Upper micrograph: the tapered distal end of an elliptical erythrocyte demonstrating the microtubules forming the marginal band (MB). Approximately 40 microtubules have been cut in cross section, TEM x 53,000. Lower micrograph: a segment of an erythrocyte sectioned parallel to its flat surface. The marginal band is cut along its long axis, TEM x 43,000. Sekhon and Beams; Wiley-Liss.
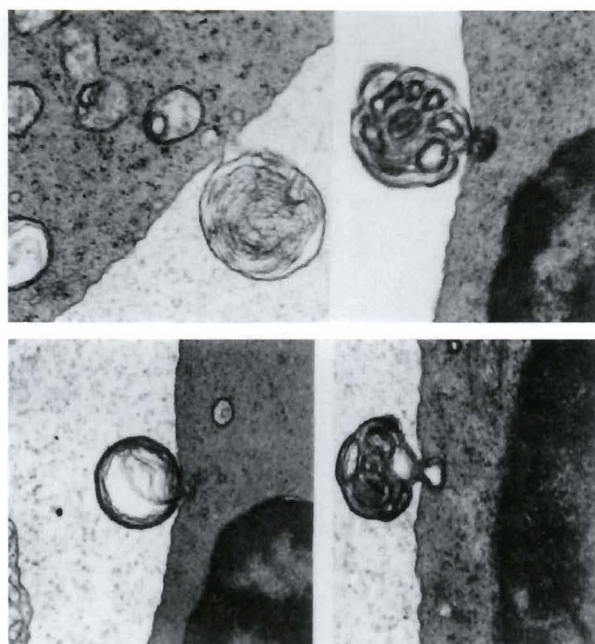
Fig. 63. *Salmo gairdneri*, rainbow trout. Erythrocytes demonstrating exocytosis of degradation products of mitochondria and other organelles. In many instances the material has a lamellar or whorled structure, TEM x 25,000. Sekhon and Beams; Wiley-Liss.

Fig. 64. Mature, definitive generation erythrocytes, *Salmo gairdneri*. The biconvexity of mature ellipsoidal cells is seen. The nuclei are condensed and exhibit prominent masses of chromatin (CR) separated by less electron-dense collections of hemoglobin (HE) which appear contiguous with the cytoplasmic hemoglobin via the nuclear pores. The cytoplasm is essentially homogenous due to the loss of the great majority of the organelles and accumulation of hemoglobin. The marginal band (MB), not ideally resolved at this magnification, is observed persisting in the mature cell, TEM x 13,000. Sekhon and Beams; Wiley-Liss.

chromatin pattern (nuclear cytoplasmic ratio ~2:1), a characteristic high density of ribosomes, identifiable Golgi complex, some mitochondria and a developing marginal band (Figs. 60 - 62). Some of the cytoplasmic organelles become degraded by lysosomal activity and converted to residual myelin-like figures which undergo exocytosis (Fig. 63). By maturity the nucleus has become oval, compact, and markedly reduced in volume; the cytoplasm, also correspondingly oval, is filled with hemoglobin and exhibits a few remnant ribosomes and mitochondria (Fig. 64).

Primitive (larval) generation erythrocytes are identifiable in nine day old larval trout (alevins). They become a population of uniform-sized red cells by larval day 16 and are the dominant circulating erythroid cells until the immature fish are about 10 day old fry. They gradually disappear thereafter (Yamamoto and Iuchi, 1975). Their development closely parallels that of the definitive series (Figs. 65 - 68). Unlike the members of the permanent series, however, these cells divide in the circulation, demonstrate synchronous maturation and terminate their development as round (14 µm diam., 1.5 µm thick) discs.

The primitive (yolk sac, larval) generation erythrocytes perform ontogenetically important functions. Their origin in the yolk sac and the embryonic intermediate cell mass has been demonstrated by histochemistry and immunohistochemistry (Iuchi and Yamamoto, 1983) (Fig. 39). This population of cells once having made its appearance in fishes has phyl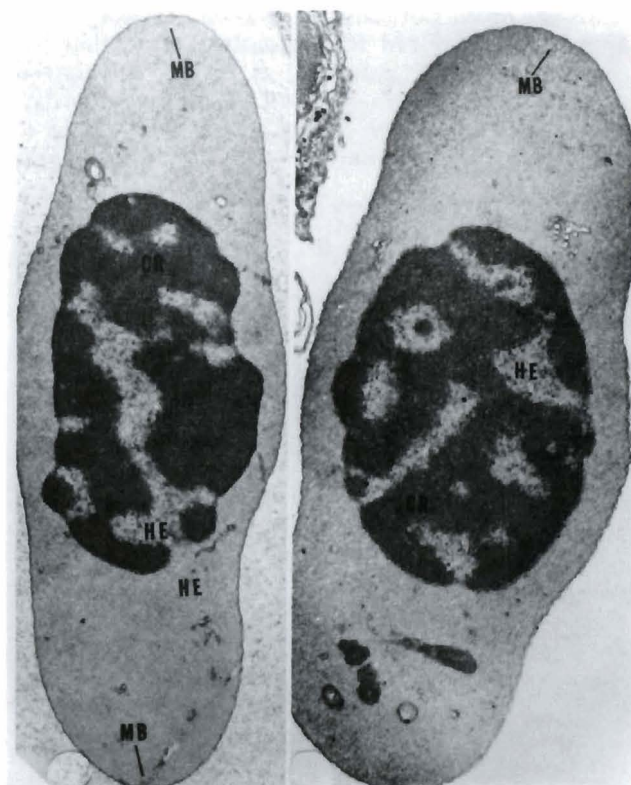ogenetically persisted in the amphibian, avian and mammalian species including man, surely expressing some biological precept. These cells arise from aggregations termed blood islands and synthesize hemoglobins other than those of the adult animal. They have a limited tenure and are replaced by cells with different morphologic characteristics and varieties of hemoglobin better suited for a new non-larval environment. Interestingly, the basis of the recently hypothesized relationship between the coelacanth and the terrestrial vertebrates rests on the similarity of its hemoglobin with the larval, and not the adult, hemoglobin of the frog *Rana catesbeiana* (Gorr et al., 1991).

The relevance of the erythrocyte cytoskeleton to the cell's dynamic activities as well as the details of its architectural design have recently become more appreciated. The cytoskeleton in smooth dogfish *Mustelus canis* erythrocytes, functionally analyzed and ultrastructurally dissected by Cohen, Joseph-Silverstein and collaborators (1982, 1984), is a model for primitive and modern fishes. It consists essentially of a marginal
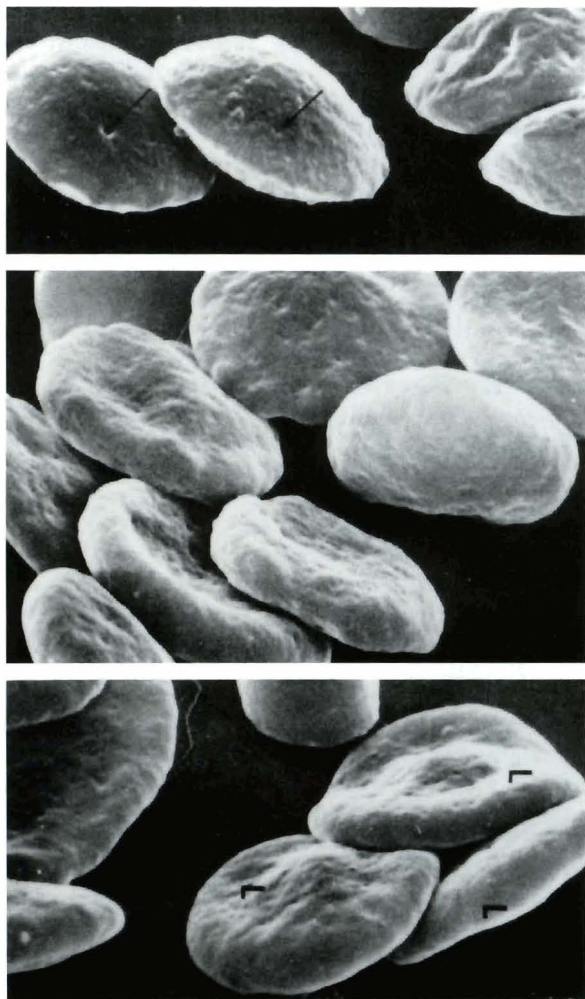
Fig. 65. *Salmo gairdneri,* larval (primitive) generation erythrocytes, scanning electron micrographs. Upper plate: immature erythrocytes. They are round, biconvex and have a sharply demarcated edge. The arrows indicate plasmalemmal pits. From 12 day-old embryo. Middle plate: erythrocytes at an intermediate level of maturation. They are round, rather thick discs with a slight central depression and have smoothly curved cell margins. The cell surfaces continue to demonstrate pits. From 16 day -old embryo. Lower plate: mature erythrocytes (L). They are thinner and flatter but retain the round discoid configuration. A central nuclear bulge is identifiable. From day 3 day-old fry. All magnifications x 3,200. Yamamoto and Iuchi; Wiley-Liss.

Fig. 66. *Salmo gairdneri,* rainbow trout, larval (primitive) generation erythrocyte. A very immature cell in the circulation of the embryo. The nucleus (N) is relatively large; its chromatin is finely dispersed, delicate and has several small clumps of heterochromatin. The cytoplasm contains a high concentration of polyribosomes seen as tiny granular material. Mitochondria (M) are present. From a 9 day-old embryo. TEM x 7,600. Yamamoto and Iuchi; Wiley-Liss.
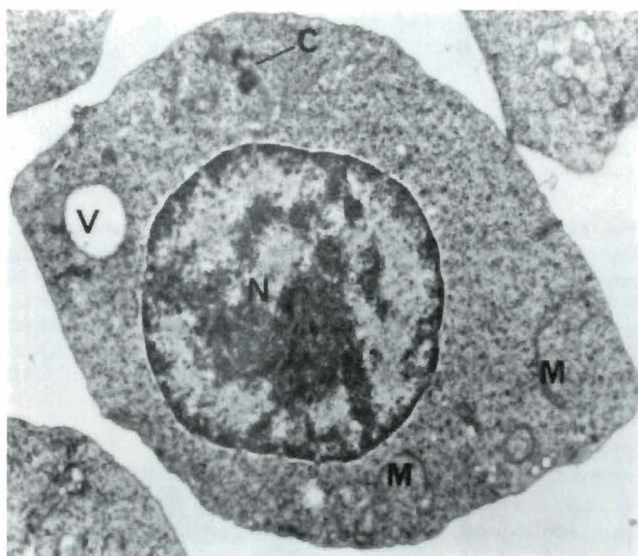
Fig. 67. *Salmo gairdneri,* larval (primitive) generaton erythrocyte. An immature cell demonstrating some maturation. The nucleus (N) is smaller than it is in the most immature progenitor (prior figure) and has prominent clumped masses of heterochromatin. The cytoplasm reveals numerous ribosomes plus amorphous material of moderate electron density (also seen in the nucleus) that is believed to represent hemoglobin. Mitochondria (M) and a vacuole (V) are identified. From 12 day-old embryo, TEM x 9,400. Yamamoto and Iuchi; Wiley-Liss.

band (MB) of microtubules and the membrane skeleton (cell surface-associated cytoskeleton, SAC). The marginal band is a bundle of continuous subplasmalemmal microtubules that encircle the cell like a belt in the plane parallel to the flat surface of the cell (Figs. 60 - 62, 64). The membrane skeleton,in turn, is an irregular network of intermediate filaments (major components actin and spectrin) which completely encloses the nucleus and the marginal band (Fig. 69). The marginal band has been identified in piscine species that embrace cyclostomic (hagfish, Fig. 6) chondrichthian (skate, ray), and teleostean (trout, goby,
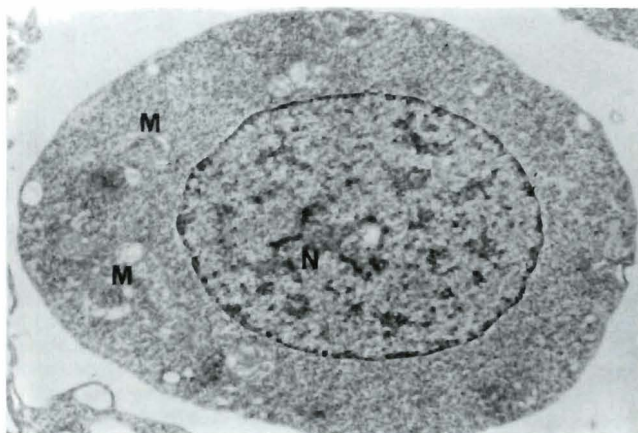
roach, toadfish, pike, goldfish) classes. It is present in both larval and definitive generation erythrocytes (Yamamoto and Iuchi, 1975). The thickness of the MB, i.e. the number of microtubules in cross section, each having a diameter of ~200-400Å, roughly parallels the size of the cell (Weinreb and Weinreb, 1965). Goniakowska-Witalinska and Witalinski (1976) have
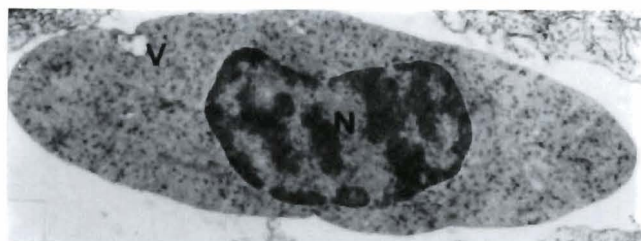
Fig. 68. *Salmo gairdneri* larval (primitive) generation erythrocyte. It demonstrates continued maturation (see prior figure). The cell has a flattened profile. The nucleus (N) is further reduced in volume and has increasingly condensed chromatin. Mitochondria are sparse and the number of ribosomes is diminished. A vacuole (V) presumably containing degraded material is present. Complete cellular development will result in a smaller, denser nucleus. The cytoplasm will become more electron opaque due to accumulation of additional hemoglobin. From 16 day-old embryo, TEM x 7,500. Yamamoto and Iuchi; Wiley-Liss.
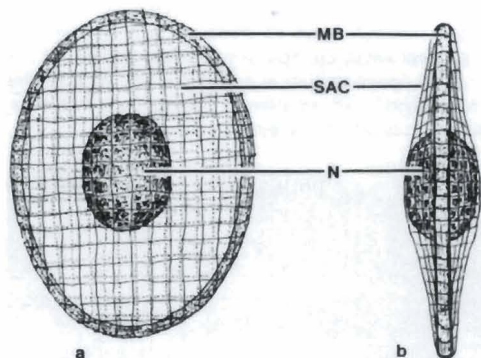

Fig. 69. Model of the nucleated erythrocyte cytoskeleton illustrating the relationship between the marginal band (MB) and the cell surface-associated cytoskeleton (SAC, membrane skeleton). Face view (a) and edge view (b). The SAC completely encloses the MB and holds it under asymmetrical tension generating an oval form. The presence of prominent nucleus (N) results in a central bulge and the biconvex profile typical of fish rbc. Joseph-Silverstein and Cohen, reproduced from the Journal of Cell Biology, 1984, Vol. 98, p 2124 by copyright permission of the Rockefeller University Press.

noted a positive linear correlation between the length of a red cell and the logarithm of the number of microtubules/MB (i.e. a semilogarthmic curve) in a spectrum of 23 piscine, amphibian, reptilian and avian species.

The membrane skeleton is normally under tension and applies forces asymmetrically across the MB. It is responsible for the red cell's maturational conversion from a sphere to an ellipse as well as for its discoid form (flatness). Experimental manipulations document that the MB and SAC function cooperatively in the continued maintenance of the discoid oval shape in the presence of external mechanical stress such as cell collisions while in circulation. Denaturization of the marginal band of dogfish erythrocytes by incubation at 0° C renders no immediate configurational change to static cells. However, when such red cells are subjected to mechanical forces they become permanently distorted (folded, buckled). Reassembly of the MB restores to the cell the property of resilience to plasmalemmal

deformation. Conversely if the SAC is digested away the cell loses its oval shape and becomes circular (Cohen, 1978). The erythrocytic MB and associated elliptical configuration made their initial phylogenetic appearance in some invertebrates´erythrocytes (e.g. blood clams), became uniformly represented in the piscine species, and have persisted throughout the submammalian vertebrates. The long term retention of these features attests to their value to the red cell. The recent verification that mammalian yolk sac-derived erythrocytes manifest a marginal band (Cohen et al., 1990), a hallmark of submammalian, but not mammalian, erythrocytes seems to underscore the evolutionary tenacity of the MB. Inasmuch as a spherical ancestral erythroid precursor i.e. a hemoblast, changes its configuration during differentiation to an oval form, it is teleologically acceptable to view the cell as «selecting» the new geometry. Benefits that accrue from the design of the cytoskeleton and the cell's ellipsoidal profile are said to be enhanced flow properties in circulation as well as the discussed resistance to deformation. In accord with this view is the observation that the submammalian thrombocyte, the only other blood cell whose entire mature existence entails residence in circulating blood is similarly an ellipsoid with a marginal band (Fawcett and Witebsky, 1964; Sekhon and Beams, 1969). The reversion of the normally round, biconcave denucleated human erythrocyte to an ellipsoid under shear stress (interpreted as mimicking flow conditions in large vessels) lends additional support for the correlation of flow benefits and oval cell form. In regard to the bristlemouths, whether a marginal band is present in the oval denucleated rbc of *M. mülleri* and *V. lucetia* or particularly the spherical cells of *V. tripunctulatus* remains to be established.

Erythrocyte-based hematologic data (erythrocyte counts, hemoglobin concentrations, hematocrit levels)
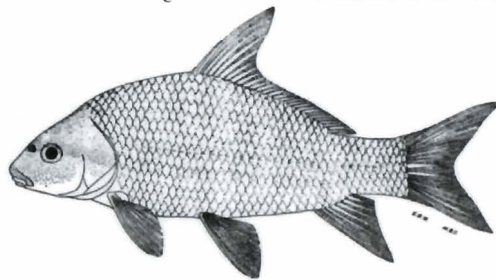

Fig. 70. *Ictiobus bubalus.* smallmouth buffalofish. Fishes of Ohio, Ohio State University Press. 1981.
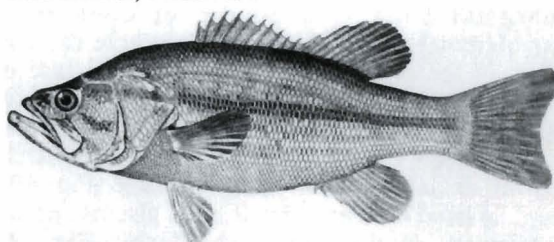

Fig. 71. *Micropterus salmoides,* largemouth blackbass.

in fishes extend over a wide base and are variably affected by numerous factors (McCarthy et al., 1973; Houston, 1990). Among the most apparent are order/family-specific characteristics, age, sex, season, spawning, migration, stress due to capture, diet, and environment. It is possible, nevertheless, to characterize the ichthyic erythroid profile. Erythrocyte counts are markedly lower in fishes than in mammals. Commonly given ranges for the two are 500,000 to 2,500,000 cells/mm^3 or µl and 5.0 - 10.0 million/µl respectively. Red cell counts for the toadfish *Opsanus tau* (0.68 x 10^6/µl), smallmouth buffalofish *Ictiobus bubalus* (1.28 x 10^6/µl), largemouth blackbass *Micropterus salmoides* (1.57 x 10^6 µl), and pipefish *Syngnathus fuscus* (2.5 x 10^6 µl) are typical for teleosts (Table 1, Figs. 29, 30, 70,
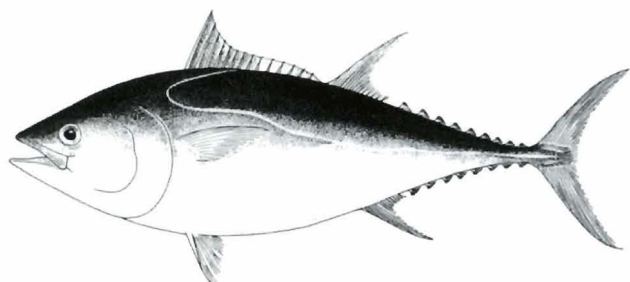

Fig. 72. *Thunnus thynnus*, bluefin tuna. The bluefin's crescentric tail is a structural indicator of a powerful swimmer. Due to its countercurrent heat exchange system this endothermic fish can maintain its muscle temperature 10 - 15º C higher than the ambient sea. The bluefin's high level of activity is supported by a particularly rich erythrocellular endowment.

71). The lowest values are observed in the cyclostomes and chondrichthyes. *Torpedo marmorata* and *T. ocellata* (Figs. 12, 13) have a mean rbc count of 200,000/µl; values for other elasmobranchs derived by various
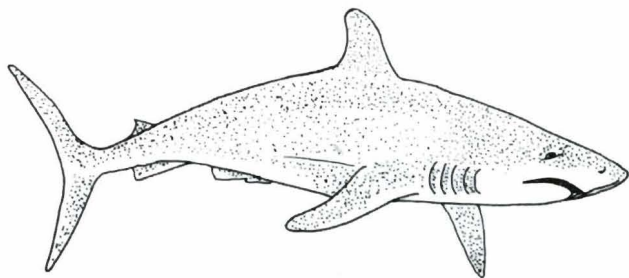

Fig. 73. *Isurus oxyrinchus*, shortfin mako shark. An active shark capable of extreme bursts of speed; it has high Hct and Hb levels.

workers are 70,000 - 680,000/µl (Table 1) (Pica et al., 1983). However, since the elasmobranchian and myxinoid erythrocytes are markedly larger than teleostean red cells (approximately four fold, ~800 fl vs. 200 fl), their hematocrits (Hct) are not necessarily markedly different from those of modern bony fishes. The highest erythrocyte counts and comparable Hb and Hct levels are obtained in certain scombrids (mackerel familiy) e.g. bluefin tuna *Thunnus thynnus,* albacore *Thunnus alalunga* and skipjack tuna *Katsuwonus*
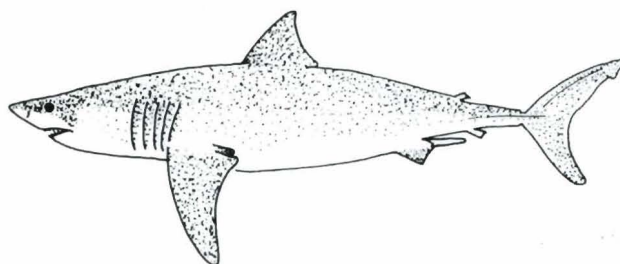

Fig. 74. *Carcharodon carcharias*, great white shark. it is an endothermic elasmobranch and consequently has a high Hb and Hct.

*pelamus (*Figs. 31, 32, 72) (Alexander et al., 1980). The physiologic basis for this is novel (among fishes) and hematologically interesting. The tunas and the lamnid sharks (Isuridae) e.g. mako shark *Isurus oxyrinchus* (Fig. 73), the great white shark *Carcharodon carcharias* (Fig. 74), and the porbeagle shark *Lamma nasus* have circulatory systems that include heat-exchaning retia mirabilia which conserve heat produced by the fishes' metabolism (Carey et al., 1982). This results in a relative elevation of the body temperature above the surrounding marine milieu. Fishes demonstrating this quality are termed endothermic. All other fishes being poikilotherms exhibit the same temperature as their aqueous environment, lose most of their body heat, and are considered ectothermic. The endothermic fishes distribute some of their retained heat to red swimming-musculature and thereby enhance its
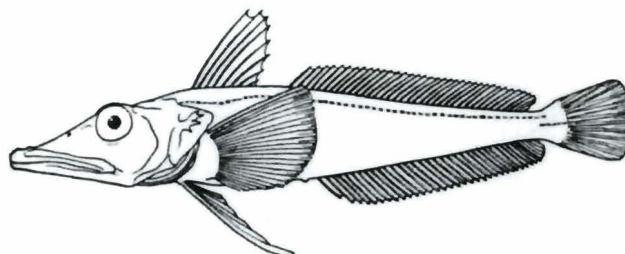
Fig. 75. *Chaenocephalus aceratus*, the ice fish (so-called because of its near transparency). This antarctic fish is remarkable because it has only rare erythrocytes and is devoid of hemoglobin.

functional capacity (Carey et al., 1982). Such fishes, in addition, are endowed with a high erythrocyte count which permits the oxygenation of body tissues at levels sufficient to support a high energy metabolism. The erythrocyte levels for the albacore, skipjack and bluefin tunas are ~2.3 - 4.1 million/µl (Alexander et al., 1980). Their hemoglobin and hematocrit values are even more impressive (Table 1).

In terms of low erythrocyte counts in fishes nothing equals the dramatic condition obtained in some icefishes (family Chaenichthyidae) (Kooyman, 1963). They are unique in that they have creamy white (hence «bloodless») gills and colorless blood that is devoid of significant numbers of erythrocytes, e.g. *Chaenocephalus aceratus* (Fig. 75), *Chaenocephalis bouvetensis*, *Champsocephalus esox.* Indeed, initial studies failed to

identify any red cells in the blood of the most highly examined species *Chaenocephalus aceratus* (Ruud, 1954, 1965). Erythrocytes, though present, are extremely rare, seldom identified in dry film blood smears, and are more frequently observed in kidney imprints and within blood vessels of sectioned kidney and spleen (Barber et al., 1981). They are nucleated, have the dimensions 12.7 x 9.9 μm and apparently do not contain hemoglobin. Their sparsity is underscored by the fact that unequivocal erythroblasts have yet to be identified in any hemopoietic tissue and erythrocytes have yet to be encountered in electron microscopy. This hemoglobinless icefish is capable of existing and attaining a significant mass (> 1kg) because of the low temperature of Antarctic waters (thus fostering oxygen solubility) and because it has a cardiac ventricle that is three times greater than comparable «red blooded» fish (Harrison et al., 1991). The latter functions as a low pressure, high volume pump which compensates for the low oxygen carrying capacity of the blood. There are also rare examples of fishes without erythrocytes during a specific period of their existence. The thin, ribbon-like leptocephalus stage of the American eel *Anguilla rostrata* (Fig. 55) is devoid of erythrocytes until it undergoes metamorphosis to an elver (glass eel) at about the end of
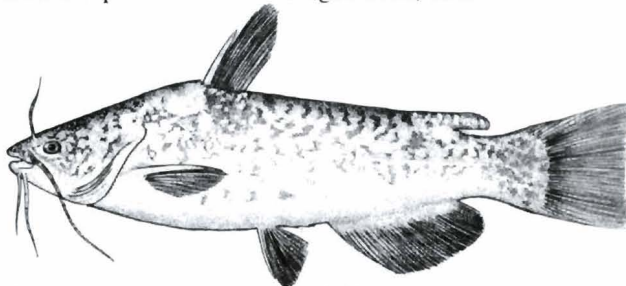

Fig. 76. *Ictalurus nebulosus*, brown bullhead. It has four pairs of barbels («whiskers») which have sense and taste receptors. They are used to touch the water bottom and objects in search for food.

its first year of life (Harder, 1975). The Atlantic herring *Clupea harengus* (Fig. 26), among the most commercially important fish in the world, has a transparent thin larva like the eel (John, 1932). It also does not develop erythrocytes until at metamorphosis (age ~3 months, ~25 mm). Primitive yolk sac generation erythrocytes are consequently deleted from these species' ontogeny.

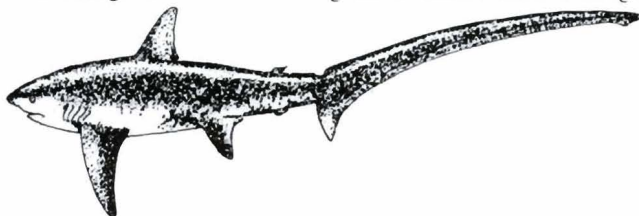Hemoglobin levels among most fishes have a range

Fig. 77. *Alopias vulpinus*, thresher shark. It has an unusual dorsal caudal lobe («tail») which is about as long as the rest of the body. The high erythrocyte-based values in this shark suggest that it may be an endothermic fish.

of 5 - 10 g/dl (deciliter), significantly lower than what is observed in mammals, e.g. the blackbass *Micropterus salmoides* (5.8 g/dl), brown bullhead *Ictalurus nebulosas* (6.9 g/dl), northern pike *Esox lucius* (8.8 g/dl) and carp *Cyprinus carpio* (10.5 g/dl) (Table 1, Figs. 41, 58, 71, 76). Hb concentrations less than 5-6 g/dl blood are standard in the chondrichthyes with the exception of the so-called endothermic sharks which have the much higher level of ~14 g/dl (Wintrobe, 1933; Kisch, 1951; Larsson et al., 1976; Pica et al., 1983; Emery, 1986). Comparably large, active, but non-endothermic selachians such as the sandbar shark *Carcharhinus plumbeus* and tiger shark *Galeocerdo cuvier* maintain a pedestrian 5 - 7 g/dl. The thresher shark's *(Alopias vulpinus)* hematologic picture (Hb 14 g/dl, Hct 37) suggests that it may also prove to be an endothermic fish (Fig. 77) (Emery, 1986). Among teleosts the greatest Hb concentrations are present in (endothermic) tunas (Table 1) (Klawe et al., 1963; Alexander et al., 1980). The bluefin tuna *Thunnus thynnus*, the world's largest teleost and avidly sought, epitomic source of sushi and sashimi, is representative of this group (Fig. 72). The mean Hb (~16.0 g/dl) established for these fishes is an example of piscine species attaining the mammalian level of hemoglobin. *Scomber scombrus*, the Atlantic mackerel (a member of the Scombridae, as are the tunas) does not possess a countercurrent heat exchange apparatus but nevertheless has a high Hb concentration, ±14 g/dl (Fig. 52, Table 1). The rich hemic endowment apparently supports this fish's very strong swimming activity. Other fishes that can demonstrate relatively high hemoglobin concentrations (±10 g/dl) are the eel *Anguilla rostrata* and the snakehead *Channa punctatus* (Table 1, Figs. 44, 54). In regard to *Channa punctatus,* its Hb level (as well as rbc count and Hct) remain consistently impressive even during seasonal fluctuations (Mahajan and Dheer, 1979; Dheer, 1988). These factors plus the small size of its erythrocytes are consistent with this
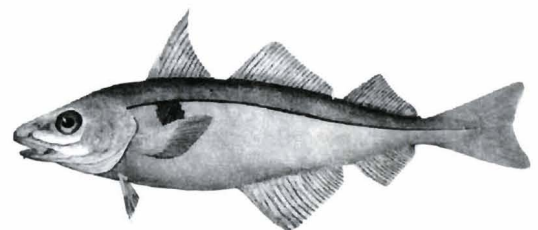
Fig. 78. *Melanogrammus aeglefinus*, haddock, eglefin. One of the most prized marine food fishes; marketed smoked as finnan haddie.

hardy, air-breathing fish's ability to prevail under adverse conditions and in the most limited volumes of water. It has been demonstrated that, in general, fishes leading an active existence possess higher hemoglobins than those maintaining a comparatively sedentary existence. The Hb level is the single best erythrocellular indicator of the oxygen carrying capacity of an ichthyic species. It avoids the limitations of the rbc count (lack
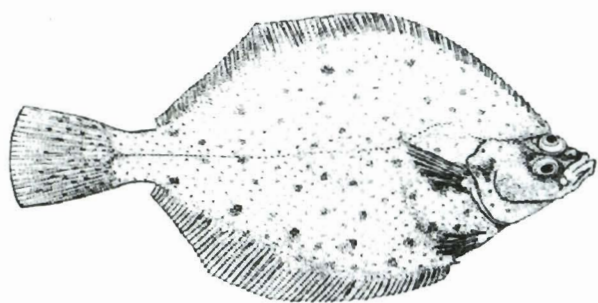
Fig. 79. *Limanda ferruginea*, yellowtail flounder. This benthic marine fish is a righteye flounder. During development its left eye migrates to the right side. The fish swims on one side and lies on the sea bottom on its (blind) left side. Atlantic fishes of Canada, Department of Fisheries and Oceans. Reproduced with permission of the Minister of Supply and Services, Canada, 1991.
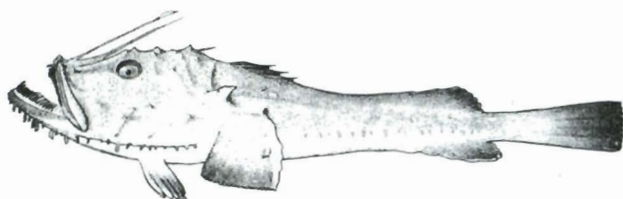
Fig. 80. *Lophius piscatorius*, angler or goose fish. An extremely inactive fish which lies on the bottom and angles for food using the fringes on the first dorsal spine as a lure. Its erythroid profile, a reflection of its activity is understandably low, Hb 3 - 4 g/dl, Hct 18.

of recognition of differences in cell volume) and the Hct (packed cell volume is not necessarily an equivalent measure of hemoglobin content since all fish rbc cannot be assumed to be fully saturated with Hb).

Typically, hematocrits in fishes range ~20 ~35 e.g. the northern searobin *Prionotus carolinus* Hct 24 (Fig. 50, Table 1) and the Atlantic haddock *Melanogrammus aeglefinus* Hct 39, Hb 6.0 g/dl (Fig. 78) (Larsson et al., 1976). Some teleosts have lower values as the yellowtail flounder *Limanda ferruginea* and the goosefish *Lophius piscatorius* (Figs. 79, 80; Table 1) (Larsson et al., 1976). Low values also tend to be offered by the cartilaginous fishes with the exception of the endothermic sharks which have significantly higher hematocrits than their ectothermic counterparts (Emery, 1986). The very highest packed cell volumes are seen in the tunas e.g. albacore (Hct 54) and skipjack (Hct 59) (Alexander et al., 1980). Among many teleosts (as with certain sharks) Hct and Hb levels are positively correlated with the level of activity i.e. more active genera exhibit richer hemic endowments. Thus the benthic, relatively static angler *Lophius piscatorius* as compared with the more active, bottom feeding haddock *Melanogrammus aeglefinus* (both inhabitants of the north Atlantic) and the polar, sedentary, benthic scamvenger *Trematomus bernachii* vs. the (also polar) pelagic *Trematomus borchgrevinki* have appropriate (relatively high or low) activity-correlated Hct and Hb

values. Two other fishes, both native to the North Sea, with higher or lower Hct and Hb levels relating to their activity are the migratory herring *Clupea harengus* (Hct 51, Hb 14 g/dl) and the cod *Gadus morhua* (Hct 32, Hb 7 g/dl). Hematocrits rarely exceed 55 regardless of the species, whether inframammalian or mammalian. Recognizing that the erythrocyte is a finite cellular mass conferred with generous but limited deformability and an inherent resistance to passive flow in suspension, the greater the mass of red cells in a given volume of plasma the greater will be the cardiac force required to propulse the blood (a «slurry» of rbc and plasma). Thus hematocrits have physiologic limits which if surpassed impose a cardiac circulatory load (blood viscosity) that exceeds the capacity of the vascular system. The hematocrits found in the tunas are uniquely high and their value probably represents a near physiologic maximum for fishes as well as all vertebrates. Cardiac failure is a recognized complication of polycythemia vera, a disease in humans in which the number of erythrocytes (and Hct) are malignantly elevated beyond rheologic tolerance.

The Wintrobe erythrocyte indices (MCV, MCH and MCHC) offer an insightful standard for the comparison of the sizes and hemoglobin content of red cells among orders, classes, families and individuals accrross the spectrum of vertebrate (and sometimes invertebrate) life. The derivation of the mean cellular volume (MCV) from the rbc count and Hct per the Wintrobe formula is a particularly effective method for establishing the size of an animal's circulating erythrocytes. It avoids one of the alternatives, i.e. the visual measurement of erythrocytes in blood smears. The latter´s disadvantages include dependency upon the quality of the blood films, variability in cell size secondary to the distribution of the cells on the smear, and limited applicability in inter-experiment comparison. Ichthyan red cells exhibit a species-dependent, several-fold range in MCV. Thus the largest erythrocytes in fishes are found in the primitive hagfish and dipnoi (±1500 fl) followed by the sharks and rays (500 - 1300 fl) and then most of the teleosts
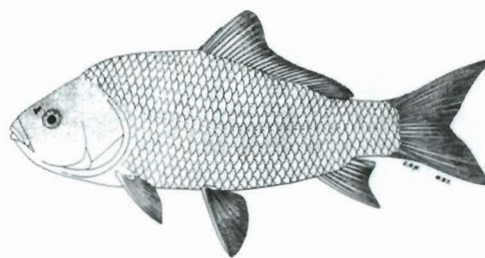

Fig. 81. *Ictiobus cyprinellus*, bigmouth buffalofish. Fishes of Ohio, Ohio State University Press, 1981.

(150 - 350 fl). Cellular volumes <100 fl, as seen in the kissing gourami *Helostoma temmincki* (Fig. 40), are uncommon (Table 1). Of interest regarding the dimensions (and by extension the MCV) of the rbc in endothermic sharks is the observation that within

Emery's data (1986), barring the one exception of the blue shark *Prionace glauca,* the adult erythrocytes in these elasmobranchs were greater than those of the compared ectothermic selachians, ~21 x 15 µm vs. 18 x 13 µm (insufficient data available for MCV). A review of shark rbc sizes as reported by Saunders (1966) neither supports nor discounts this proposed tendency. Closely related species can be expected to express similar mean corpuscular volumes but this is not always the case. Concurrent examination of two members of a genus can sometimes reveal notable differences. Two suckers, the smallmouth and bigmouth buffalofishes *Ictiobus bubalus* and *I. cyprinellus* (Figs. 70, 81; Table 1) have generated data yielding reasonably similar MCV's of 257 and 320 fl respectively (20% difference) (Chlebeck and Phillips, 1969). However, the channel catfish *Ictalurus punctatus* and the brown bullhead *I. nebulosus* (Figs. 56, 76; Table 1) present values of 136 and 228 fl (40% difference) (Haws and Goodnight, 1962). When present, significant intraspecies differences in MCV most likely reflect the comparison of unequivalent populations (due to nutrition, age, etc).

Smaller erythrocytes offer in aggregate a greater total cell surface for oxygen uptake/release than an equivalent packed cell volume composed of larger rbc. It is therefore understandable why active fishes may advantageously have smaller red cells than relatively sedentary ones, e.g. the benthic, shoal water toadfish *Opsanus tau,* and the pelagic mackerel *Scomber scombrus* (MCV 400 vs. 150 fl respectively). Still not all «active» fishes have the smallest red cells; rainbow and brown trout *Salmo gairdneri* and *S. trutta* have a relatively large MCV, ~350 fl.

The mean cellular hemoglobin (MCH) expresses the amount of hemoglobin present in an average circulating erythrocyte (Table 1). Since the size of a piscine red cell can vary widely so can its total content of hemoglobin, range ±30 -300 pg. The MCH for modern bony fish is usually <100 pg, that of cyclostomes, elasmobranchs and primitive bony fish >100 pg.

The mean cellular hemoglobin concentration (MCHC) indicates what proportion of an average erythrocyte can be considered devoted to hemoglobin (wt/vol, g Hb/100 ml rbc). The MCHC in fish erythrocytes is somewhat low, typically 18 - 25%. In some cases it may attain 30% or higher, a value more likely to be seen among avians. The endothermic and other large active sharks apparently have unusually high MCHC's (±34%) in sharp contrast with their fellow chondrichthians, the skates and electric rays (~20%) (Emery, 1986; Pica et al., 1983). The very lowest MCHC's are seen in the (family) Nototheniidae, the most commonly caught fishes that live in the frozen antarctic McMurdo Sound (Wells et al., 1980). The MCHC for the representatives *Trematomus borchgrevinki, T. hansoni* and *T. bernacchii* is ~13% (Table 1). (Hb levels in this group are correspondingly basal ~3.3 g/dl, while the MCV is in the teleostean mode, 275-450 fl). An analysis of MCHC of fishes

residing at different latitudes i.e. tropical (<20° latitude), temperate, cold north temperate, and polar (>65° latitude) reveals «a remarkable association in which MCHC is decreased with increasing latitudes» (Wells et al., 1980). It has been theorized that this reduction represents a mechanism of adaptation to the temperatures of different geographic bodies of water. That is, oceans within the latitudes having low temperatures, e.g. the polar regions induce a temperature-enhanced solubilization of oxygen in the plasma and a reciprocally diminished dependance on erythrocytic hemoglobin for oxygen transport. The net result has led to the generation of lower erythrocytic MCHC's in such regional fishes. The reverse holds true for tropical zone fishes. Typical MCHC ranges are ~30 - 35% and <15% for tropical and polar fishes respectively. Fishes that have a large population of immature circulating red cells (bearing less Hb than mature rbc) as seen in the chondrichthyes would be expected to generate low MCHC values (Härdig and Höglund, 1983). Because piscine erythrocytes have a nucleus and since it represents a space-occupying mass within the cell, the MCHC of fish erythroid cells usually does not reach the maximum levels seen in denucleated (mammalian) red cells (~32 -34%).
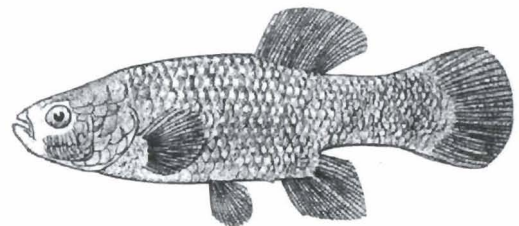
Fig. 82. *Fundulus heteroclitus,* mummichog or killifish. This marine fish can exist in a wide range of salinity and is very tolerant of low oxygen levels. It is often used as a subject in embryologic and physiologic studies.

Erythrocyte-based values often manifest a sex-related bias. Mean Hct as well as Hb values and rbc counts are frequently higher in male than in female teleosts. However, the ranges overlap and a sex-related difference even in those fishes cited as manifesting this phenomenon is not identifiable under all circumstances. Typically one or two but not all rbc-based data exhibit a gender based variance. For example, in Mulcahy's investigation although Hct's were higher in male
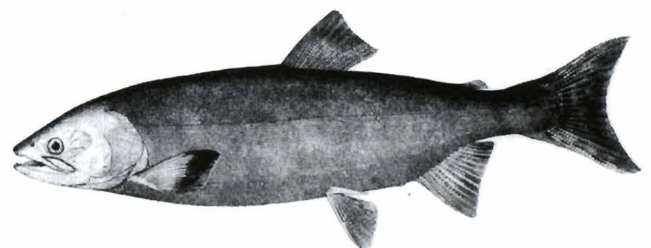
Fig. 83. *Oncorhynchus gorbuscha,* Alaskan pink salmon.

northern pike *Esox lucius* (♂33.9, ♀27.9) the rbc and Hb levels did not show any mean differences. Higher hematocrits have been reported for male largemouth blackbass *Micropterus salmoides* (50 vs. 42.2) while significantly different sex-linked Hct's (♂32.5, ♀29.4) and Hb concentrations (♂8.8, ♀7.8 g/dl) have also been attributed to the goldfish *Carassius auratus* (Steucke and Atherton, 1965; Summerfelt, 1967). The same observation has been made in the marine *Fundulus heteroclitus* regarding erythrocyte counts and Hb concentrations (Fig. 82) (Slicher, 1958). Among the most firmly documented differences are the hematocrits observed in adult rainbow *Salmo gairdneri* (♂44; ♀42.2), brook *Salvelinus fontinalis* (♂45.2, ♀38) and brown trout *Salmo trutta* (♂44; ♀38.3) (Snieszko, 1961). Hemoglobin levels in mature brown trout have been reported higher in males during the period approaching highest sexual development i.e. spawning (Poston, 1966). Parallel temporal elevations in the erythrocyte counts also occur in male, (but not female adult, and ♂ & ♀ immature), brown trout (Pickering, 1986). Another salmonid, the wild Alaskan pink salmon *Oncorhynchus gorbuscha* presents a male-dominant blood picture at spawning in rbc, Hb, Hct and MCV determinations (Fig. 83) (Hutton, 1966). In a six month study of mature rainbow trout prior to and through the spawning period even though rbc, Hct and Hb levels significantly decreased across time the mean values for males persistently remained higher (Lane, 1979). Of particular interest in the latter study was the fact that the MCV increased with time, though not linearly, with the males always maintaining a greater mean value. The MCH increased and reflected the same sex variance. Intraperitoneal injections of methyl testosterone neutralize the erythrocytopenia induced by hypophysectomy of the male *Fundulus heteroclitus* (Slicher, 1958). Gender-related differences in the erythrocytic MCV has been recognized in some invertebrates (estimated per microscopy), avians (chicken), and mammals (gerbil) as noted in our laboratory, some swine, rhesus monkey, and man). It is reasonable to assume that this may also prove to be the case in a significant number of piscine species.

The age and hence the size of a fish also influence the erythroid blood picture. In the American plaice *Hippoglossoides platessoides* (a flounder) the Hb, Hct and rbc levels are directly correlatable with the weight of the specimens (Fig. 48) (Smith, 1977). In addition the smaller flounders have, throughout most of the year, a lower MCV than their larger cohorts. The well known plaice *Pleuronectes platessa* exhibits an increase in mean Hct with increased weight up to 120 g, at which point the hematocrit stabilizes (Fig. 57) (Preston, 1960). Adult Atlantic herrings *Clupea harengus* have twice as many immature red cells in their peripheral blood than their juvenile cohorts, ±25% vs. 12% (Fig. 26) (Boyar, 1962). Salmon parr (5 - 6 months old) have 15 - 20% immature circulating red cells. The latter increase in hemoglobin content as well as cell size with maturity

(Härdig, 1978). The size of a fish can also be a determining factor in the erythrocellular response (if any) to changes in the environment e.g. temperature (Houston, 1990). Other factors responsible for differences in intraspecies erythroid profiles include season, spawning (as implied by the discussion of sex-linked hematologic characteristics), nutrition, quality of the environment and stress (including that associated with capture). Juvenile Baltic salmon *Salmo salar* sampled over an annual cycle demonstrated significant seasonal changes (Hct increased November - February, fell below initial level July, returned to basal value August; Hb demonstrated peaks in November and May; rbc count increased in September and returned to initial level May - June). Immature rbc attained maximum representation, ±15%, in April - June (Härdig and Höglund, 1984). An analysis of the data supported the premise that the seasons exert a greater influence upon the erythroid picture than the age of the fish. Among the oft-cited seasonal and environmental initiators that modify the erythropoietic status are temperature, photoperiod and availability of oxygen. In regard to the two former conditions, hematologic variation among salmonids appears to be more correlated with the photoperiod (day length) than with the temperature of the water. A seasonal rhythm for Hct and Hb has been observed in mature female perch *Perca fluviatilis* residing in Baltic brackish water (Sandström, 1989). The period of peak erythropoiesis apparently coincides with that of regional salmon. During a year-long survey of *Channa punctatus* (Fig. 44) the levels of rbc, Hb and Hct were seen to decrease during the breeding season and return to previous levels thereafter (Mahajan and Dheer, 1979). In a nine month analysis of the blood picture of the plaice *Pleuronectes platessa* (wt. 140 g) the Hct and rbc count were lowest at the end of winter and highest during early summer (Hct 21.4 vs. 27.0; rbc 2.05 vs. 2.28 x 10^6/μl) (Preston, 1960). Stress due to capture in this species resulted in an elevated Hct and decreased MCHC in the presence of a stable red cell count; following acclimatization in an aquarium the values returned to normal (Bourne, 1986). A similar study of the winter flounder *Pseudopleuronectes americanus* sustaining capture or laboratory-defined stress similarly revealed an increased Hct and MCV accompanied by a diminished MCHC (Fletcher, 1975). The erythrocyte count and MCH usually did not differ from the control subjects. It has been suggested that catecholamines or other hormones released at the time of stress induce fish red blood cells to swell secondary to alterations in ionic and intracellular fluid equilibrium thereby resulting in a rise in Hct and MCV accompanied by a fall in MCHC. Hematologic responses to increased temperature are identifiable in many teleosts (DeWilde and Houston, 1967; Houston, 1980; Murad et al., 1990). Exposure of the hardy, air breathing *Channa punctatus* to elevated temperatures of 30° and 35° C resulted in an increase of all primary and derived erythrocytic quanta while an aqueous environment maintained at 25° C did not affect
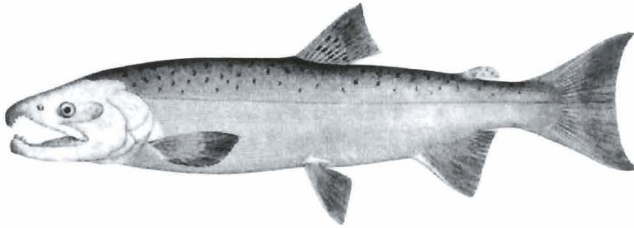
Fig. 84. *Oncorhynchus kisutch*, coho or silver salmon. A fish native to the Pacific area.

the hematopoietic equilibrium (Dheer, 1988). An erythrocytosis and related rbc responses to thermal stress have also been observed in the goldfish *Carassius auratus*, carp *Cyprinus carpio* and rainbow trout *Salmo gairdneri*. The observed elevations in Hb concentration, rbc count, and MCHC accompanied by a diminished MCV (via altered cell molarity) have been interpreted as adaptations to increased oxygen requirements. The reduction in MCV offers an advantage since the rate of oxygen combination with Hb varies inversely with cell volume. The latter can be considered a short term, environmental restatement of the dictum that in general the size of the erythrocyte tends to diminish with higher metabolic demands.

A dietary deficiency of folic acid that results in a specific, classic, morphologically recognizable alteration of developing erythroid cells is elicitable in fishes. Coho salmon *Oncorhynchus kitsutch* (Fig. 84) maintained on a folate-deficient diet develop an anemia signified by a lowered erythrocyte count, hematocrit and hemoglobin concentration, macrocytosis (increased MCV and MCH), anisocytosis, poikilocytosis (rbc nuclear segmentation), renal erythroid hyperplasia and (most important diagnostically) megaloblastic alteration of the erythroblasts (Smith, 1968; Smith and Halver, 1969). The term «megaloblastic» characterizes the morphology of the atypical nuclei in the developing erythroid cells (open, immature-appearing chromatin pattern, minimal chromatin clumping, increased interheterochromatin spaces) and reflects the nuclear/cytoplasmic maturational asynchrony and impaired synthesis of DNA. The abnormal process results in the generation of very large erythroblasts and macrocytic mature erythrocytes as a result of failure to undergo mitosis during maturation. The nuclear pattern is virtually identical with that observed in megaloblastic anemia of man that is also induced by a deficiency of folic acid. Folate and folate-cyanocobalamin deficiencies also result in a macrocytic anemia while lack of pyridoxine is associated with a microcytic hypochromic anemia (decreased MCV and MCHC) in *Channa punctatus* (Mahajan and John, 1981; Agrawal and Mahajan, 1983). Another condition demonstrating altered erythroid cytology is the Heinz body hemolytic anemia of coho salmon exposed to chlorinated water (Buckley, 1976).

Erythropoiesis in a fish is influenced by a species'

phylogenetic niche, ontogenic status, habitat, age/size, adaptative state vis-à-vis its immediate environment, and other factors. The huge number of species that comprise the agnathic, chondrichthian, and osteichthian fishes offers a panoply of erythrocytic profiles. The study of the piscine erythrocyte affords a view of the form and function of this cell, identifies some of its restraints and limitations, and gives an insight into its expression in evolutionarily higher species.

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