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Morphological characteristics of blood cells in species of cartilaginous fish from the Adriatic Sea

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This study was performed to evaluate the morphological characteristics of erythrocytes, erythroblasts, thrombocytes and leucocytes in six species of cartilaginous fish from the Adriatic Sea. The staining characteristics, cell shapes and diameters, cell surface areas and nuclear characteristics were obtained for each cell type in each of the six species. The prevalence of each type of blood cell in each species was also provided.

Key words: Cartilaginous fish, erythrocytes, erythroblasts, thrombocytes, leucocytes

INTRODUCTION

DAWSON (1933) and ANDREW (1959) were among the first to record the morphological characteristics of cartilaginous fish erythrocytes. Later, TIMET (1956) and ŠVOB M. and T. (1962) studied the erythrocytes of cartilaginous fish from the Adriatic Sea.

SIAWCILLO (1895) and GRÜMBERG (1901) were the first to describe granular leucocytes in the blood of rays (*Raja denticulata*). Eosinophils were subsequently described by GRÜMBERG (*op.cit.*) in the blood of catfish (*Scyliorhinus canicula* L.) and by JOLLY (1923) in the blood of fish of the genus *Scyliorhinus, Raja, Torpedo.*

ANDREW (1959) noted leucocytes with rough and fine acidophilic granulation in the blood of the dogfish (*Mustelus* sp.) while ŠVOB M. and T. (1962) noted in the blood of rayfish (*Raja clavata* L.).

JOLLY (*op.cit.*) and ŠVOB M. and T. described lymphocytes in the blood of cartilaginous fish *Torpedo* sp., *Raja* sp. and *Scyliorhinus* sp. GRÜMBERG (1901) and RAWITZ (1900) described mononuclear leucocytes or monocytes in the blood of cartilaginous fish of the species *Scyliorhinus canicula* L.

ŠVOB M. and T. (*op.cit.*) described neutrophilic granulocytes and possible basophilic granulocytes in the blood of four investigated cartilaginous fish.

Unfortunately, descriptions of fish blood cells are generally fragmented and often contradictory. A comprehensive review of this topic is lacking in the literature. Conspicuously absent are modern hematological criteria for differentiating the various cell types present in fish blood. There is also an urgent need for a common terminology if study in this field is to continue.

MATERIAL AND METHODS

Six species of cartilaginous fish caught in the channel of Brač portion of the Adriatic Sea were included in the study. Blood from between three and six normal-appearing specimen of each of the six species provided the pool from which individual cells were taken for examination. Fish blood was harvested with an eyedropper from a cardiac ventricle prior to death, immediately after the fish were caught. Air-dried blood smears were used for cellular analysis since this became the most commonly employed method for routine blood examination. Slides were prepared using the MAY-GRÜNWALD GIEMSA method with distilled water at a pH of 6.8. Cell measurements were made with a light microscope on a low magnification setting after calibrating the microscope using REICHERT's scale on the immersion objective (100x). All, cell and nuclear surface area measurements in this paper, were calculated from the measured short and long axes of one ellipse without taking cell width or surface curvature into consideration. The surface areas of cells and nuclei were calculated according to the formula P=(a 2)x(b 2)p where a and b represented the short and long axes of the ellipse of the erythrocyte, multiplied by two to include both sides.

RESULTS

Morphology of blood cells of cartilaginous fish

The erythrocytes of all six studied species; *Squalus acanthias* L., *Scyliorhinus canicula* L., *Scyliorhinus stellaris* L., *Raja miraletus* L., *Raja clavata* L. and *Myliobatis aquila* DUM. were elliptical in shape. The nuclei of these erythrocytes were typically oval and located eccentrically in the cell. The shape of the nuclei was less commonly round or curvilinear. Occasionally, two nuclei were observed. The dimensions of the erythrocytes of every species investigated are represented in Tables 1, 2, 3 and 4, Fig. 2.

Table 1. Dimensions of the erythrocytes: R = range, x = mean, s = standard deviation, Sx = standard error, V = coefficient of variability

Fish species	LENGTH OF LONG AXIS OF ERYTHROCYTES (microns)					LENGTH OF SHORT AXIS OF ERYTHROCYTES (microns)				
	R	x	s	S_x	V	R	x	s	S_x	V
Squalus acanthias L.	28,50-34,20	30,93	1,58	0,29	5,18	20,52-23,94	21,84	1.06	0,20	4,94
Scyliorhinus canicula L.	27,36-39,90	34,02	2,81	0,36	8,26	17,10-29,64	23,91	2,74	0,35	11,46
Scyliorhinus stellaris L.	27,70-40,10	30,20	2,40	0,35	7,21	20,70-27,62	25,1	2,12	0,30	7,30
Raja miraletus L.	28,45-34,20	31,52	2,12	0,37	6,72	19,38-23,08	21,73	1,42	0,25	6,53
Raja miraletus L.	27,36-38,76	32,43	2,88	0,52	8,88	17,10-25,08	21,33	2,15	0,39	10,08
Myliobatis aquila DUM.	22,80-31,92	25,04	2,56	0,46	10,20	15,96-20,56	16,40	1,33	0,24	8,11

Table 2. R = range, x = mean, s = standard deviation, Sx = standard error, V = coefficient of variability

Fish species	LENGTH OF THE LONG AXIS OF THE NUCLEI OF ERYTHROCYTES (microns)					LENGTH OF THE SHORT AXIS OF THE NUCLEI OF ERYTHROCYTES (microns)				
	R	x	s	S_x	V	R	x	s	S_x	V
Squalus acanthias L.	9,12-12,54	10,98	0,82	0,15	7,47	6,84-9,12	8,09	0,72	0,13	8,9
Scyliorhinus canicula L.	11,40-15,96	13,26	1,30	0,24	9,79	7,98-11,4	9,85	1,19	0,22	12,07
Scyliorhinus stellaris L.	13,20-17,50	14,4	1,63	0,29	11,31	8,20-12,10	10,8	1,23	0,22	11,38
Raja miraletus L.	7,98-11,4	9,30	1,07	0,19	11,5	5,70-9,12	6,88	1,04	0,19	15,12
Raja miraletus L.	9,12-12,54	10,85	1,79	0,32	21,26	5,70-11,40	8,42	1,79	0,32	21,26
Myliobatis aquila DUM.	7,98-13,68	9,93	1,26	0,23	12,39	4,54-6,84	5,70	0,66	0,12	11,56

Table 3. R = range, x = mean, s = standard deviation, Sx = standard error, V = coefficient of variability

Fish species	SURFACE OF ERYTHROCYTES (microns ^{2})						SURFACE OF NUCLEUS (microns ^{2})	
	R	x	s	S_x	V	2 x	x	2 x
Squalus acanthias L.	447,7-592,01	530,96	32,08	5,85	6,04	1061,92	70,53	141,06
Scyliorhinus canicula L.	459,32-703	642,26	66,09	11,85	10,26	1284,53	123,30	246,60
Scyliorhinus stellaris L.	450,0-869,0	710,0	60,09	10,79	8,46	1420,85	122,08	244,16
Raja miraletus L.	416,0-612,42	547,10	56,48	10,14	10,33	1094,20	50,22	100,44
Raja miraletus L.	408-694,0	544,93	80,31	14,42	14,74	1089,86	71,75	143,50
Myliobatis aquila DUM.	260,0-382,0	322,14	31,65	5,78	9,82	644,28	44,50	89,0

Table 4. a/b = Relation between two diameters of elliptic shape of erythrocytes, Sc/Sn = surface of the erythrocytes in relation to surface of the nucleus,. Sc-Sn = erythrocyte surface area minus nuclear surface area

Fish species	a/b	Sc/Sn	Sc - Sn
Squalus acanthias L.	1.41	7.53	920.86
Scyliorhinus canicula L.	1.42	5.22	1037.93
Scyliorhinus stellaris L.	1.44	3.81	1176.37
Raja miraletus L.	1.45	10.89	993.76
Raja miraletus L.	1.52	7.59	946.36
Myliobatis aquila DUM.	1.52	7.23	555.28

Cartilaginous blood smears exhibited erythrocyte anisocytosis as a consequence of normal maturation (Fig. 1B) as well as several transitional erythrocyte forms including basophilic proerythroblasts, trough basophilic erythroblasts, polychromatophilic erythroblasts, orthochromatophilic erythroblasts, and mature erythrocytes (Fig. 1C, D, E).

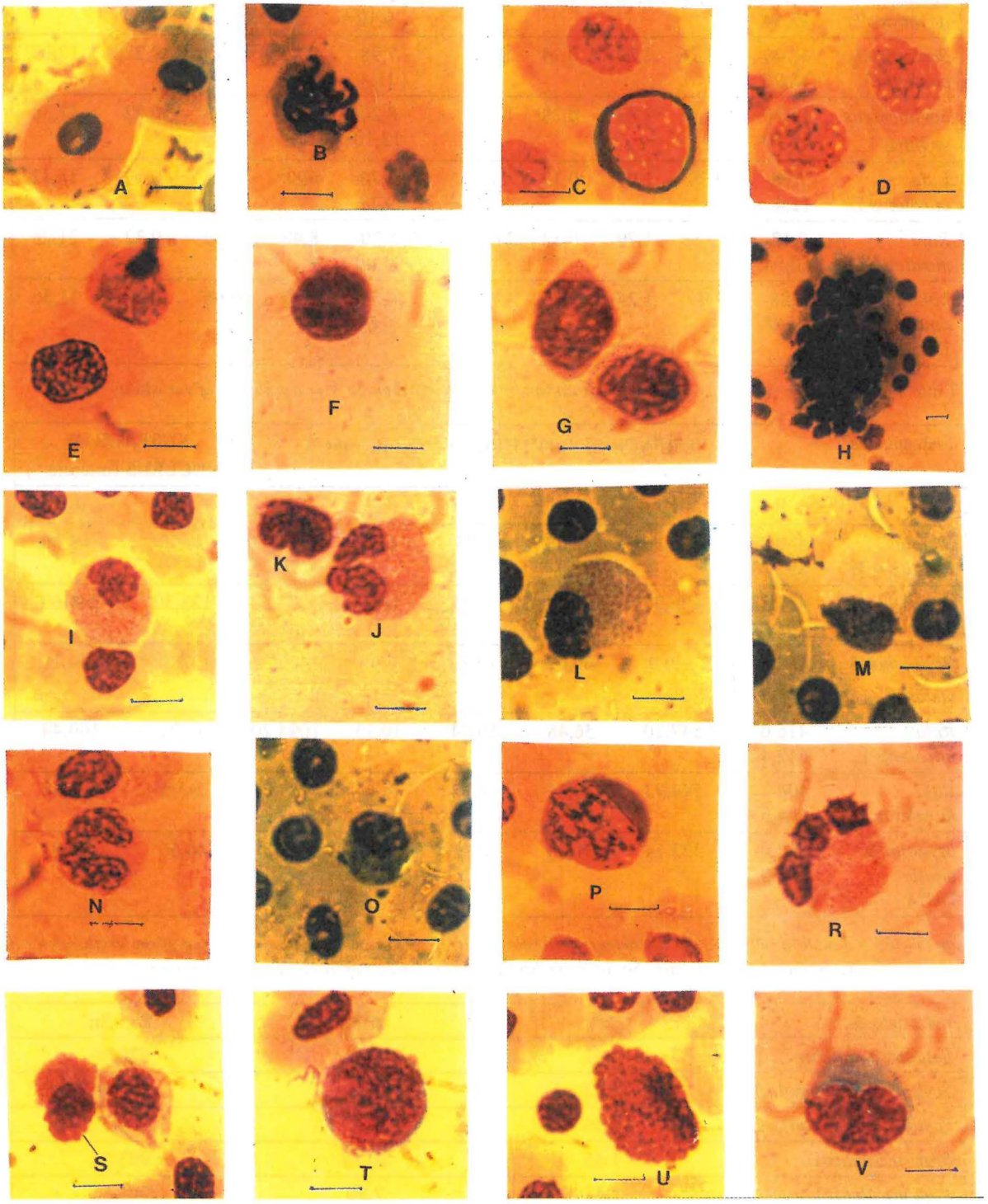


Fig.1.

Fig.1.

A - Acanthias vulgaris *L., erythrocytes*
B - Acanthias vulgaris *L., promethaphase of mitosis in an erythrocyte*
C - Acanthias vulgaris *L., erythroblast basophilic*
D - Acanthias vulgaris *L., erythroblasts polychromatic*
E - Acanthias vulgaris *L., erythroblasts ortochromatic*
F - Scyliorhinus canicula *L., round thrombocyte*
G - Scyliorhinus canicula *L., spindle chaped thrombocytes*
H - Acanthias vulgaris *L., accumulation of thrombocytes*
I - Raja clavata *L., acidophilic granulocyte with round chaped nucleus*
J - Scyliorhinus canicula *L., acidophilic granulocyte with two lobed nucleus*
K - Scyliorhinus canicula *L., thrombocyte*
L - Acanthias vulgaris *L., granular neutrophilic polymorph*
M - Acanthias vulgaris *L., neutrophilic leucocyte*
N - *Acanthias vulgaris L., acidophilic metamyelocyte*
O - Acanthias vulgaris *L., plasma cell*
P - Acanthias vulgaris *L., lymphocyte*
Q - Scyliorhinus canicula *L., acidophilic granulocyte with three lobed nucleus*
R - Scyliorhinus canicula *L., acidophilic granulocite, the young form*
Š - Scyliorhinus canicula *L., lymphoblast*
T - Raja clavata *L., acidophilic granulocite like eosinophil leucocytes in mammals blood*
U - Scyliorhinus canicula *L., monocyte*

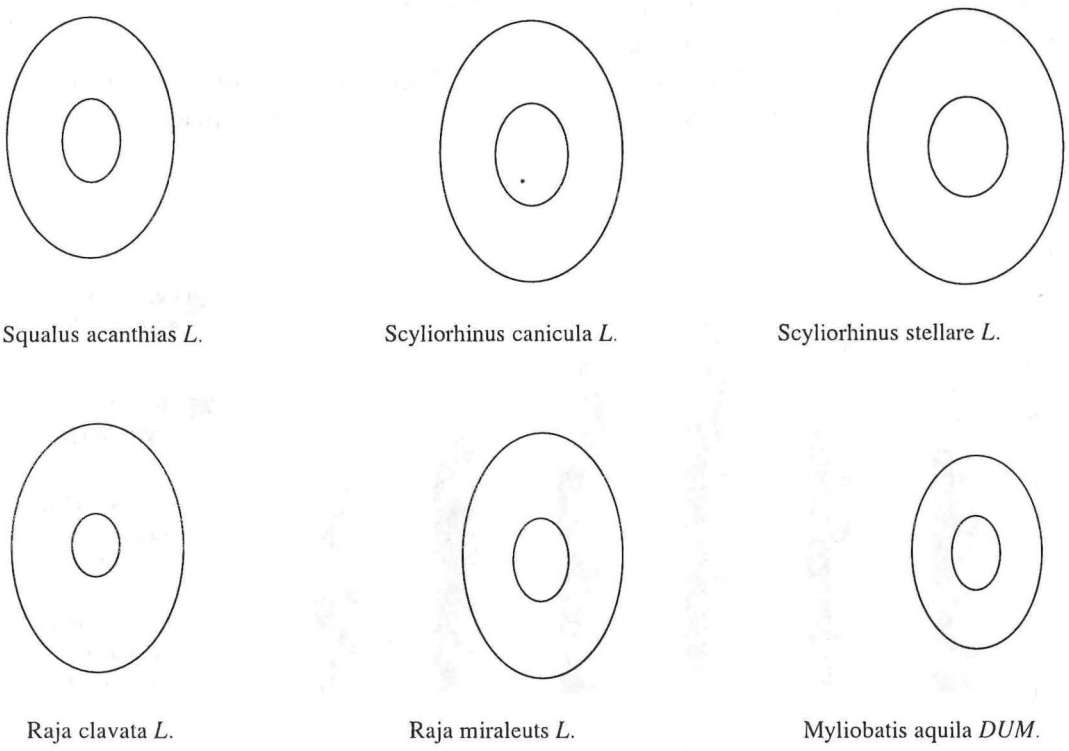


Fig. 2. Schematic drawing of erythrocytes of 6 species of cartilaginous fish (magnified 103 x)

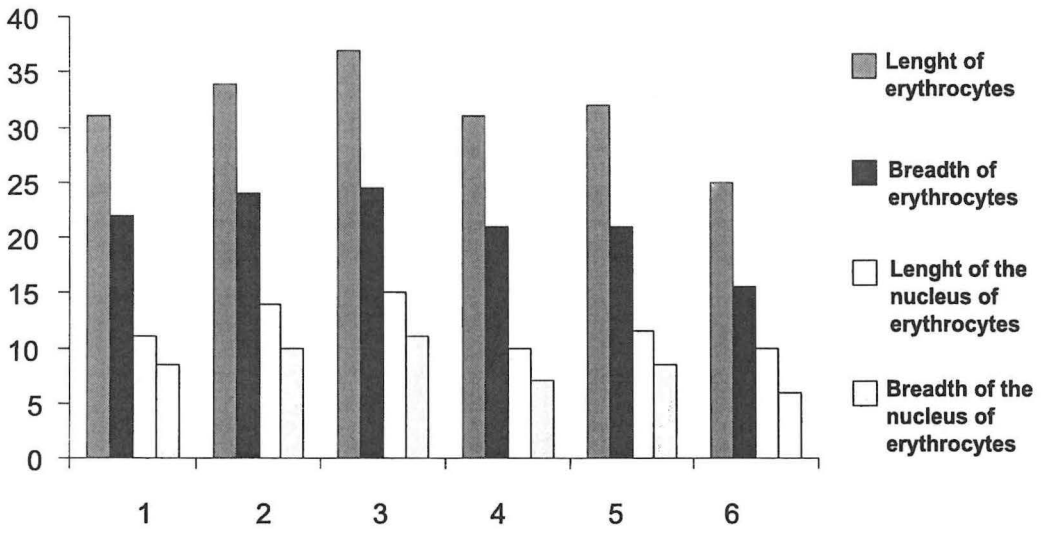
The erythroblasts were round but sometimes elliptically shaped. The proerythroblasts exhibited dark basophilic cytoplasm and differed from lymphoblasts in their breadth, the transparency of their chromatin structure and the greater conspicuity of their nucleoli. The nuclei of erythroblasts were uniformly centrally positioned within cells, typical for immature forms. The cytoplasm of erythroblasts was more or less basophilic depending upon their stage of matu-

ration. The stages of mitotic division of erythroblasts are depicted in Fig. 1B.

Occasionally, lightly stained vacuoles were observed in the cytoplasm of erythroblasts. The centrally positioned nuclei were initially round and later became elliptical as erythroblast maturation progressed. The structure of the chromatin with granular nucleoli was observed chiefly in younger erythroblasts. The long axes of erythroblasts were a function of fish species and erythroblast age, found to range between 25 and 30 microns. The long axes of nuclei varied between 18 and 25 microns. The cytoplasm of young forms of cells exhibited lighter staining characteristics and greater transparency than mature cells. These young cells also changed shape from round to elliptical with progressive cell maturation. The nucleus/cytoplasm ratio decreased with erythroblast maturation. Orthochromatic erythroblasts could be differentiated from mature erythrocytes by their enlarged nuclei and lightly stained cytoplasm including a light ring around the nuclei. This ring was also observed in young erythrocytes (Fig. 1E). In the final phase of maturation, the cytoplasm stained acidophilic instead of basophilic, consistent with it's growing hemoglobin content. In basophils, polychromatophils and erythroblasts, acidophilic-staining granulations were rarely observed and occasionally persisted in mature erythrocytes.

Catfish: *Scyliorhinus canicula* L. In the peripheral blood of this species, we noted 500 mature erythrocytes, 8 basophils, 16 polychromatophils, and 19 orthochromatophilic erythroblasts (Fig. 3).

Thrombocytes varied in size and shape in all six investigated cartilaginous fish species.



1. Squalus acanthias *L.*
2. Scyliorhinus canicula *L.*
3. Scyliorhinus stellare *L.*
4. Raja clavata *L.*
5. Raja miraleuts *L.*
6. Myliobatis aquila *DUM.*

Fig. 3A. The presentation of the length of long and short axis of erythrocytes and their nuclei

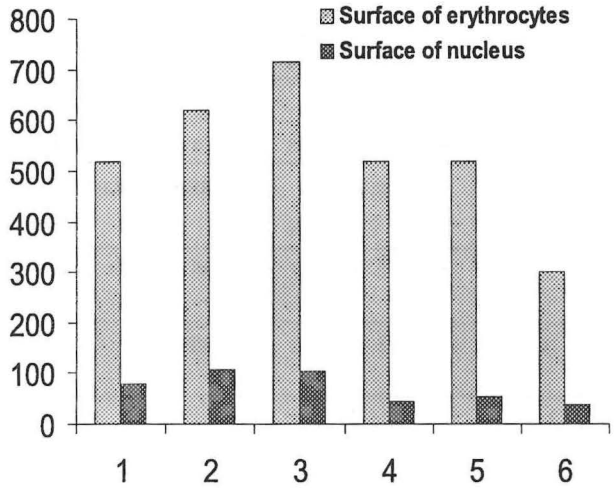


Fig. 3B. The presentation of the surfaces of the erythrocytes in relation to surfaces of their nuclei

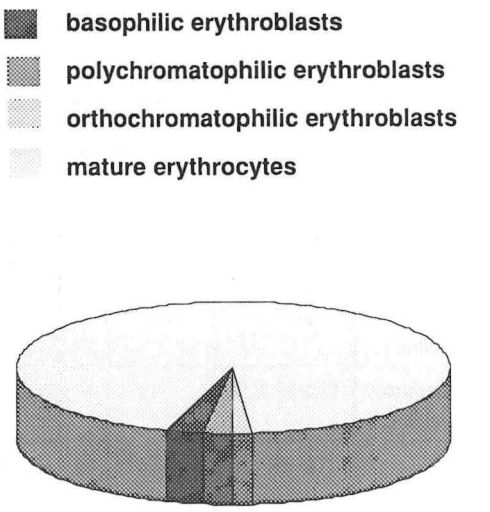


Fig. 3C. The relation between mature erythrocytes and erythroblasts in the peripheral blood of Scyliorhinus canicula *L.*

They were observed as round, elliptical, elongated, fusiform, and occasionally baton or kidney-shaped. The maximum diameter (long axis) of round thrombocytes ranged between 6.1 and 12.3 microns. With regards to elongated thrombocytes, the long axis averaged 28 microns while the short axis averaged 4.8 microns. We observed changes associated with the stages of division of thrombocytes. The narrow cytoplasm was initially basophilic and later filled with dense granulation which surrounded the nucleus closely (Fig. 1F, 1G). In the early stages of development, thrombocytes of all investigated fish species were similar to erythroblasts in appearance. The cytoplasm of thrombocytes stained more darkly than the one of erythroblasts, later becoming light gray and filling with fine granulation. In peripheral smears, the cytoplasm of mature erythroblasts was typically broken down. Specifically, 90% of smears depicted only the naked nucleus of the mature thrombocyte involved with a quantity of fibrin (Fig. 1H). This phenomena is consistent with normal blood clotting in fish. Thrombocytes are the second most numerous cells in fish blood following erythrocytes.

Polymorphonuclear granulocytes varied in number depending on the species of fish considered.

Shark: ***Squalus acanthias* L.** In the peripheral blood of this species, polymorphonuclear granulocytes were the most numerous form of leucocyte and could be subdivided into two types: acidophilic granulocytes and neutrophilic granulocytes.

In acidophilic granulocytes, the cytoplasm was lightly basophilic and filled with baton-like acidophilic granulation. These granulations were less than a micron in length and contained one or more deeply staining and light-defracting centers. The nucleus was either a single unit or subdivided into two or three segments residing primarily at the periphery of the cells. Polymorphonuclear acidophilic granulocytes constituted 60% of all leucocytes in the species *Squalus acanthias* L. (Table 5).

Polymorphonuclear neutrophilic leucocytes were characterized by their lightly neutrophilic or marginally basophilic cytoplasm (Fig. 1L,1M). The nuclei were predominantly singular, oval structures of densely packed

Table 5. Morphology of leucocytes in some cartilaginous fishes

Fish species	Polymorphonuclear leucocytes						Mononuclear leucocytes							
	Acidophylic granulocytes		Eosinophylic granulocytes		Neutrophylic granulocytes		Lymphocytes		Lymphoblasts		Plasma cells		Monocytes	
	Long axes R	%	Long axes R	%	Long axes R	%	Long axes R	%	Long axes R	%	Long axes R	%	Long axes R	%
Squalus acanthias L.	16-23	59	-	-	17.1-22.8	18	12.5-14.8	15	16-20	2	14-16	1	16-20	5
Scyliothinus canicula L	19,5-21.5	50	-	-	16-18	1	14-20.8	44	18-22	2	16-20	2	18-21	1
Raja clavata L.	16-18	20	17.2-21.39	51	-	-	13.5-17.6	28	17.5-20	-	-	-	19-21	1
Raja miraletus L.	17-19	22	17-20	50	-	-	14-17.3	27	-	-	-	-	18-21	1
Myliobatis aquila L.	13.2-19.1	60	-	-	-	-	-	29	-	9	-	1	-	1

chromatin residing at the periphery of cells. Occasionally, the nuclei of polymorphonuclear neutrophilic leucocytes were subdivided into two or three segments. In the cytoplasm, rare, very small dust-like purple-blue granulations were noted. These granulocytes gradually disappeared during the maturation of these cells.

The mononuclear leucocytes of *Squalus acanthias* L. were oval-shaped with a narrow basophilic cytoplasm and an oval or kidney-shaped nucleus of dense chromatin (Fig. 1P). The monocytes of *Squalus acanthias* L. had long axes ranging between 16.5 and 21 microns. The cytoplasm was only very lightly basophilic. Occasionally, azurophilic granules were noted. The nuclei of *Squalus acanthias* L. were broad, kidney-shaped, structures of fine chromatin which usually appeared transparent. Monocytes constituted 5% of all circulating blood cells in *Squalus acanthias* L. Immature leucoblasts were also noted. Leucoblasts were characterized by basophilic cytoplasm, but most immature forms of leucoblast were difficult to differentiate from immature forms of erythroblast. With maturity, the absence of the irregular-shaped nuclei and cytoplasmic granules in leucocytes enabled their differentiation from granulocytes. Their dark basophilic cytoplasm, density and coarseness of their nuclear chromatin characterized the plasma cells of *Squalus acanthias* L. (Fig.1 0).

Catfish: *Scyliorhinus canicula* L. *et stellaris* L. In the circulating blood of this species, we observed polymorphonuclear leucocytes with acidophilic granulations. These granulations were baton-shaped with one or more densely staining and light-defracting centers. The granules were highly sensitive to the alcohol used in the cell preparations. Following alcohol, the granules disappeared and the cytoplasm assumed a diffusely ruddy appearance. Typically, all that remained were the cytoplasmic central bodies (crystals), similar in appearance to minute round granules.

The nuclei of mature acidophilic granulocytes were either round, baton-shaped, horse-shoe-shaped, or segmented into two, three, or rarely four parts. The latter segments were either detached or narrowly connected. The nuclei of immature granulocytes were large and round, encompassing one-third to one-half of

the cell. The structure of chromatin in the nuclei of immature acidophilic granulocytes was loosely packed and the cytoplasm often deeply red-brown stained due to dissolved granules. These richly staining granules gave the cytoplasm an overall spotty appearance. The size and prevalence of acidophilic granulocytes is presented in (Table 5, Fig. 1S).

The nuclei of neutrophilic granulocytes in *Scyliorhinus canicula* L. were segmented. The cytoplasm of neutrophilic granulocytes stained light pink with occasional violaceous granules.

Mononuclear forms in the blood of catfish *Scyliorhinus canicula* L. were similar in appearance to the mononuclear leucocytes described in the blood of a shark (*Squalus acanthias* L.) (Fig. 1P). In *Scyliorhinus canicula* L. lymphoblasts constituted 2-4% of all lymphocytes. These lymphoblasts had large, round nuclei with a thin rim of cytoplasm that usually stained dark blue but in a few spots was colorless. Nuclei were eccentrically positioned in these cells.

Ray fish: *Raja clavata* L. *et Raja miraletus* L. In the circulating blood of these species, two forms of the polymorphonuclear acidophilic granulocytes could be differentiated. The first form was similar to the acidophilic granulocytes described in the blood of the catfish (*Scyliorhinus canicula* L.) The nuclei of immature polymorphonuclear acidophilic granulocytes were oval, later becoming baton-shaped and segmented. Cytoplasmic granules in *Raja clavata* L. were also baton-shaped and had central bodies that persisted after the granules had been dissolved during slide preparation.

The second form of acidophilic granulocytes in the blood of *Raja* species, *R. clavata* L. and *R. miraletus* L., had large, densely packed granules filling the entire cytoplasm. Single granules ranged between 3.2 to 3.8 microns in diameter and every cell contained 25 to 50 granules. The nuclei were sometimes difficult to visualize because of these densely packed granules. The nuclei were round, oval, or irregular in shape and eccentrically positioned in the cell. The chromatin of these cells appeared coarse and granular with unstained spaces between the accumulations of chromatin. The size and prevalence of these cells compared to other leucocytes are presented in Table 5, Fig.4. The leucocytes found in *R. clavata* L. *et R. miraletus* L. were similar in appearance to the description given above for catfish lymphocytes. They had diameters ranging from 3.5 to 17.6 microns. The prevalence of lymphoblasts was 2%.

Raja monocytes had gray-blue cytoplasms, irregular or kidney-shaped nuclei and a loose chromatin structure. Monocytes were very rare with a prevalence of between 0-1% as a percentage of all leucocytes.

Eagle Ray: *Myliobatis aquila* DUM. Acidophilic granulocytes in the blood of the eagle ray were uniform in appearance analogous to catfish. The cytoplasmic granules were baton-shaped. The polymorphonuclear granulocytes in the eagle ray were smaller than those found in catfish and also smaller than those found in *Raja* species (Table 5). There were no differences in appearance between eagle ray lymphocytes and catfish lymphocytes. In the eagle ray, the prevalence of lymphoblasts reached 10% (Table 5, Fig. 1T and Fig.4).

DISCUSSION

Terminology

Although it is hard to establish homology when comparing blood cells of fish with those of mammals, we have used the terminology, which has been modified for precisely that purpose. In describing the blood cells of our six species of cartilaginous fish, we emphasized morphological similarities with blood cells found in mammals.

The erythrocyte series were categorized according to RAUNICH (1947). Specifically, we used the terms proerythroblast, basophilic

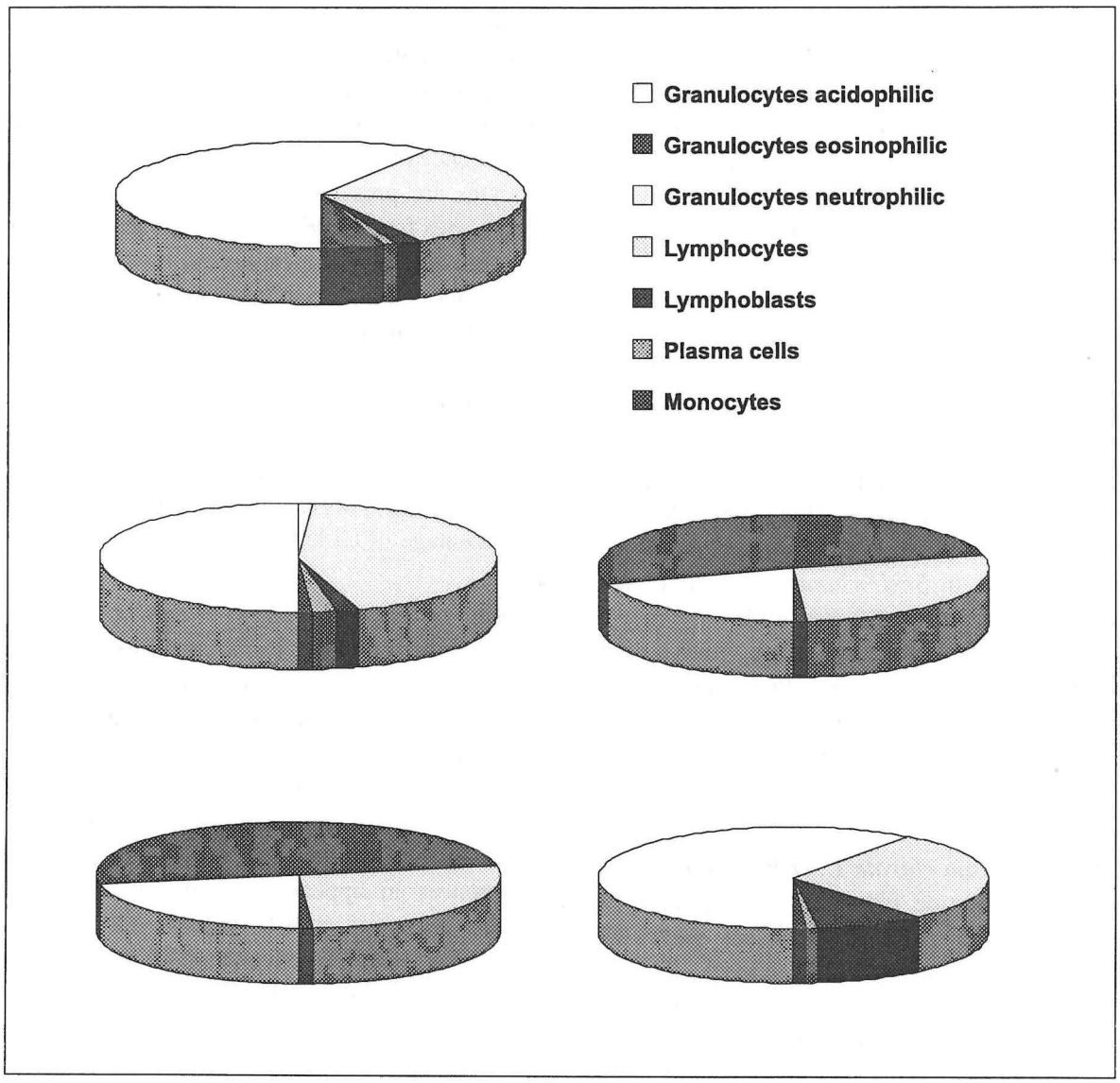


Fig. 4. Percentage of each form of leucocytes

erythroblast, polychromatophilic erythroblast, orthochromatic erythroblast, and erythrocyte.

We categorized leucocytes in accordance with terminology used in mammalian hematology: polymorphonuclear granulocytes, neutrophilic granulocytes, basophilic granulocytes, monomorphonuclear lymphocytes and monocytes. Immature forms of monocytes and lymphocytes were all referred to monoblasts and lymphoblasts for the purpose of this study even though some of these immature forms were closer to maturity than others.

All the studied cartilaginous fish blood cells were categorized according to the above-described terminology aware of the fact that this classification system cannot be expected to represent a complete nomenclature for vertebrates, much less accord with many accepted theories of hematology.

Blood cell morphology in the investigated species

Erythrocytes in cartilaginous fish were similar in appearance to megaloblasts found in fetuses of mammals or in the embryos of bird eggs after 10 to 15 days of incubation. This stage of development in higher vertebrates at the time when their megaloblasts resemble mature fish erythrocytes is referred to as the ichthyoid stage.

DAWSON (1933) studied many premature forms of erythrocytes in the blood of cartilaginous fish. He described the mitotic division of circulating erythrocytes and thus established that erythrocytes in the shark species, *Mustelus canis* MITCH. and in the ray species, *Raja diaphanes* L., erythrocytes mature in circulation. We confirmed this phenomenon in our six species by documenting the presence of identical chromosome garnitures in the nuclei of both erythroblasts and erythrocytes (Fig. 1B, 1C, 1D). It is noteworthy that megaloblasts in embryonic blood vessels multiply by way of division.

RAWITZ (1900) described leucocytes in the blood of catfish (*Scylium canicula* L.) as round, oval, or spindle-like. The spindle-like forms described by RAWITZ were probably thrombocytes. GRÜMBERG (1901) described leucocytes with kidney-shaped nuclei in *Scyliorhinus canicula* L. that, he claimed, gave rise to "polymorphonuclear leucocytes". It is speculated that these "kidney-shaped nuclei" were really in metamyelocytes prior to their maturation into polymorphonuclear granulocytes.

In the catfish *Scylium canicula* L. and *Scylium stellare* GTHR, ŠVOB M. and T. (*op.cit.*) described "cells with reddish-brown fine granulated cytoplasm" as being heterophile granulocytes. It is speculated that these heterophile granulocytes were, in fact, acidophilic granulocytes and the reddish-brown cytoplasm. ŠVOB M. and T. described those as a consequence of dilution of the baton-like acidophilic granulocytes during slide preparation. The fine cytoplasmic granules mentioned by ŠVOB M. and T. might have been central bodies that persisted following dissolution of the baton-like granules.

In the circulating blood of the ray species (*Raja clavata* L. and *Raja miraletus* L.), we described granulocytes with baton-shaped granules as well as granulocytes with cytoplasmic granules of great dimensions and intense orange-reddish staining by the MAY-GRÜNWALD GIEMSA method. We named these leucocytes eosinophilic granulocytes to distinguish them from cells with small, fine acidophilic granulations.

ŠVOB M. and T. (1962) described cells in the circulating blood of the shark *Acanthias blainvillii* (RISSO) and catfish (*Scylium canicula* CUV.) which were supposed to represent basophilic granulocytes based upon the presence of extensive lightly stained fields on microscopy following dissolution of basophilic granulations.

In some forms of leucocytes with neutrophilic cytoplasm, we observed cytoplasmic vacuoles containing blue-violet neutrophilic granules.

ŠVOB M. and T. (1962) found monocytes in the circulating blood of a catfish (*Scylium canicula* CUV.) and *Scylium stellare* GTHR monocytes but none in the smears of the shark *Acanthias blainvillii* (RISSO).

CONCLUSIONS

By comparing erythrocyte diameters, surface areas, nuclei and cytoplasms, important differences between the six species of cartilaginous fish were established.

The means (x) of the long and short axes of the elliptical surfaces of the species *Myliobatis aquila* L. varied from 25.04 x 16.40 to 36.2 x 25.1 for the species *Scyliorhinus canicula* L.

Although all the investigated erythrocytes were elliptical, the mean values obtained for the ratio of the length of the long and short axes of

the erythrocyte varied between species from 1.41 for *Squalus acanthias* L. to 1.52 for the species *Raja miraletus* L. and *Myliobatis aquila* L.

The mean of the length of the long and short axes of erythrocyte nuclei varied from 9.2 x 6.88 for *Raja clavata* L. to 14.4 x 10.8 for the species *Scyliorhinus stellaris* L.

The surface areas of erythrocytes average 644.28 microns for *Myliobatis aquila* L. and 1420.85 microns for *Scyliorhinus stellaris* L.

There appeared to be direct proportion only between erythrocyte surface area and cytoplasmic volume for given fish species. The ratios of erythrocyte surface area to nuclear surface area varied from 5.22 for the species *Scyliorhinus canicula* L. to 10.89 for the species *Raja clavata* L.

The peripheral blood smears of the various studied cartilaginous fish revealed large numbers of erythroblasts in different stages of maturity. For example, in the smear from *Scyliorhinus canicula* L., we observed 500 mature erythroblasts, 8 basophilic, 16 polychromatophilic and 19 orthochromatophilic erythroblasts.

In smears of all investigated cartilaginous fish species, thrombocytes were uniformly the most numerous cell types. Thrombocyte diameters measured between 5 and 12 microns with granulated-appearing chromatin in the nucleus and frequent acidophilic granules in the cytoplasm. The thrombocytes were typically round or spindle-shaped.

Four types of leucocytes were revealed in the fish species studied: a. polymorphonuclear leucocyte with acidophilic cytoplasmic granules; b. forms with neutrophilic-stained cytoplasm and bluish-violet cytoplasmic granules; c. lymphocytes; d. monocytes.

Large numbers of polynuclear forms with acidophilic cytoplasmic granules were found in the blood of all investigated fish. In ray species, we describe two forms of these cells, the diameters of which range from 17 to 24.39 microns. The first form was the polymorphonuclear leucocytes with fine acidophilic granulation similar to the acidophilic granulated leucocytes found in other investigated cartilaginous fish. The second form had large granules similar to the eosinophilic granulated leucocytes found in the blood of mammals.

In the cytoplasm of neutrophilic forms of the shark *Squalus acanthias* L., we observed bluish granules. The diameter of these cells measured from 17.1 to 22.8 microns.

In the blood of the fish species, *Raja clavata* L. and *Raja miraletus* L., no neutrophilic leucocytes were observed.

Lymphocytes were common in the blood of cartilaginous fish. The diameters of the lymphocytes varied from 12.5 to 20.8 microns. The nuclei were dense, round, or kidney-shaped structures. The thin rim of surrounding cytoplasm was basophilic. We also noted plasma cells in the early stages of immature lymphoblasts.

Monocyte-like cells were rare in the blood of cartilaginous fish.

In the circulating blood of cartilaginous fish, immature forms of all of the described types of leucocytes and leucoblasts were observed.

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Morfološke značajke krvnih stanica u nekih vrsta hrskavičnih riba

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SAŽETAK

Prikazane su morfološke značajke krvnih stanica u 6 vrsta riba.

Crvena krvna zrnca, eritrociti, u svih istraživanih vrsta su eliptičnog oblika s centralno postavljenom jezgrom. Vrijednosti aritmetičke sredine (x) za dužinu dužeg diametra eritrocita dosežu od 25.04 mikrona za vrstu *Myliobatis aquila* L. do 36.2 mikrona za vrstu *Scyliorhinus canicula* L.

Srednje vrijednosti (x) odnosa između dva dijametra elipse eritrocita variraju s obzirom na riblju vrstu, između 1.52 za vrste *Myliobatis aquila* DUM. i *Raja miraletus* L., do 1.41 za vrstu *Squalus acanthias* L.

Sukladno većem i manjem dijametru eliptičnog oblika eritocita, površina eritrocita, uzevši u obzir dvije plohe eritrocita iznosi od 644.28 μ^2 za vrstu *Myliobatis aquila* DUM. do 1420 μ^2 za vrstu *Scyliorhinus stellaris*. U razmazima periferne krvi ispitivanih vrsta zapažen je znatan broj eritroblasta na raznim stupnjevima zrelosti.

U krvi vrste *Scyliorhinus canicula* L. na 500 zrelih eritrocita zabilježeno je 8 bazofilnih, 16 polikromatofilnih i 19 ortokromatofilnih eritroblasta.

Trombociti su vrlo česte stanice u krvi ispitivanih vrsta. Promjeri trombocita u ispitivanih vrsta iznose od 5-12 mikrona, a oblik im može biti okrugao ili vretenast s bazofilnom citoplazmom te može sadržavati fine acidofilne granulacije.

U krvi ispitivanih vrsta zapaženi su slijedeći oblici leukocita:

Polimorfnonuklearni oblik granulocita s acidofilnim granulacijama u citoplazmi nazočan je u krvi hrskavičnih riba u velikom postotku. U krvi Rajidae su opisana dva oblika tih stanica i to s obzirom na oblik i veličinu granulocita. Jedan je oblik s velikim zrnatim granulacijama do 50%, a drugi sa sitnim granulacijama, oko 22 %. Prve su označene kao eozinofilne granulacije s obzirom na sličnost s eozinofilnim stanicama viših kralježnjaka.

Neutrofilni granulociti su zabilježeni u krvi vrsta *Squalus acanthias* L. Citoplazma u nezrelim granulocitima ispunjena je ljubičastim granulacijama, koje sazrijevanjem tih stanica blijede.

Monociti su rijetke stanice u krvi ispitanih hrskavičnih riba. Citoplazma je svijetloplava. Od metamielocita se razlikuju po tome što metamielociti imaju ružičasto obojenu citoplazmu.

Limfociti su česte stanice u perifernoj krvi. Nukleus ima gustu kromatinsku strukturu, a uska citoplazma je jako bazofilna.

U perifernoj krvi istraženih hrskavičnjaka zabilježene su još i plazma stanice te razni mladi stadiji leukocita.