

CELL TYPES, DIFFERENTIAL CELL COUNTS, AND BLOOD CELL MEASUREMENTS OF A PORTUGUESE SHARK, *CENTROSCYMNUS COELOLEPIS*, CAPTURED AT 700 FATHOMS

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ABSTRACT

A Portuguese shark, *Centroscymnus coelolepis*, 620 mm (24.4 inches) in total length captured in a lobster trap 700 fm deep at Hudson Canyon in February 1971 had a mean erythrocyte size of $33.4 \times 23.6 \mu$, which is larger than any previously recorded for the class Chondrichthyes. Both neutrophils and heterophils were found in the blood, whereas earlier reports describe only one or the other from the same elasmobranch.

Most sharks are shallowwater forms, with relatively few representatives from the deeper parts of the ocean. Bigelow and Schroeder (1948:494-499) reported that the Portuguese shark is strictly a deepwater shark, found chiefly in depths greater than 200 fm; it has been recorded down to 1,487 fm. The smallest recorded specimen is about 9 inches long, and the largest, about 44 inches. Off the North American coast it occurs mostly in temperatures of 5° to 6°C; off Portugal, between 4° and 11°C; and in the Mediterranean, at 12° to 13°C.

Few detailed studies have been made of either the blood or the blood-forming organs in elasmobranchs, probably because of difficulty in obtaining the necessary material (Saunders, 1966a). In this report I describe the cell types, differential cell counts, and measurements of both the erythrocytes and leukocytes in the blood of a Portuguese shark, *Centroscymnus coelolepis*. I report on this specimen because of its unusually large cell sizes in all cell categories. Cell measurements revealed erythrocytes larger than those in all but a few of the vertebrates, and the cells

appear to be the largest reported for the class Chondrichthyes.

A search of the literature reveals no previous blood work has been reported on this species.

MATERIALS AND METHODS

The Portuguese shark examined for this report was a female, 620 mm (24.4 inches) in total length, and was captured in a lobster trap 700 fm deep at Hudson Canyon on 21 February 1971.

Smears were made with blood obtained by direct heart puncture of the live shark. A small incision was made over the heart area through which a heparinized capillary tube 75 × 1.1-1.2 mm was inserted into the heart and allowed to fill three-quarters full. A small drop of blood from the tube was placed on a microscope slide, the tube sealed with plastic clay and the smear made. Three capillary tubes were used to make four blood smears and the microhematocrit determination. The sealed tubes were centrifuged for 3 min at 11,000 rpm. Both a microcapillary reader and a millimeter rule were used in measuring the packed red cell volume, buffy coat, and plasma volumes.

The slides were air-dried and stained with Wright's and with Wright's and Giemsa's stains, and the staining times were varied.

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The best stain was obtained with 1½ min in Wright's followed by 20 min in Giemsa's stain; that slide was used in this study.

Cells were examined under oil immersion at 800 and 1200 power. Cells were measured at 800 power. Cell classification followed that of Saunders (1966a) for elasmobranch blood cells.

RESULTS AND DISCUSSION

This deepwater Portuguese shark had larger blood cell sizes, a lower hematocrit, and a different white cell differential count than those reported for other sharks.

Cell Differentials

Six classifications of cells were present: mature erythrocytes, immature erythrocytes, lymphocytes, heterophils, neutrophils, and thrombocytes. I found no monocytes, eosinophils, or basophils in the material I studied.

Mitotic division occurs in the circulating blood, for mitosis, in all stages, was present in a few cells in the blood smear (Figures 1, 2).

An erythrocyte differential count of a random sample of 100 red cells revealed that mature erythrocytes constituted 92% of the total, immatures 7%, and erythroblasts 1%.

The percentage of mature red cells agrees closely with Saunders' (1966a, b) findings for other elasmobranchs, but the white cell differential of this shark differed substantially from those of other elasmobranchs reported by Saunders. This shark had 73% lymphocytes, 22% thrombocytes, and 5% heterophils; whereas in 13 species of elasmobranchs Saunders found a maximum of 32% lymphocytes and 20-85% thrombocytes, and in 9 species, 3-54% heterophils. The large difference in lymphocyte frequency may be an individual characteristic of this specimen, for Saunders found a substantial difference between the white cell differentials of two adult specimens of sandbar shark, *Carcharhinus milberti*. Saunders also reported that either heterophils or neutrophils, but not both, are found in any given species of elasmobranch; however, I found a few neutrophils in addition to heterophils on close examination

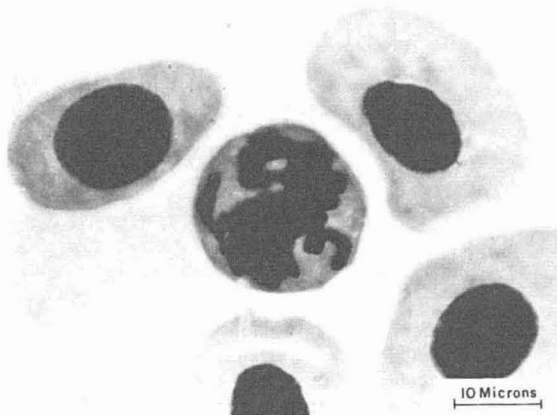


FIGURE 1.—Mitosis-Prophase.

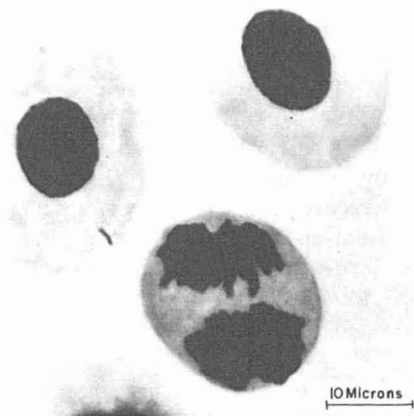


FIGURE 2.—Mitosis-Anaphase.

of the blood in this shark. Several were located at the end of the smear where a differential would not normally be made because of mechanical distortion of the cells; others were discovered in the area where a differential might be made, though the neutrophils could be easily overlooked because of their scarcity.

Table 1 lists the measurements of all cells described in this report.

Mature Erythrocytes

The mean size of the Portuguese shark mature erythrocytes (Figure 3) is the largest

TABLE 1.—Blood cell measurements in microns of a Portuguese shark, the mean \pm standard deviation and range in parentheses.

Cell type	Number of cells measured	Cytosome		Nucleus	
		Length	Width	Length	Width
Mature erythrocyte	200	33.4 \pm 2.5 (26.5 - 40.6)	23.6 \pm 1.8 (18.7 - 28.1)	13.4 \pm 0.7 (12.2 - 15.4)	10.2 \pm 0.6 (9.1 - 11.7)
Immature erythrocyte	10	25.8 \pm 2.2 (21.8 - 27.9)	16.4 \pm 2.2 (14.0 - 20.1)	15.0 \pm 0.7 (14.0 - 16.1)	12.8 \pm 1.0 (10.9 - 14.0)
Erythroblast	1	28.1	17.5	18.7	15.9
Heterophil	10	23.8 \pm 1.5 (22.3 - 25.0)	22.6 \pm 1.3 (21.8 - 25.0)	10.8 \pm 1.2 (9.4 - 12.5)	15.2 \pm 1.5 (12.8 - 17.2)
Lymphocyte	10	17.8 \pm 2.2 (15.6 - 20.3)	16.8 \pm 1.2 (14.0 - 18.7)	16.2 \pm 2.0 (14.0 - 19.5)	14.7 \pm 1.1 (12.5 - 16.4)
Thrombocyte	20	23.4 \pm 2.7 (18.7 - 28.0)	13.2 \pm 1.6 (10.9 - 17.2)	16.6 \pm 1.1 (14.5 - 18.7)	9.4 \pm 1.0 (7.8 - 11.1)
Neutrophil	5	23.6 \pm 1.2 (21.8 - 25.0)	23.2 \pm 0.9 (21.8 - 24.2)	11.2 \pm 1.3 (9.4 - 12.5)	18.9 \pm 2.7 (15.6 - 21.7)

reported for the Chondrichthyes, including the rays, which characteristically possess larger cells than sharks. A comparison of some of the largest recorded erythrocyte dimensions for the Chondrichthyes (Altman and Dittmer, 1961) with the Portuguese shark is listed below.

Species	Erythrocyte dimensions (Dry films)
Portuguese shark, <i>Centroscymnus coelolepis</i>	33.4 \times 23.6 μ
Electric ray, <i>Torpedo nobiliana</i>	29.8 \times 23.1 μ
Little skate, <i>Raja erinacea</i>	24.3 \times 13.9 μ
Spiny dogfish, <i>Squalus acanthias</i>	22.7 \times 15.2 μ
Hammerhead shark, <i>Sphyrna zygaena</i>	15.2 \times 11.2 μ

Immature Erythrocytes

One thousand erythrocytes were counted on the slide and 8.6% of the cells were immature. A few erythroblasts were seen, and measurements of a typical one are listed in Table 1. Immature erythrocytes are easily distinguished from the mature cells by their larger nucleus and smaller cytosome (Figure 3).

Heterophils

Heterophils stained clearly to show purplish nuclear material and eosinophilic rods. The

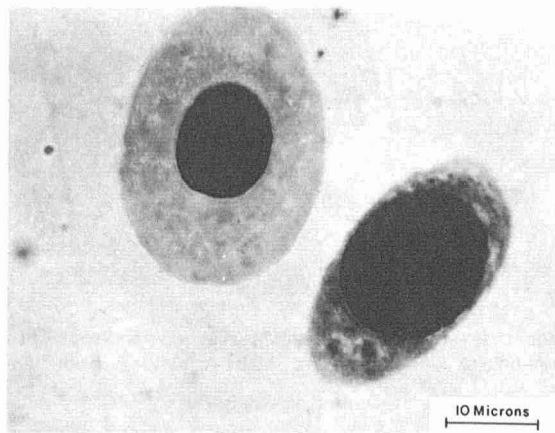


FIGURE 3.—Mature erythrocyte on the left, immature erythrocyte on the right.

heterophils are quite round and similar in size (Figure 4).

Lymphocytes

Lymphocytes stained well with the purplish nucleus taking up most of the cell space. The cytoplasm stains a medium to deep blue and often shows blebs extending from the cell wall (Figure 5).

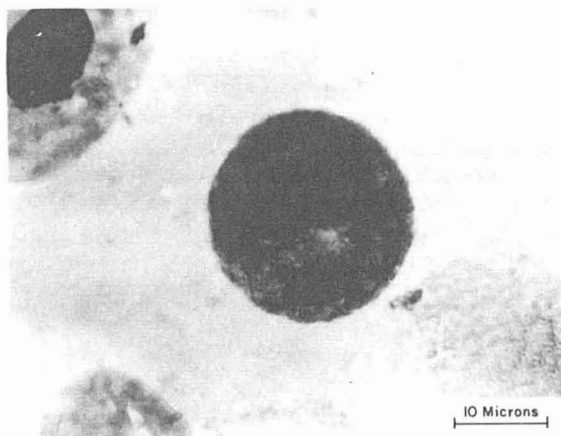


FIGURE 4.—Heterophil showing rod-shaped granules.

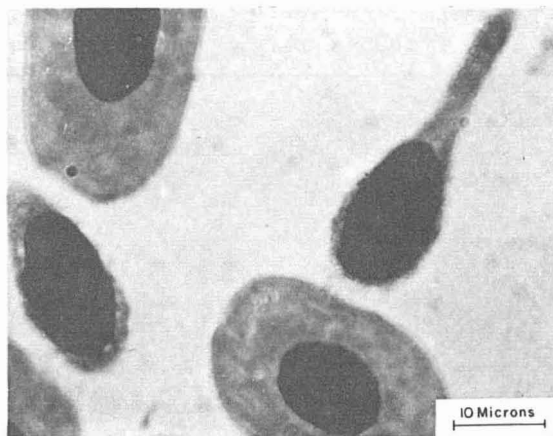


FIGURE 6.—Two thrombocytes, one with a fingerlike cytoplasmic projection.

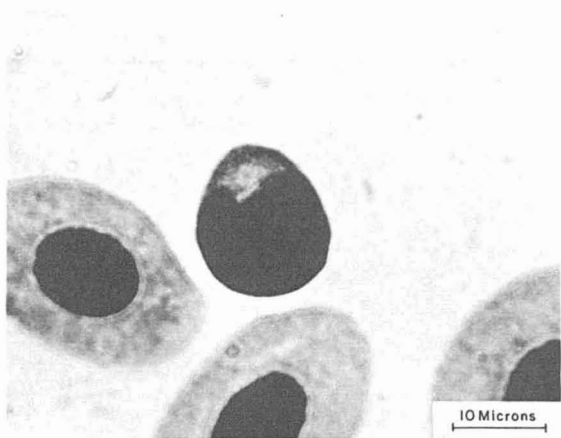


FIGURE 5.—Lymphocyte and three erythrocytes. This lymphocyte does not have blebs extending from the cell wall.

Thrombocytes

Thrombocytes, which are characteristically some of the smallest cells found in the peripheral circulation, were as large in this shark as many mature erythrocytes in other vertebrate species. The stained nucleus is ovoid with pink to red dotted cytoplasm. Since there is quite a range in the length of some of these cells due to fingerlike cytoplasmic projections, I measured 20 ovoid thrombocytes (Figure 6).

Neutrophils

Neutrophils were scarce and were discovered only when the whole slide was studied in detail. They are within the same size range as the heterophils but are distinguished by the absence of eosinophilic rod-shaped granules and the colorless cytoplasm (Figures 7, 8).

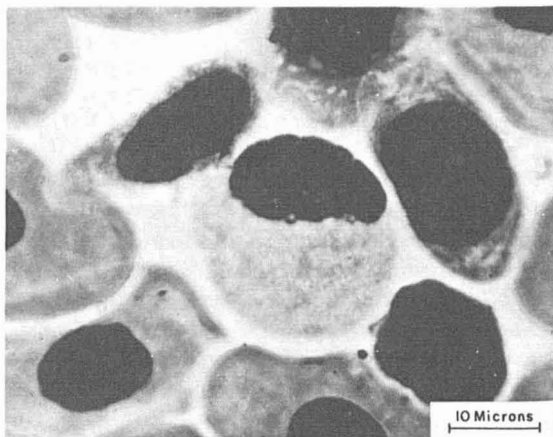


FIGURE 7.—Center: A neutrophil located at the end of the smear where other cells have been mechanically distorted. Clockwise: Immature erythrocyte, lymphocyte, mature erythrocyte, and thrombocyte.

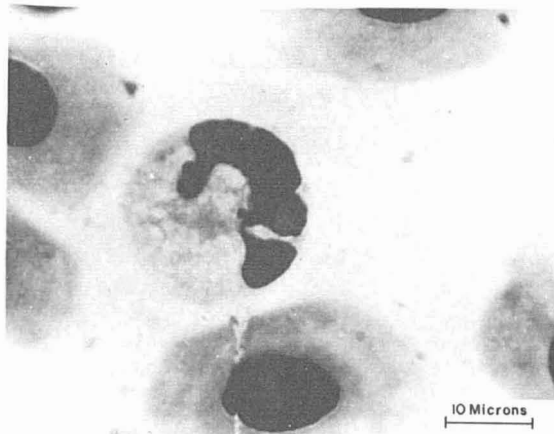


FIGURE 8.—Neutrophil showing a segmented nucleus.

Hematocrit

The packed cell volume of this deepwater shark was less than that reported for other sharks. In a 62 mm whole blood column, 52 mm was clear plasma, 2 mm buffy coat, and 8 mm packed erythrocytes. This was a 13% hematocrit compared with 17-32% from 11 wild dogfish (Burger, 1967) and 23.1% for the hammerhead shark (Altman and Dittmer, 1961).

The blood of the Portuguese shark examined showed no evidence of abnormality. There was no hemolysis in the plasma, and though the absolute cell sizes were larger than previously

reported for other sharks, the staining and morphological characteristics of each cell category were typical of shark cells.

ACKNOWLEDGMENT

I wish to thank Gareth W. Coffin of the Northeast Fisheries Center, Boothbay Harbor Laboratory, National Marine Fisheries Service for the photomicrographic work.

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