Veterinary Clinical Pathology ISSN 0275-6382

BRIEF COMMUNICATION

Ultrastructural characteristics of blood cells in the Yellow-Bellied Slider Turtle (*Trachemys scripta scripta*)

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Key Words
Electron microscopy, erythrocytes, hematology, hemoglobin, leukocytes, reptile

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DOI:10.1111/vcp.12325

Background: The classification of blood cells from the Yellow-Bellied Slider Turtle (*Trachemys scripta scripta*) is relevant due to their increasing importance as pets and as object of study in clinical settings and research projects. However, no previous ultrastructural characterization of blood cells from turtles of the genus *Trachemys* has been reported.
Objectives: The objective of this study was to provide an ultrastructural characterization of blood cells of the Yellow-Bellied Slider Turtle.
Methods: Blood samples from 10 healthy adult turtles (5 males and 5 females) were obtained and processed for transmission electron microscopy using standard methods.
Results: Some erythrocytes had intracytoplasmic inclusions compatible with hemoglobin precipitates; mitochondria and ribosomes in the cytoplasm of erythrocytes were also observed. Five types of white blood cells were ultrastructurally identified: heterophils, eosinophils, basophils, lymphocytes, and monocytes. Heterophils were similar to those described from Sea Turtles, with only one morphologic variation of this cell. Eosinophils were homogeneous in size and had intracytoplasmic granules without crystalline structures. Basophils were ultrastructurally described for the first time for a turtle and had heterogeneous intracytoplasmic granules. Lymphocytes and monocytes were similar to those described from other chelonians. Some thrombocytes had an irregularly lobulated nucleus and intracytoplasmic canalicular structures.
Conclusion: This study provides the first ultrastructural classification of blood cells in *Trachemys scripta scripta*, as a baseline for further hematologic studies in this species.

The classification of blood cells in reptiles is controversial either because variable criteria have been used to categorize cells or because cellular lineages are uncertain.[1] Ultrastructural descriptions of the morphologic characteristics of blood cells of chelonians are scarce, and they include studies on some sea turtle species such as the Green Turtle (*Chelonia mydas*)[1] and the Loggerhead Turtle (*Caretta caretta*)[2], and some fresh water turtles such as the Chinese Pond Turtle (*Geoclemys reevesii*)[3] and the South American Turtle (*Phrynopys hilarii*).[4]

To our knowledge, no previous ultrastructural characterization of blood cells in turtles of the genus *Trachemys* has been reported. The classification of blood cells from the Yellow-Bellied Slider Turtle (*Trachemys scripta scripta*) is relevant because of the increasing importance of these reptiles as pets and as patients presented at veterinary hospitals. In addition, both subspecies *Trachemys scripta scripta* and *Trachemys scripta elegans* are intensely studied in captivity in clinical settings and research projects.[5,6] This article provides an ultrastructural characterization of blood cells of the Yellow-Bellied Slider (*Trachemys scripta scripta*) as a useful reference for future hematologic studies in this species.

Five male and 5 female adult Yellow-Bellied Slider Turtles (mean straight carapace length: 14.8 ± 3.8 cm; mean weight: 0.6 ± 0.4 kg) were used. All the specimens were obtained from private collections from Gran Canaria Island; turtles had been maintained in outdoor facilities under natural conditions and fed mainly com-

mercial turtle diets (Supermenú tortugas; Moly, Murcia, Spain). The animals were treated according to the guidelines specified by the Spanish Ministry of Agriculture, Food and Environment, and under the control of the ethical commission of Veterinary Medicine of the University of Las Palmas de Gran Canaria (agreement MV-2014/06).

A blood volume of 0.1 mL was collected from the dorsal coccygeal vein of each turtle, avoiding the extraction of lymphatic liquid; contaminated samples were discarded. Four micro-tubes with ammonium heparin (Statspin; Iris Sample Processing, Chatsworth, CA, USA) were filled with the blood of each turtle. Samples were centrifuged immediately after collection at 1880 g for 5 min, plasma was discarded, and 2 cell layers were identified. The layer of WBC and thrombocytes (buffy coat) and a small fraction of the RBC layer were removed separately and first fixed in phosphated buffered glutaraldehyde 2% (0.1 M, pH 7.4) for 12 h at 6°C. Samples were gently centrifuged and washed thrice for 5 min in the same solution. Buffered 2% osmium tetroxide was used as the second fixative for 4 h, and subsequently cells were washed thrice in distilled water for 5 min, and then placed in 1% uranyl acetate solution for 4 h. Samples were dehydrated in an ethanol series and passed through 100% ethanol–propylene oxide, propylene oxide, and propylene oxide–Embed812 resin. After polymerization in fresh Embed812 resin at 70°C for 48 h, they were ultrasectioned at 80 nm in an Ultracut S microtome (Leica, Vienna, Austria). The sections were stained with uranyl acetate in 1% methanol and lead citrate solution. The observation and microphotographs were carried out using a JEOL JEM-1400 electron microscope (JEOL Ltd., Tokyo, Japan).

Ultrastructurally erythrocytes appeared oval to fusiform, with a round/oval nucleus with dense heterochromatin. Some erythrocytes had single small round and electron-dense intracytoplasmic inclusions near the nucleus and without recognizable organelles. Some erythrocytes had also mitochondria and ribosomes in the cytoplasm (Figure 1A). Ultrastructurally erythrocytes were similar to those described in Sea Turtles.[1,2] The intracytoplasmic inclusions identified in some erythrocytes were not considered to be bacteria because they lacked a cell membrane or pili, and were too big to be viral particles. These inclusions have also been described in several species of reptiles.[7] Some authors considered them as degenerating organelles.[1,2] A direct relationship between the presence of the inclusions in the erythrocytes of the Eastern Painted Turtle (*Chrysemys picta picta*) and the maturation of the cells has been established.[8] Recent studies described them as hemoglobin precipitates, resembling human Heinz bodies.[9] The presence of mitochondria and ribosomes in some erythrocytes is a rare finding, and it is probably associated with immature cells.[10]

In this study, 5 types of WBC were ultrastructurally identified: heterophils, eosinophils, basophils, lymphocytes, and monocytes. Heterophils were round cells with abundant cytoplasm. The nucleus was oval to fusiform, often eccentric, containing variable but moderate amounts of heterochromatin. Numerous oval to elongated granules were found in the cytoplasm, showing homogeneous and usually moderate electron density. Some smaller and round granules of variable electron density were also seen (Figures 1A and 1B). Mitochondria and smooth endoplasmic reticulum were the most commonly observed organelles, but rough endoplasmic reticulum and Golgi complex were also seen. Beta glycogen granules were scarce. Heterophils were similar to those described in Sea Turtles.[1,2] Although some heterophils showed few granules with higher electron density than regular ones, suggesting different development stages, we did not identify 2 morphologic variations of this cell. However, other studies on the Tuatara (*Sphenodon punctatus*)[11] and on several species of snakes[12,13] described 2 morphologic variations of heterophils.

Eosinophils presented a homogeneous round shape similar to that of heterophils. The eccentric nucleus was oval to fusiform with variable amounts of heterochromatin. Intracytoplasmic granules were round to oval, with higher electron density than that of heterophils and without crystalline structures (Figure 1C). Small granular inclusions were also observed, as well as β glycogen granules. Mitochondria and both smooth and rough endoplasmic reticulum were easily found, but Golgi complex and vacuoles were rare. Ultrastructural characteristics of eosinophils were similar to those described in Loggerhead Turtles.[2] They were also similar to small eosinophils from Green Sea Turtles[1], except for the absence of crystalline structures. Large and small eosinophils are uncommonly reported in reptiles. However, large and small eosinophils have been described in Kemp's Ridley Turtles (*Lepidochelys kempii*)[14] and Green Turtles.[1]

Basophils were round cells, smaller than heterophils and eosinophils, with a big round eccentric nucleus, showing moderate amounts of heterochromatin and low electron density (Figure 1D). The cytoplasm was usually scarce, clumped with round granules which sometimes appeared pleomorphic. Most of them showed high electron density and a well-defined shape, while others appeared to be undergoing a degeneration process. A smaller number of granules

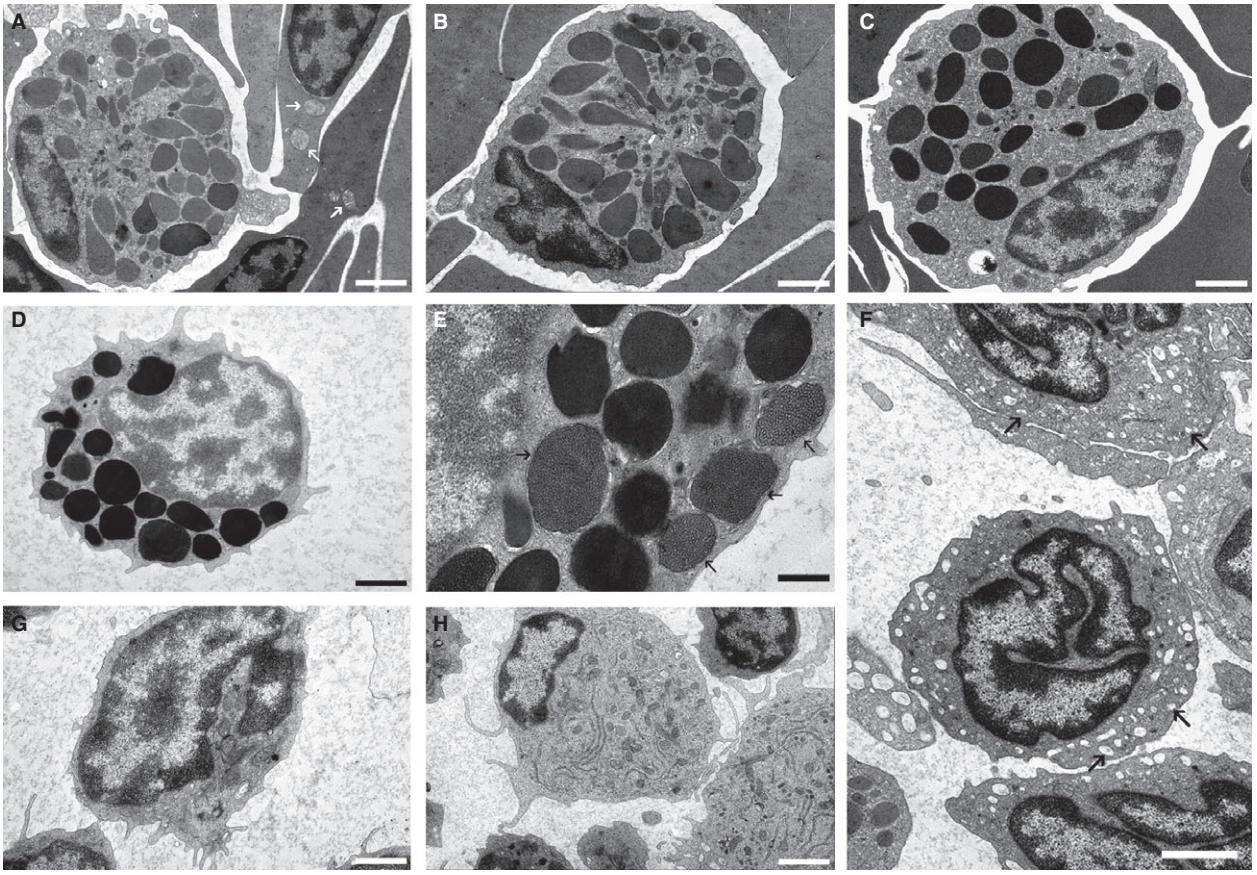

Figure 1. Transmission electron micrographs of blood cells of a Yellow-Bellied Slider Turtle (*Trachemys scripta scripta*). Uranyl acetate and lead citrate stains. (**A**) Two erythrocytes (right) with intracytoplasmic mitochondria (arrows) and a heterophil (left). Bar = 1.3 μm. (**B**) Heterophil. Bar = 1.3 μm. (**C**) Eosinophil. Bar = 1.3 μm. (**D**) Basophil. Bar = 1.3 μm. (**E**) Intracytoplasmic granules in a basophil. Note the presence of granules rich in microtubules (arrows). Bar = 0.5 μm. (**F**) Lymphocyte. Bar = 1 μm. (**G**) Two monocytes (central image and lower right corner), and a thrombocyte (upper right corner). Bar = 1.6 μm. (**H**) Thrombocytes. Note the irregularly lobulated nucleus and the intracytoplasmic canalicular structures (arrows). Bar = 1.8 μm.

exhibited low electron density with an inner structure rich in microtubules (Figure 1E). Mitochondria, both smooth and rough endoplasmic reticulum and Golgi complex were usually found, and β glycogen granules were abundant. Ultrastructural characteristics of basophils could not be compared with those of other turtles because there are no reports on the ultrastructural description for basophils in these species.[1,2] However, the cell morphology and the characteristics of the intracytoplasmic granules were similar to those described for basophils from the Tuatara (*Sphenodon punctatus*), with some granules appearing altered and clumped with microtubules.[11]

Lymphocytes had a round but often indented or segmented nucleus, with abundant amounts of heterochromatin. The lymphocyte usually appeared as a round cell, but frequently exhibited irregular margins and cytoplasmic projections (Figure 1F). Mitochondria, polyribosomes, and also rough endoplasmic reticulum were abundant. Smooth endoplasmic reticulum, Golgi complex, and some small electron-dense granules were also observed. Ultrastructural characteristics of lymphocytes were similar to those described for lymphocytes from Sea Turtles[1,2] and several snakes.[12,13]

Monocytes were generally big, round to fusiform or amoeboid cells with a proportionally small nucleus, which presented a fusiform shape, was usually indented, and contained less heterochromatin than lymphocytes (Figure 1G). The most frequent organelles were mitochondria, both rough and smooth endoplasmic reticulum and some small vacuoles. Small pleomorphic heterogeneously electron-dense granules were also observed. Ultrastructural characteristics of monocytes were similar to those described for monocytes from Green Turtles[1] and Loggerhead Turtles.[2]

Thrombocytes were generally small round to oval cells with scant cytoplasm. occasional finger-like projections, and thrombocyte aggregates were common. The nucleus was round, although sometimes irregularly lobulated, and heterochromatin was peripheral with higher electron density than in lymphocyte nuclei. Polyribosomes and smooth and rough endoplasmic reticulum and mitochondria were also seen. Golgi complex, when present, was adjacent to the centrosome. Some thrombocytes had canalicular structures resembling small vacuoles, and small heterogeneous granules (Figure 1H). Ultrastructural characteristics of thrombocytes were similar to those described for thrombocytes from Loggerhead Turtles.[2] However, the irregularly lobulated nucleus observed in some thrombocytes was also described in thrombocytes from Green Turtles[1] and South American Fresh Water Turtles.[4] These differences between thrombocytes could be explained by the presence of reactive or activated cells.[4] Open canalicular systems and cytoplasmic projections are characteristic of this cell type in many reptiles[1–4], and have also been described in mammalian platelets.[15]

In conclusion, this study provides the first ultrastructural classification of blood cells for the Yellow-Bellied Slider Turtle (*Trachemys scripta scripta*). The data serve as a baseline for future hematologic studies in this species.

Acknowledgments

The authors thank Patricia Ramírez (Serviexotic) for her unconditional support, and the staff of the Electronic Microscopy Service of the ULPGC for technical assistance. They are also grateful to the turtle owners and all colleagues of the Veterinary Hospital Benartemi (Vencindario, Las Palmas) and the Veterinary Clinic Atlántico (Las Palmas de Gran Canaria).

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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