Morphologic, cytochemical staining, and ultrastructural characteristics of blood cells from eastern diamondback rattlesnakes (*Crotalus adamanteus*)

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Objective—To evaluate light microscopic, cytochemical, and ultrastructural characteristics of blood cells from eastern diamondback rattlesnakes.

Animals—10 healthy snakes.

Procedure—Various stains, including Wright-Giemsa, benzidine peroxidase, Sudan black B, chloroacetate esterase, α-naphthyl butyrate esterase, acid phosphatase, leukocyte alkaline phosphatase, periodic acid-Schiff with diastase, and toluidine blue, were used to stain leukocytes differentially on multiple blood smears. Electron microscopy also was performed.

Results—Lymphocytes were the most commonly observed leukocyte and could be distinguished from thrombocytes, using periodic acid-Schiff stain with diastase. Azurophils also were commonly observed; their granules stained with peroxidase. Eosinophils were not identified; however, 2 morphologic variations of heterophils were seen in the blood of all snakes and were considered the same cell type at different stages of cytoplasmic granule development. Heterophil granules were better preserved, using a one-step Wright-Giemsa method that did not require alcohol fixation prior to staining. Degranulated heterophils were observed in all preparations.

Conclusions—Most leukocytes of eastern diamondback rattlesnakes can be identified easily on Wright-Giemsa-stained preparations. However, hematologic stains that do not require alcohol fixing prior to staining may be preferred for leukocyte evaluation in certain reptiles. A limited degree of heterophil maturation may continue in the blood of healthy snakes. This, along with degranulation of heterophils, may result in a variable staining pattern in this cell type, regardless of the stain used.

Clinical Relevance—Results provide baseline data for use in hematologic testing in diagnosis of disease and monitoring of treatment of sick or injured snakes. (*Am J Vet Res* 1999;60:507–514)

Circulating blood cells of reptiles may be grouped into RBC, WBC, and thrombocytes (counterpart of mammalian platelets).[1] Erythrocytes are morphologically similar among various species of reptiles. Thrombocytes and lymphocytes are also similar among various species, even though in most reptiles, distinction between the 2 cell types is difficult. However, appreciable morphologic differences exist between granulocytes and monocytes, making it important to characterize the cell types for various species of interest. In addition, the relative numbers of various leukocytes (lymphocytes, heterophils, eosinophils. basophils, monocytes, and azurophils) in the blood varies considerably among different reptile species. For example, azurophils are a commonly observed leukocyte in Squamata (snakes and lizards) and Crocodilia (alligators, crocodiles, caymans, and gavials), but are seen only occasionally in Chelonia (turtles, tortoises, and terrapins). Eosinophils are observed commonly in Crocodilia and Chelonia, but existence of this leukocyte in the blood of different Squamata, particularly snakes, is controversial.[2-4]

The role of various leukocytes in the inflammatory process and immune response of snakes also is understood poorly. To the authors' knowledge, criteria that may be useful in recognizing typical leukocyte populations in blood and diseased tissues have not been firmly established. Recently, pulmonary lesions of rattlesnakes experimentally infected with a paramyxovirus were evaluated.[5] The inflammatory response was characterized; however, 1 cell type, believed to be a type of granulocyte, remained unidentified. As a result of this finding, the study reported here was designed to characterize the light microscopic, cytochemical, and ultrastructural features of blood cells from healthy eastern diamondback rattlesnakes. This information is also essential to recognize changes in the blood of diseased snakes, identify inflammatory cell types in damaged tissues, and understand the potential role of various blood cells in resisting infection.

Material and Methods

Samples—Blood samples were collected from 10 healthy adult captive rattlesnakes located in the St. Augustine Alligator Farm, St. Augustine, Fla. Each snake was manually restrained, and a 22-gauge needle was inserted into the tail vein. Blood was drawn into a syringe and placed in lithium heparin-containing microcollection tubes. Blood smears for Wright-Giemsa and cytochemical staining were prepared immediately and air dried.

Light microscopic examination—Blood smears were fixed in methanol and stained with Wright-Giemsa for determination of differential leukocyte counts and morphologic evaluation of erythrocytes, thrombocytes, and leukocytes. A minimum of 400 leukocytes were counted

Received for publication May 8, 1998.
Accepted Aug 11, 1998.
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The authors thank Dr. R. Avery Bennett and the St Augustine Alligator Farm for providing blood samples from the rattlesnakes, and Karen Vaughn and the Electron Microscopy Core for technical assistance.