Morphologic and Cytochemical Characteristics of Blood Cells from the Desert Tortoise (*Gopherus agassizii*)


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Originally published in the American Journal of Veterinary Research.

INTRODUCTION

The morphologic and cytochemical staining characteristics of erythrocytes, leukocytes, and thrombocytes of the desert tortoise (*Gopherus agassizii*) were evaluated using blood smears prepared from 23 healthy tortoises from Kern County, California. Special emphasis was placed on differentiating features of the various leukocytes and thrombocytes using a variety of cytochemical stains.

Circulating blood cells of reptiles can be grouped into RBC, WBC, and thrombocytes. Red blood cells, are morphologically similar among various species of reptiles. They are nucleated and oval shaped, and represent most of the circulating blood cells. Thrombocytes (counterpart of mammalian platelets), which are next in abundance, are also nucleated, but morphologic variability exists among the various reptiles. In blood smears, thrombocytes may be confused with lymphocytes because they appear morphologically similar.

Lymphocytes, monocytes, and basophils are morphologically similar among various species of reptiles; however, acidophils (heterophils and eosinophils) may vary. In Wright-Giemsa stained smears, depending on the group of reptile, 1 or 2 types of acidophils can be seen. Pienaar referred to them as type-1 and type-2 eosinophils. While Girons also considered these cells as morphologic variants of a single cell line, evidence exists that these acidophils are each derived from separate lines. In most squamates, only 1 type of acidophil, the heterophil, can be recognized. This corresponds to Pienaar's type-1 eosinophil. In chelonians and crocodilians, in addition to the heterophil, an eosinophil corresponding to the mammalian eosinophil may also be seen. This latter cell corresponds to Pienaar's type-2 eosinophil.

In addition to the aforementioned cell types, some reptiles also have a unique leukocyte, the azurophil, which has morphologic features of monocytes and acidophils. A distinguishing feature of azurophils is the metachromatic reaction of their cytoplasm to Romanowsky stains. The azurophil appears to be derived from a monocyte and contains peroxidase-positive granules similar to those of mammalian monocytes.
A wide variety of research projects have been conducted and are ongoing with the desert tortoise, *Gopherus agassizii*, a species native to the Mojave and Sonoran Deserts of western United States and adjacent areas of northern Mexico. Partially, because of a respiratory tract disease and high mortality in populations of desert tortoises in the Mojave Desert, these animals north and west of the Colorado River were classified by the United States Federal Government as threatened or endangered species on April 2, 1990.6

Although blood samples are routinely collected from desert tortoises for determination of CBC, morphologic criteria for distinguishing the various circulating blood cells have not been established to our knowledge. Thus, the objective of the study reported here was to define and describe morphologic features and cytochemical staining characteristics of circulating blood cells of the desert tortoise.

**MATERIALS AND METHODS**

Wright-Giemsa stained smears were prepared from blood samples of 23 clinically healthy, free ranging, desert tortoises located in the Desert Tortoise National Area, Kern County, California. Each tortoise was manually restrained, and a 23 gauge butterfly catheter was inserted into the jugular vein. Blood was drawn into a syringe and placed in lithium heparin containing microtainer tubes. Tubes were then placed on ice and transported to the College of Veterinary Medicine, University of Florida. It was approximately 18 hours from the time the samples were collected until blood smears were prepared.

Blood smears were prepared, using a coverslip technique, and were stained, using Wright-Giemsa, for determination of differential leukocyte counts and morphologic evaluation of erythrocytes, thrombocytes, and leukocytes. Differentials were performed by categorizing leukocytes into one of 5 groups; heterophils, lymphocytes, monocytes (including azurophilic monocytes), eosinophils, and basophils.

Air-dried smears were prepared for cytochemical staining with benzidine peroxidase, Sudan black B, chloroacetate esterase (CAE), α-naphthyl butyrate esterase (NBE), acid phosphatase, leukocyte alkaline phosphatase, periodic acid-Schiff (PAS) with diastase, and toluidine blue.7 Presence and pattern of stain within individual leukocytes and thrombocytes were recorded.

**RESULTS**

To be presented.

**DISCUSSION**

The morphologic features of blood cells from the desert tortoise, *Gopherus agassizii*, were similar to those of other species of tortoises, with minor differences observed in numbers and shape of the granules observed in heterophils and eosinophils. The remaining blood cells resembled those from other species of tortoise.4

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Heparin was used as an anticoagulant because EDTA has been reported to cause lysis of chelonian blood. It is, therefore, recommended that blood smears from these species be prepared from non-anticoagulated blood or heparinized samples. Although heparin is the recommended anticoagulant for avian and reptile species, it should be noted that heparin may impart a blue tinge to blood smears treated with Romanowsky stains and may cause clumping of platelets and leukocytes.

The *Haemogregarina sp* parasite seen in 2 of the tortoises examined has been commonly seen in blood smears of a variety of reptiles. This parasite is considered nonpathogenic and generally does not cause clinical signs of disease.

Special cytochemical stains were used in this study to document the staining characteristics of circulating leukocytes and thrombocytes as an aid in their identification. Special stains may be particularly useful in serving as markers for leukocytes in cytologic preparations. Cytochemical stains may be useful in identifying inflammatory cell types in cytologic samples, as well as differentiating thrombocytes from lymphocytes in specimens from blood and bone marrow.

Reptiles are a heterogenous group of vertebrates with regard to their blood cell morphology. The desert tortoise has similarities and differences in morphology and cytochemical staining characteristics of blood cells, as reported for other reptiles. Because differential blood cell counts are important in determining the health status of animals, normal blood morphology needs to be described for representative species of the 4 major orders of reptiles. The results of this study may be used as a guideline for categorizing the various blood cells of the other 39 species of tortoises. However, this does not preclude existence of minor differences in blood cell morphology among the various species.

Additional results to be discussed.

**Acknowledgments:** The authors thank Dr. J. Jenkins, Armed Forces Institute of Pathology, Washington DC for technical assistance.
Table 1

Mean ± SD Percentage of the Leukocytes Seen in 23 Healthy Desert Tortoises

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>Mean %</th>
<th>SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterophils</td>
<td>33.0</td>
<td>15</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>23.0</td>
<td>7</td>
</tr>
<tr>
<td>Monocytes</td>
<td>11.0</td>
<td>7</td>
</tr>
<tr>
<td>Azurophilic monocytes</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Basophils</td>
<td>30.0</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2

Cytochemical Staining Features of Blood Leukocytes From the Desert Tortoise

<table>
<thead>
<tr>
<th>Stain</th>
<th>Heterophil</th>
<th>Lymphocyte</th>
<th>Monocyte</th>
<th>Eosinophil</th>
<th>Basophil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzidine peroxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroacetate esterase</td>
<td>Foc +</td>
<td>-</td>
<td>Stip ±</td>
<td>Stip +</td>
<td>-</td>
</tr>
<tr>
<td>α-Naphthyl butyrate esterase</td>
<td>Foc +</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Foc +</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Stip ±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Periodic acid-Schiff (diastase)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+)= diffuse positive staining; (Foc +)= large focal area of positive staining; (Stip +)= stippled positive staining in the cytoplasm of all cells; (Stip ±)= stippled positive staining in some cells.

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LITERATURE CITED/REFERENCES


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