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Transactions of the American Microscopical Society, Vol. 100, No. 2. (Apr., 1981), pp. 210-215.

Stable URL:
http://links.jstor.org/sici?sici=0003-0023%28198104%29100%3A2%3C210%3APBCIAM%3E2.0.CO%3B2-R

Transactions of the American Microscopical Society is currently published by American Microscopical Society.

Peripheral Blood Components in *Alligator mississippiensis*[1]

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Abstract. Cells of the peripheral blood of *Alligator mississippiensis* were examined and classified by light microscopy. Erythrocyte (RBC) numbers, hematocrits, hemoglobin, other RBC indices, white blood cell (WBC) percentages and thrombocyte numbers were determined. These hematologic values are reported for 45 wild healthy animals.

In the course of routine studies conducted at the Savannah River Ecology Laboratory (SREL) during the spring and summer of 1975, nine American alligators (*Alligator mississippiensis* (Daudin, 1803)) died suddenly, and without apparent cause. At necropsy, *Aeromonas hydrophila* (Kluyver & van Niel, 1936) was isolated in each case from internal organs such as the liver, lungs, and kidneys. At the SREL, studies on large-mouth bass located in the same fresh-water environment have since shown a direct relationship between thermal stress and incidence of red-sore disease caused by *A. hydrophila*. The effect of thermal stress on large-mouth bass has been measured by changes in various hematological parameters (Hazen et al., 1978; Esch & Hazen, 1978).

A study was undertaken to determine the susceptibility of alligators to infection with *A. hydrophila* and the role of thermal stress in the disease process. As a part of the study, a method for objective measurement of stress response by alligators was designed to determine whether changes in blood elements might be correlated with infection, disease, and recovery.

Previously published work described general biochemical data on serum (Coulson & Hernandez, 1974), but lacked a description of formed elements of the peripheral blood and serum protein electrophoretic components of alligators. Reese (1917) evaluated and partially characterized peripheral blood components of alligators by light microscopy but did not quantitate the WBC elements. Pienaar (1962) and others have described in detail the blood of *Crocodilus niloticus*, but no description of the peripheral blood leukocytes in *A. mississippiensis* was given. However, Glassman & Bennett (1978) dis-

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[1] Dr. Robert Gorden, Director, Aquatic Ecology Division, Illinois Natural History Survey, Urbana, Illinois, served as project coordinator and principal investigator. This study was supported by Contract EY-76-S-09-0965 between the Energy Research and Development Administration and Wake Forest University. We are grateful to Dr. Carl Fliermans and the Savannah River Laboratory for providing space, equipment, and assistance. Dr. Gerald Esch, Department of Biology, Wake Forest University, made major contributions to overall experimental design.

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TRANS. AM. MICROSC. SOC., 100(2): 210–215. 1981.

cussed the effects of thermal stress in detail and offered a general description of peripheral blood. The purpose of this study is to identify, quantitate, and describe, by light microscopy and cytochemical techniques, the cells of the peripheral blood of wild alligators.

Materials and Methods

Normal values for hemoglobin, hematocrit, other RBC indices, total white blood cells (WBC), and peripheral blood differential cell counts, were determined from wild alligators (n = 45) caught in their natural habitat off the coasts of South Carolina and Louisiana. Animals were healthy and non-infected; i.e., they had no visible skin lesions, were actively feeding, and gained weight during the study.

Blood was collected in 1.5-ml EDTA tubes from the dorsal vein of the tail. Peripheral blood cells were studied in thin films stained with Wright-Giemsa in order to perform differential counts. Cells were divided into series of erythrocytic, thrombocytic, myelocytic, and lymphocytic cells. In each specimen, 100 cells of both the myelocytic series (including granulocytes, thrombocytes, and monocytes) and lymphocytic series were counted to determine the percentages of each individual cell type in the peripheral circulating blood. Microhematocrit values were determined for each of the anticoagulated specimens. RBC's and WBC's were counted on an automated electronic counting device.

Additional information determined for the red cells included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hemoglobin concentration was determined by a modified cyanmethemoglobin method using an automated hemoglobinometer. Thrombocyte counts were determined using a modified reticulocyte counting method (Dacie & Lewis, 1968).

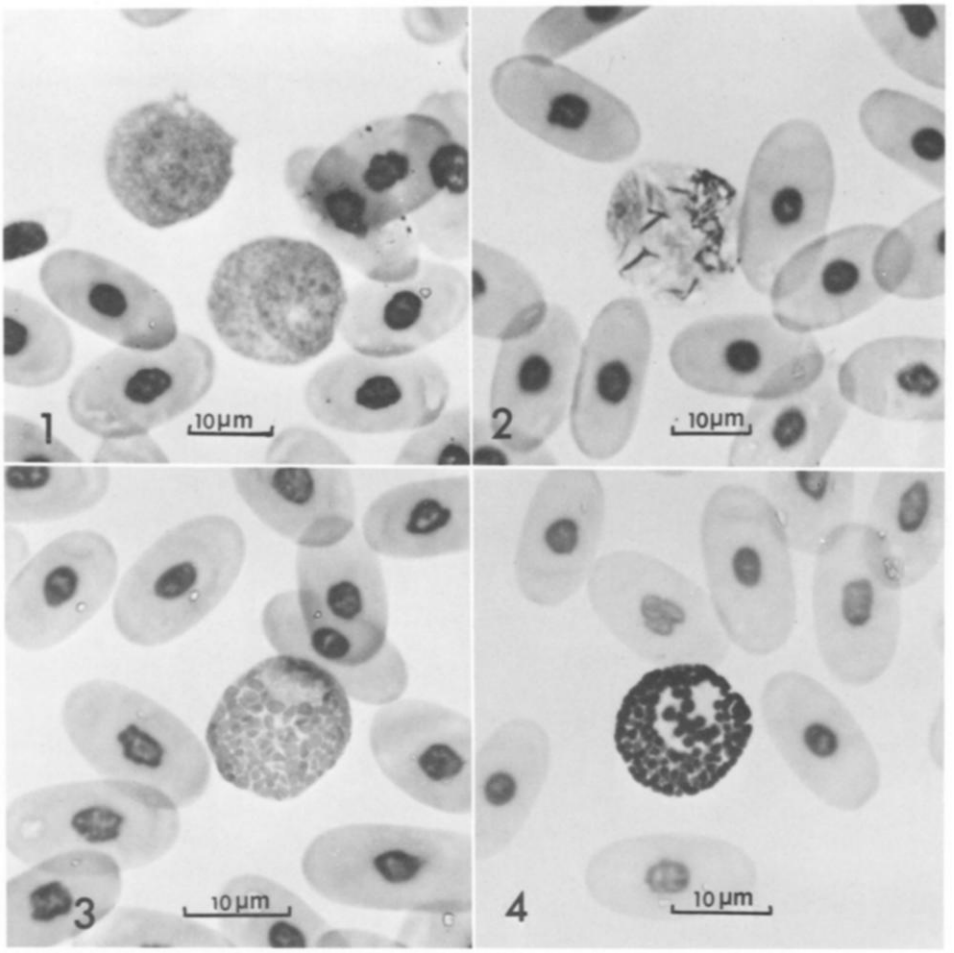
Cytochemical stains including periodic-acid-Schiff (PAS) as described by Lillie (1965), peroxidase by the Kaplow method (1965), and leukocyte alkaline phosphatase (LAP) as reported by Kaplow (1968) were used to aid in the characterization of certain WBC's by determining the products of specific cytochemical reactions.

Results and Discussion

Erythrocytes (Figs. 1–5) measured $17.9 \pm 2.0 \times 9.7 \pm 1.5$ μm ($n = 100$). The cytoplasm was homogeneously dark pink in color. The nucleus was placed centrally within the cell, had prominent chromatin condensation, and measured 2.8 ± 0.6 μm ($n = 21$).

The lymphocyte series (Fig. 1) was composed of cells measuring 12.8 ± 1.3 μm in diameter with a rounded ovoid nucleus which measured $10.1 \pm 2.4 \times 7.7 \pm 1.8$ μm ($n = 45$). The nucleoplasm showed evidence of chromatin condensation and a "reticulated" pattern. The cytoplasm contained occasional granules and stained blue in Wright-Giemsa preparations.

Neutrophils or heterophils (Fig. 2) measured 16.5 ± 2.9 μm ($n = 51$) and contained varying numbers of blue-stained organelles. The nucleus was non-



FIGS. 1–4. Wright-Giemsa-stained cells from the peripheral blood of *Alligator mississippiensis*. Fig. 1. A lymphocyte with dark blue-purple cytoplasm and a slightly ovoid nucleus. The red blood cells have a homogeneous light red or dark pink cytoplasm and a dark condensed centrally-placed nucleus. Fig. 2. Heterophils have varying degrees of cytoplasmic granulation with an eccentric purple nucleus. Fig. 3. An eosinophil with dark orange or light red pleomorphic granules within the cytoplasm. A dark-stained eccentric nucleus is discernible. Fig. 4. A basophil with orange-purple granules filling the cytoplasm and obscuring portions of the nucleus.

segmented and measured $7.3 \pm 2.4 \times 5.7 \pm 2.1$ μm ($n = 35$). Organelles seemed to overlap when present in large numbers. Some of these cells contained cytoplasmic inclusions which were similar to Döhle bodies (Hyun et al., 1975). These cells are considered to be phagocytic because they increased in numbers during bacterial infection and decreased after successful host response or adequate antibiotic therapy (Glassman & Bennett, 1978).

Eosinophils (Fig. 3) are ovoid cells measuring 12.5 ± 2.7 μm ($n = 37$) in

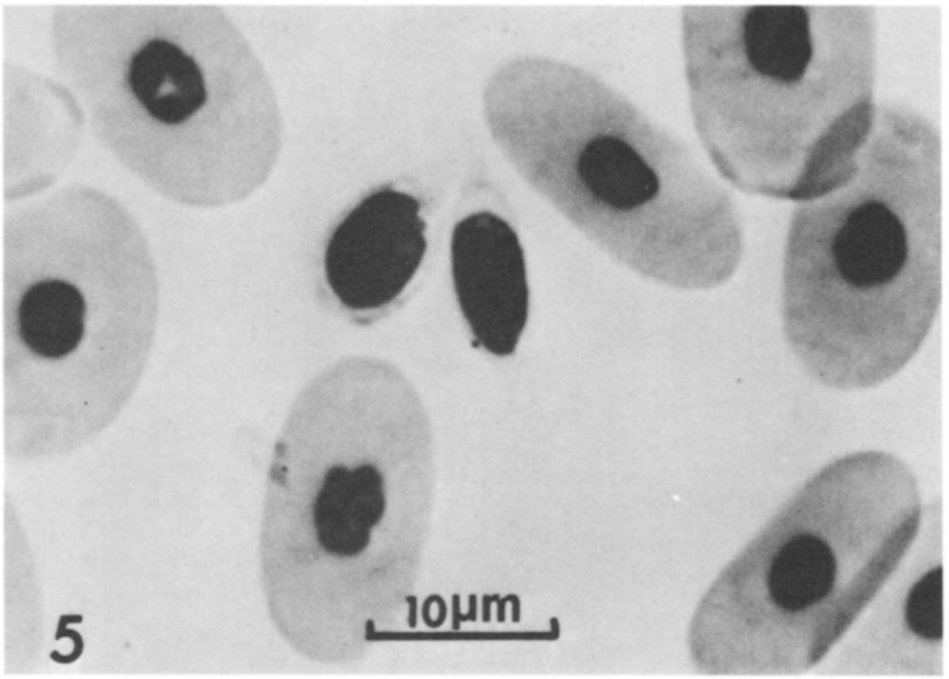


FIG. 5. Nucleated, somewhat spindle-shaped thrombocytes.

diameter with a nucleus which measured 7.7 ± 0.6 × 3.4 ± 1.0 μm (n = 20). The cytoplasm was almost filled completely with angular inclusions which stained yellow-orange in Wright-Giemsa preparations.

Basophils (Fig. 4) measured 11.9 ± 2.5 μm (n = 41) in diameter with a small, rounded nucleus 5.4 ± 1.1 μm (n = 12) and had dark purple, spherical intracytoplasmic granules often obscuring portions of the nucleus.

Thrombocytes (Fig. 5) measured 9.4 ± 1.3 μm (n = 79) in diameter, contained a rounded nucleus 6.7 ± 1.1 × 4.9 ± 1.2 μm (n = 30) and had little cytoplasm. Granules rarely could be seen within the cytoplasm of some cells.

Heterophils differ from neutrophils (PMN's) of higher vertebrates in that they have a single non-segmented nucleus. The increase of heterophils seen in alligators in response to infection with *A. hydrophila* is similar to the granulocyte response seen in higher vertebrates exposed to pyogenic organisms. A decrease in leukocytes, particularly heterophils, follows spontaneous or therapeutic resolution of lesions caused by *A. hydrophila*. The role played by these cells during inflammation and host defense is believed to phagocytic. Cytochemical stains revealed that heterophils were positive for PAS, LAP, and peroxidase. This suggests that these cells are of the myelocytic series and probably analogous to neutrophils seen in mammals.

Hematologic values for 45 wild uninfected alligators were compared with those of 25 *A. hydrophila*-infected animals (Table I). An increase in the total

TABLE I

Hematologic data for uninfected, free-living alligators as compared to infected animals

Parameter	Uninfected wild (n = 45)	Infected (n = 25)
RBC ($\times 10^5$/mm^3)	4.0 ± 0.1	4.0 ± 0.08
WBC ($\times 10^3$/mm^3)	5.3 ± 1.9	8.1 ± 3.4
Hemoglobin (g/dl)	7.2 ± 0.5	7.9 ± 0.3
Hematocrit (%)	18.6 ± 1.6	17.8 ± 1.1
MCV (μm^3)	516.0 ± 83.0	506.0 ± 94.3
MCH (pg)	185.0 ± 41.6	160.0 ± 37.9
MCHC (g/dl)	36.1 ± 2.8	36.3 ± 4.9
Thrombocyte count ($\times 10^4$/mm^3)	2.4 ± 1.0	1.9 ± 0.5
WBC (%)		
Neutrophilic macrophages or "heterophils"	37.4 ± 10.6	72.9 ± 13.8
Eosinophils	5.5 ± 3.0	6.1 ± 3.3
Basophils	3.5 ± 2.7	2.0 ± 1.7
Monocytes	3.0 ± 6.6	3.0 ± 2.4
Lymphocytes	50.6 ± 16.4	16.0 ± 10.5

WBC's was noted in response to infection (Table I). Heterophil counts increased from a normal range of 37.4% ± 10.6 to 72.9% ± 13.8 in alligators exposed to *A. hydrophila*. The percentage of heterophils and WBC's returned to a normal level 1–2 weeks after the host responded adequately to the infection, or the lesions were treated with antibiotics (Glassman & Bennett, 1978). A change in lymphocyte percentages was noted. This change may reflect the effect of combined stresses of captivity, temperature, and bacterial infection (Bennett & Reap, 1978).

Host responses to *A. hydrophila* infection suggests analogous function in alligators of erythrocytic, myelocytic, thrombocytic, and lymphocytic series to those of mammals. This model has been used successfully to monitor experimental infection and recovery under varied thermal stresses.

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Dallas Meetings

The annual meeting of the American Microscopical Society will be held in conjunction with meetings of the American Society of Zoologists, Animal Behavior Society, Crustacean Society, Society of Protozoologists, and Society of Systematic Zoologists at the Hyatt Regency Hotel, Dallas, Texas, 27–30 December 1981. The host institution is Southern Methodist University with John L. McCarthy and John E. Ubelaker serving as co-chairpersons of the Local Arrangements Committee. All abstracts will be due in August (AMS abstracts due 28 August). A symposium entitled "Meiofauna ecology: present concepts and future directions" (organized by Susan S. Bell) will be co-sponsored by the American Microscopical Society and the Divisions of Ecology and Invertebrate Zoology of the American Society of Zoologists. Other symposia include: (1) The teaching of protozoology; (2) Phylogeny within the Crustacea; (3) Developmental biology of the ascidians; (4) Chromatophores and color changes; (5) Research developments in arthropod water relations; (6) Epithelial-mesenchymal interactions; (7) Interface of quantitative genetics, life-history evolution and whole-organism ontogeny; (8) Optimization of behavior; (9) Comparative aspects of inflammation; and (10) Adaptive radiation within a highly-specialized system: the diversity of feeding mechanisms of snakes. Additional titles will be announced. The AMS Past-Presidential Address by John Cairns, Jr., speaking on "Microbial colonization processes," is scheduled for the morning of 29 December and is to be followed by a luncheon in his honor. Other planned features of the meetings include socials, special programs, commercial exhibits, and job placement service. For further information, contact Mary Wiley, ASZ Business Manager, Box 2739, California Lutheran College, Thousand Oaks, California 91360. For AMS abstract forms and brochure, send a legal-size self-addressed envelope to Dr. Dee S. Dundee, AMS Secretary, Department of Biological Sciences, University of New Orleans, New Orleans, Louisiana 70122.