HISTOLOGY OF THE CIRCULATING BLOOD OF <u>GERRHONOTUS</u> <u>COERULEUS</u> (BAIRD AND GIRARD), THE NORTHERN ALLIGATOR LIZARD

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HISTOLOGY OF THE CIRCULATING BLOOD OF GERRHONOTUS COERULEUS (BAIRD AND GIRARD), THE NORTHERN ALLIGATOR LIZARD

INTRODUCTION

The northern alligator lizard *Gerrhonotus coeruleus* (Baird and Girard), is distributed throughout parts of Montana, Idaho, British Columbia, Washington, Oregon and California (21, p. 329). The average adult size varies from 3½ to 5¼ inches in snout-vent length. The blood which was examined came from animals collected a few miles inland from the Oregon coast, approximately nine miles south of Coos Bay. Lizards were collected in November of 1959 and in July and November of 1960, with animals from the latter two groups used for the detailed examinations. Those collected in July were found on the south slope of a low, rolling hill covered with tall grass, brush, young coniferous trees and rotten stumps. The lizards collected in November were found on the same hillside, but were pseudo-hibernating in the numerous large, rotten stumps present.

The investigation includes descriptions, differential counts, measurements of the circulating blood cells, and determination of the number of blood cells present per cubic millimeter.

The literature on the blood of the lizard, and of other reptiles, is very scanty, and on review it reveals confusion regarding the nomenclature of the leucocytes and differences of opinion as to the varieties normally present. Gulliver (9, p. 110) mentions only "pale globules", in addition to red blood cells, in his early paper on the blood cell dimensions of British ophidians. Werzberg (22), cited from Wood (23, p. 10), tabulated the leucocytes of the gecko, Tarentola mauritanica, as spindle cells, mast cells, eosinophils, mononuclear leucocytes, lymphocytes, leucocytoid lymphocytes and micromyeloblasts. Alder and Huber (1, p. 1-22) listed the leucocytes for the same species of gecko and for Anguis fragilis, Lacerta agilis, and Emys orbicularis as hemocytoblasts, thrombocytes, myelocytes, neutrophils, eosinophils, and basophils. Lowenthal (15, 16), cited from Wood (23, p. 15), lists the leucocytes for reptiles in general as lymphocytes, mononuclears, neutrophils, eosinophils, and basophils. Babuder (2), cited from Wood (23, p. 15), uses the same classification, except that monocytes replace mononuclears. Bernstein (3, p. 329) lists the leucocytes of Testudo geometrica as thrombocytes, monocytes, lymphocytes, macrocytes, neutrophils, basophils and eosinophils. Jordan and Flippin (13, p. 4-9) describe erythrocytes, eosinophilic granulocytes, mast cells or basophilic granulocytes, non-granular mononuclear leucocytes, lymphocytes, and spindle cells present in the blood of adult Chelydra serpentina (snapping turtle) and Cistudo (Terrapene) carolina (common box-turtle). Concerning the cells called leucocytoid lymphocytes by Werzberg, and which Jordan and Flippin call mononuclear leucocytes, the latter authors write (13, p. 8), "As Werzberg's designation implies, it is probably a modified (aged beyond mitotic capacity) large lymphocyte. At any rate such derivation is readily conceivable, and would seem to involve only slight alteration."

When the nomenclature of cell types is reduced to a uniform system, most authors seem to be in general agreement that lizards and other reptiles have at least the following leucocytic elements present in their circulating blood: lymphocytes, basophilic granulocytes, acidophilic granulocytes, thrombocytes or spindle cells, and some type of hemoblast. Monocytes and neutrophilic granulocytes are reported in many forms.

METHODS AND MATERIALS

Two techniques were utilized in examining the circulating blood of the northern alligator lizard, Gerrhonotus coeruleus. Blood-smears were prepared and used to ascertain the histology of the formed elements present. The cells present were identified and differential counts were made of them. An average was also determined for the length and width of each cell type. A hemocytometer was used to approximate the number of cells per cubic millimeter of blood.

Smears were prepared with blood obtained directly from the heart of decapitated animals. Blood was taken from the ventricle with an eyedropper and several slides were prepared, using the slide to slide technique for smearing. The slide used for spreading the smear had a small corner broken off (7, p. 438) to keep the blood smear narrow enough to be entirely covered with a cover slip. This was deemed necessary because leucocytes tend to be drawn towards the edges of the smear (7, p. 436; 8, p. 123), in which case many would not be observed during the differential counting.

The smeared slides were air-dried overnight, cleared in xylol and stained with Wright's stain. Cover slips were mounted on them with Canada balsam.

Differential counts of the blood cells were conducted on one blood-smear slide from each of nine winter (pseudo-hibernating) lizards and nine summer (active) lizards. The counts were made in regions of each slide where the smear was even and little or no overlapping was observed. Starting at one edge of the smear, a strip of contiguous oil-immersion (970X) microscopic fields was counted until the opposite edge was reached.

Measurements of each blood cell type were made from the slides used for differential counts. The measurements employed a calibrated ocular micrometer. The length and width of ten cells of each type were measured per slide. The average length and width was then computed for each cell type of each lizard, and the group average for each type was determined from these figures.

At the same time that smears were prepared, blood was also obtained for the determination of cell concentrations. The blood was pipetted directly from the exposed ventricle with the special dilution pipettes commonly used for this procedure. Hayem's solution was the diluting fluid and counts were made with a Neubauer double-lined counting chamber. A small drop of diluted blood was allowed to flow under the cover slip onto each counting grid. The counts were made at high-power (430X) magnification.

No separation of the leucocytes from the red blood cells was attempted in the counting chamber. However, indirect white blood cell counts were calculated by the method Forkner (8, p. 122) used for the domestic fowl. Forkner computed the number of white blood cells per cubic millimeter from the total number of blood cells determined in the counting chamber and the ratio between red and white blood cells from the differential counting of blood smears.

OBSERVATIONS

Description of Cell Types

Erythrocytes (Plate I, er). The mature erythrocyte is oval to elliptical in shape, with a centrally placed elliptical nucleus usually having a slightly irregular outline. The agranular cytoplasm of the erythrocyte is homogeneous and, when well stained with Wright's stain, appears buff colored. The nucleus stains medium blue with randomly scattered blocks of chromatin of irregular shape staining bluish-black. Occasionally an anucleated erythrocyte (erythroplastid) may be seen (Plate III, erpl).

Small numbers of immature erythrocytes (Plate II, i-er), distinguished by having rounder nuclei and grayer cytoplasm than mature cells, were present in each smear, as were a few degenerating erythrocytes (Plate I, d-er). The latter are slightly larger than mature erythrocytes and are nearly round. Their nucleus appears homogeneous and stains a lilac color, while the cytoplasm becomes faintly stained.

The average erythrocyte size for each of the summer lizards is presented in Table I. The overall average for summer lizards is 17.5 X 10.0μ, with an average nuclear dimension of 7.0 X 4.1μ. The extremes were 21.3 X 11.6μ, containing a nucleus 8.8 X 4.7μ; and 14.4 X 7.9μ, having a nucleus 6.3 X 3.6μ.

The average erythrocyte size for each of the winter lizards is tabulated in Table II. The overall average is 17.5 X 10.6μ, with nucleus measuring 6.5 X 4.1μ. The range in erythrocyte size was from 21.3 X 13.0μ, with a nucleus 8.5 X 4.9μ, to 12.6 X 9.1μ, containing a nucleus 6.0 X 4.0μ.

Erythroblasts (Plate I, erb; Plate III, e-erb, i-erb, l-erb, m-erb). The erythroblast is usually oval to round and contains a relatively large nucleus. The often-vacuolated, bluish-gray staining cytoplasm is present in meager or abundant quantity, depending upon whether the cell is young or old, respectively. The cytoplasm stains dark bluish-gray in the younger forms and progressively lighter as development proceeds. The comparatively large nucleus is usually round and stains light purple. The numerous irregular particles of chromatin stain dark bluish-purple and are scattered throughout the nucleus.

The average erythroblast size is listed in Table I for each of the summer lizards. The average erythroblast size in summer lizards is 13.9 X 11.4μ, with nuclear dimension of 8.6 X 7.7μ. The extremes were 18.3 X 12.0μ, having a nucleus 12.7 X 10.5μ, and 10.2 X 9.9μ, containing a nucleus 7.1 X 6.0μ.

The average erythroblast size in winter lizards (Table II) is 13.5 X 11.8μ, with nucleus 9.9 X 9.2μ. The extremes were 18.4 X 14.8μ, having a nucleus 10.5 X 9.8μ, and 9.9 X 9.2μ, containing a nucleus 7.3 X 6.4μ.

Hemoblasts (Plate I, hem; Plate IV, hem). The hemoblast is a comparatively large cell that is usually round, rarely oval. The scanty, deeply basophilic cytoplasm appears as a thin border surrounding the nucleus. The round nucleus stains lilac, with small particles of slightly darker staining chromatin evenly distributed throughout it. The almost granular appearing cytoplasm is sometimes extended in one or more pseudopod-like structures.

The average hemoblast size is recorded in Table I for each of the summer lizards. The overall average for summer lizards is 13.9 X 12.4μ, with nucleus 11.8 X 10.8μ. The largest hemoblast measured was 22.4 X 21.6μ, with a nucleus 19.6 X 19.2μ. The smallest one measured was 10.0 X 9.4μ, having an 8.5 X 8.3μ nucleus.

The average hemoblast size for each of the winter lizards is listed in Table II. The overall average for winter lizards is 12.1 X 11.6μ, with nucleus 10.6 X 9.9μ. The variation in hemoblast size of winter lizards was from 16.0 X 14.8μ, containing a 14.4 X 13.2μ nucleus, to 9.8 X 9.1μ, with a nucleus 8.5 X 7.6μ.

Lymphocytes (Plate I, ly; Plate IV, l-ly, m-ly, s-ly). The lymphocyte is usually round and may vary markedly in size. The eccentric nucleus is round in small lymphocytes, but usually becomes oval to kidney-shaped in intermediate and large cells. In small lymphocytes, the bluish-purple staining nucleus usually contains many darkly staining coarse chromatin blotches. In the intermediate and large forms, the nucleus stains red-violet and the slightly darker chromatin is diffuse. The cytoplasm is sparse in small lymphocytes, but the relative amount increases as the cells become larger. It stains medium blue in small cells and light grayish-blue in larger lymphocytes. A few azure granules are often present in the cytoplasm of large lymphocytes.

No average lymphocyte sizes were computed because the disparity in size between individual cells would make an average size meaningless. However, the lymphocyte extremes for summer lizards were from 23.8 X 23.1μ, with a nucleus 18.8 X 11.4μ, to 5.8 X 5.6μ, having a 5.8 X 4.5μ nucleus. For winter lizards, the extremes were from 17.5 X 16.1μ, having a nucleus 16.1 X 14.0μ, to 5.9 X 5.2μ, containing a nucleus 5.2 X 5.0μ.

Basophils (Plate II, b; Plate IV, b; Plate V, b). The basophil, or mast cell, is a round, medium-sized cell possessing numerous spherical, bluish-purple staining cytoplasmic granules. The round or oval basophil nucleus stains light blue to lilac. Light-staining irregular chromatin blotches are scattered throughout the nucleus. The cytoplasm, usually almost obscured by granules, stains light blue to light gray.

The average basophil size for each of the summer lizards is listed in Table I. The overall average for summer lizards is 11.6 X 10.6μ, with nucleus 7.6 X 7.1μ. Extremes in basophil size for summer lizards were 14.1 X 13.2μ, containing a nucleus 11.1 X 9.8μ; and 9.2 X 8.7μ, having a nucleus 6.1 X 5.9μ. Granule sizes varied from 0.5 X 0.5μ to 1.9 X 1.6μ in these cells.

The average basophil size for each of the winter lizards is presented in Table II. The overall average in winter lizards is 10.9 X 10.1μ, with the nucleus 7.3 X 6.9μ. Extremes ranged from 14.5 X 12.9μ, with a nucleus 9.5 X 8.4μ; to 8.4 X 7.6μ, with a nucleus 6.1 X 5.6μ. Granules ranged in size from 0.4 X 0.4μ to 1.5 X 1.5μ.

<u>Heterophils</u> (Plate II, het; Plate V, het, r-het). The heterophils are round, relatively large cells having numerous acidophilic granules. These granules range from almost spherical to nearly fusiform in shape, but the majority are irregularly shaped. These granules are so numerous that usually the cytoplasm is obscured and the blue-violet staining, oval nucleus is relegated to a polar position. Darker staining chromatin strands are distributed

throughout the nucleus. Part of the nucleus is often covered by acidophilic granules, thus making it almost impossible to arrive at an accurate size for the nucleus. When the nucleus is exposed (Plate V, r-het), it is usually bilobed, although in intact heterophils this is not obvious.

The average size for the heterophils in each of the summer lizards is listed in Table I. For all of these heterophils the average is 15.6 X 15.0μ with a nucleus 9.3 X 4.4μ. Extremes in size were from 20.1 X 19.8μ to 11.6 X 10.8μ, with nuclei 12.8 X 7.1μ and 8.4 X 3.4μ, respectively. The acidophilic granules present in these cells varied from 0.8 X 0.9μ to 1.6 X 2.9μ in size.

The average heterophil size for each of the winter lizards is listed in Table II. For all of these heterophils the average is 17.2 X 16.1μ, with nucleus 10.1 X 4.5μ. Extremes for heterophils were from 20.1 X 19.8μ, containing a 12.8 X 7.1μ nucleus, to 11.6 X 10.8μ, with a nucleus 8.4 X 3.4μ. Acidophilic granules varied in size from 0.8 X 0.8μ to 3.4 X 2.8μ.

Spindle Cells (Plate II, s; Plate V, s). Spindle cells are narrow, elongated cells having, after staining, remarkably clear cytoplasm and a dark-violet nucleus. The nucleus is roughly elliptical and usually has an obvious groove or depression. Small, irregular particles of dark bluish-purple staining chromatin are scattered throughout the nucleus. From two to twenty or more spindle cells are often found clumped together in the smears.

The average spindle cell size for each of the summer lizards is tabulated in Table I. Overall, the spindle cells average 11.1 X 5.9μ, with nucleus 7.6 X 5.3μ. The extremes were 16.5 X 7.6μ, containing a nucleus 11.2 X 6.8μ, and 8.6 X 4.6μ, including a nucleus 5.8 X 4.1μ.

The average spindle cell size for each of the winter lizards appears in Table II. Overall, the spindle cells average 13.9 X 5.2μ, with nucleus 8.8 X 4.7μ. The extreme sizes measured 16.7 X 6.0μ, with a 10.6 X 5.4μ nucleus; and 10.4 X 4.8μ, containing a 6.6 X 4.3μ nucleus.

Many ghost (degenerated and partially cytolized) cells were found on all slides (Plate II, g; Plate V, g). No average dimensions were computed for these cells.

Cell Concentrations

Blood cell counts were made on nine lizards from each group. The average cell concentration per mm^3 of blood for each lizard is tabulated in Table III. The concentration of white blood cells, computed by the method of Forkner (8, p. 122), is also given.

For five summer females, the cell concentration varied from 2,021,000 to 2,428,000 per mm^3 and averaged 2,222,000 per mm^3. These figures represent a range from 48,000 to

77,000 white blood cells per mm^3, and an average of 63,000 per mm^3, using Forkner's method of computation. Four summer males had a range in cell concentration from 1,772,000 to 1,996,000 cells per mm^3 and an average of 1,870,000 cells per mm^3. The computed white cell numbers ranged from 39,000 to 114,000 and averaged 90,000 per mm^3.

The cell concentrations for four winter females varied from 1,018,000 to 1,253,000 and averaged 1,169,000 cells per mm^3. The computed leucocyte range was 54,000 to 113,000 and averaged 78,000 cells per mm^3. The blood cell concentration per mm^3 of five winter males varied from 989,000 to 1,147,000 and averaged 1,068,000 cells. The computed leucocyte numbers ranged from 57,000 to 71,000 and averaged 65,000 cells per mm^3.

Differential Counts

The cells counted from smears were classified into three separate groups; red blood cells, white blood cells (hemoblasts arbitrarily included) and ghost or degenerating cells. The results are given in Table III.

The average percentages (rounded to nearest 0.1 percent) for each cell group in the nine summer lizards were 94.8 percent for the red blood cells, 3.7 percent for the white blood cells and 1.5 percent for the ghost cells.

The extremes for individual animals were 92.7 and 96.5 percent for the red blood cells; 2.1 and 6.1 percent for the white blood cells; 0.5 and 2.3 percent for the ghost cells. For the nine winter lizards, the average percentages were 93.0 percent for the red cells, 6.4 percent for the white cells and 0.7 percent for the ghost cells. The extremes for individual lizards were 90.0 and 95.1 percent for the red blood cells; 4.3 and 9.7 percent for the white blood cells; 0.3 and 1.4 percent for the ghost cells.

The relative percentages of cell types within the red cell series and within the white cell series were computed from the differential counts. These percentages are presented in Table IV.

Within the red cell series, the average percentage of erythrocytes was 95.4 percent in summer lizards and 98.3 percent in winter lizards. The individual extremes for summer lizards were 94.2 and 97.6 percent. Individual extremes in winter lizards were 97.3 and 99.1 percent.

The average relative percentage of erythroblasts was 4.6 percent in summer lizards and 1.7 percent in winter lizards. The individual extremes for erythroblasts in summer lizards were 2.4 and 5.8 percent. For winter lizards, the erythroblast extremes were 0.9 and 2.7 percent.

Within the white cell series, the average relative percentage of hemoblasts was 2.2 percent in summer lizards and 1.0 percent in winter lizards. The range in percentage was 0 to 5.3 percent in summer lizards and 0 to 1.5 percent in winter lizards.

The lymphocyte percentages were 35.7 percent for summer lizards and 13.3 percent for winter lizards. The extremes were 14.4 and 54.3 percent in summer lizards; 8.4 and 17.6 percent in winter lizards.

The average relative percentage of basophils was 6.5 percent in summer lizards and 5.5 percent in winter lizards. The extremes were 3.4 and 12.6 percent in summer lizards; 1.3 and 7.7 percent in winter lizards.

Heterophil percentages were 5.1 percent in summer lizards and 7.3 percent in winter lizards. The range was 1.4 to 13.0 percent in the summer lizards and 2.6 to 14.0 percent in the winter lizards.

Spindle cell percentages were 50.7 percent for summer lizards and 73.0 percent for winter lizards. The extremes were 31.2 and 76.4 percent for the summer lizards; 63.1 and 77.9 percent for the winter lizards.

DISCUSSION

Cell Types

The blood cell sizes, measured from air-dried smears as is conventional, are slightly smaller than their actual sizes while circulating. This was recognized as early as 1842 by Gulliver (9, p. 108),

> "Though the blood-discs of birds and reptiles preserve their shape very clearly when rapidly dried on a slip of glass, they generally appear in this state slightly but distinctly smaller than when suspended in the serum of recent blood."

There is no apparent correlation between the size of the different cell types and the sex of the lizards. Nor do the slight differences between the average cell dimensions of summer lizards and winter lizards seem significant in the case of red cells. Erythrocytes averaged 17.5 X 10.0μ in summer and 17.5 X 10.6μ in winter. Erythroblasts averaged 13.9 X 11.4μ in summer and 13.5 X 11.8μ in winter.

The size of the <u>Gerrhonotus</u> erythrocyte compared to that of other reptiles may be seen in the table on the following page.

As noted in the introduction, there is confusion regarding the nomenclature of the leucocytes present in reptilian blood, and a difference (in part real, in part interpretive) as to the varieties normally present. Most

DIMENSIONS OF ERYTHROCYTES IN REPTILES

Turtles			
Chelonia mydas	20.3 X 13.3μ	(fresh)	Gulliver (10)
Emys orbicularis	17.0 X 11.3μ	(dry)	Alder and Huber (1)
Testudo geometrica	16.0 X 10.0μ	(dry)	Bernstein (3)
Lizards			
Anguis fragilis	21.2 X 9.4μ	(fresh)	Gulliver (9)
Anguis fragilis	17.7 X 9.3μ	(dry)	Alder and Huber (1)
Anolis carolinensis	17.5 X 10.0μ	(fresh)	Rabalais (24)
Gerrhonotus coeruleus	17.5 x 10.0μ	(dry)	This thesis
Lacerta agilis	14.8 X 6.3μ	(dry)	Alder and Huber (1)
Lacerta muralis	13.3 X 6.2μ	(dry)	Alder and Huber (1)
Tarentola mauritanica	17.0 X 10.0μ	(dry)	Alder and Huber (1)
Snakes			
Coluber berus	20.0 X 14.0μ	(fresh)	Gulliver (9)
Natrix clarkii	16.8 X 11.4μ	(fresh)	Rabalais (24)
Natrix cyclopion	17.5 X 12.5μ	(fresh)	Rabalais (24)
Natrix torquata	18.2 X 11.6μ	(fresh)	Gulliver (9)
Thamnophis sauritius	17.0 X 11.7μ	(fresh)	Rabalais (24)
Crocodiles			
Alligator sp.	18.9 X 11.8μ	(fresh)	Gulliver (10)
Crocodilus acutus	20.3 X 10.9μ	(fresh)	Gulliver (10)
Crocodilus lucius	22.2 X 11.3μ	(fresh)	Gulliver (10)

authors agree that lymphocytes, basophils (mast cells) and spindle cells (thrombocytes) are present. Alder and Huber (1, p. 2), however, state that the blood cells in Amphibia and Reptilia which appear to be lymphocytes are not homologous to mammalian lymphocytes. They call these cells hemocytoblasts, believing they are mother cells for the white and red cell series (1, p. 4). It is their opinion that genuine lymphocytes do not occur in forms phylogenetically below birds. Jordan (11, p. 380) also uses the term hemocytoblast to designate the cells which can differentiate into lymphocytes or into red blood cells. Jordan and Speidel (14, p. 85), however, state "the horned toad (Phrynosoma solare), like other reptiles and like amphibians, has no other hemoblast than the lymphocyte."

Lymphocytes were found to be abundant in Gerrhonotus coeruleus. Measured lymphocytes ranged from 5.9 X 5.2μ to 23.8 X 23.1μ. No average lymphocyte size was computed for lizard blood because the great disparity in size between individual cells would render an average size meaningless. However, the majority of lymphocytes were small. Recorded average lymphocyte (hemocytoblast) sizes for reptiles are close to the smaller figures.

Alder and Huber (1, p. 11-13) list average diameters for the cells they call hemocytoblasts as 5 to 7μ in

Lacerta muralis, 5.5 to 9.5μ for *Tarentola mauritanica* and 6 to 8.5μ for *Emys orbicularis*. Bernstein (3, p. 329) lists an average lymphocyte diameter of 7 X 7μ for *Testudo geometrica*.

The cell type which has been the basis of the most controversy is the leucocyte with acidophilic granules. This cell is called eosinophil, heterophil, pseudo-eosinophil or oxyphil, depending upon the author. Various reptiles may be lacking acidophilic granulocytes completely, have one type, or have two types of this leucocyte. The two types are distinguished by having either elongated granules or spherical granules. Alder and Huber (1) list only one type of eosinophil for the blood of the reptiles they examined. These reptiles were *Lacerta agilis*, *Anguis fragilis*, *Tarentola mauritanica*, and *Emys orbicularis*. Jordan lists no eosinophils for *Ophisaurus ventralis* and two types of eosinophils for *Phrynosoma solare* (12, p. 788). Among the reptiles Werzberg (22, p. 125-126) listed as having acidophilic granulocytes with rounded granules were *Emys*, *Anguis*, *Ophisaurus*, *Testudo*, and *Lacerta*. The reptiles with spindle-shaped granules were *Scincus*, *Chalcides*, *Hemidactylus*, *Anolis*, *Agama*, *Gongylus* and *Chameleon*. Werzberg listed no reptile which possessed acidophilic granulocytes of both types or which had both types of granules within the same cell.

Charipper and Davis (5, p. 381) considered turtle eosinophils having spherical granules to be young cells and those with ellipsoidal or rod-like granules to be older cells. This conclusion was based on the fact that experimental injections of both nucleic acid and thyroxine brought about an increase in the number of eosinophils with spherical granules, while the number of eosinophils with fusiform granules remained unaffected. Ryerson (19, p. 37) lists six authors who have concluded from their observations that the acidophilic granulocytes possessed by reptiles belong to a single series. These opinions rest, according to Ryerson, "...generally on the basis of their occasional observations of the so-called intermediate forms (cells containing both spheroidal and fusiform granules)." Lundquist and Hedlund (17) proposed that the osmotic pressure of the medium could be responsible for changes in the shape of the fusiform granules. This might explain a change in the shape of the granules within cells over a period of time, but it seems inadequate to explain how a single cell can contain both types of granules, or the fact that of two adjacent acidophilic granulocytes, one may have mainly fusiform granules while the other possesses a majority of spherical granules.

Ryerson (25, p. 39) points out that "up to the present time it has apparently not been recognized that the blood pictures of lizards and snakes are distinctly different from those of turtles." He states that after detailed observations of his blood slides of chickens, pigeons, turtles, alligators and lizards, the four former animals have both heterophils (fusiform granules) and eosinophils (spherical granules), while lizards have only heterophils.

My observations indicate that Gerrhonotus coeruleus has only one type of acidophilic granulocyte. The granules are usually irregular in shape, although some are spherical and others are elongated. Because the predominant granule shape is elongated, this cell type has been called heterophil, rather than eosinophil. The average heterophil size for summer lizards is 15.6 X 15.0μ and the average for winter lizards is 17.2 X 16.4μ. No explanation can be made for the markedly larger average heterophil size in the winter animals.

Alder and Huber (1, p. 11-13) recorded the average eosinophil size for several animals. The eosinophil size is 10 to 12.5μ in Lacerta agilis, 11 to 12.5μ in Tarentola mauritanica; and 11 to 12.5μ for eosinophils with spheroidal granules and 12.5 to 15μ for eosinophils with fusiform granules in Emys orbicularis.

Jordan and Speidel (14, p. 33) contend that the basophil is a young or abortive eosinophil. However, they could find no basophils with a nucleus similar to that of a young eosinophil. Charipper and Davis (5, p. 372) discuss a point of view which maintains that the basophil and eosinophil are fundamentally the same cell. The proposal is that nutritional factors account for the difference in appearance of the two cell types. If nutritional factors cause this difference in appearance, an explanation which would satisfactorily show how certain cells could be affected by these factors, while adjacent cells are not influenced, would seem quite difficult.

In Gerrhonotus coeruleus, average basophil size for summer lizards is 11.6 X 10.6μ. For winter lizards the average is 10.9 X 10.1μ. Alder and Huber (1, p. 11-13) report the average basophil diameter as 7 to 8.5μ for Lacerta agilis, 7 to 9μ for Lacerta muralis and 10 to 14μ for Emys orbicularis. The average basophil size listed by Bernstein (3, p. 329) for Testudo geometrica is 15 X 15μ.

There is disagreement with respect to the occurrence of true neutrophils in reptilian blood. Lowenthal (15, 16) and Babuder (2), cited from Wood (23, p. 10), give general figures for neutrophils in reptiles as 0-22.5 percent and 0-53 percent of the leucocytes, respectively. Alder and Huber (1, p. 20) report the presence of neutrophils in Lacerta agilis (6 percent), Anguis fragilis (5 percent)

Tarentola _mauritanica_ (1 percent) and _Emys_ _orbicularis_ (2 percent). Bernstein (3, p. 328) tabulated neutrophils as 0.3 percent of the leucocytes in _Testudo_ _geometrica_. Jordan and Flippin (13) do not report neutrophils in their description of the blood of _Chelydra_ _serpentina_ and _Cistudo_ (_Terrapene_) _carolina_. Ryerson (19, p. 42) contends that the neutrophil in reptiles described by Lowenthal (15), Babuder (2), Jordan (12) and others is "more closely related to the monocytes of other classes than it is to the finely granular leucocytes." He lists five lengthy reasons supporting this contention. Evidently, true neutrophils do not occur in _Gerrhonotus_ _coeruleus_. It might be noted that Aves have no neutrophils. Shaw (20) does not list neutrophils in his paper on the leucocytes of the pigeon. Among authors reporting the absence of neutrophils in birds are Bradley (4, p. 994), Cullen (6, p. 353), and Ryerson (19, p. 25). The place of the neutrophil is reportedly taken by a granulocyte with coarse, fusiform acidophilic granules (the heterophil of this paper). For numerous Pisces, Aves, Reptilia and Mammalia which he examined, Bradley (4, p. 994) reports no occurrence of both neutrophils and heterophils in the same animal.

Spindle cells (thrombocytes) are listed for various reptiles by practically all authors. These cells are thought to function in blood clotting, and in animals where this function is probable, the cells are called thrombocytes.

In Gerrhonotus coeruleus the average spindle cell for summer lizards measures 11.1 X 5.9μ. In winter animals it measures 13.9 X 5.2μ. Alder and Huber (1, p. 11-13) list the average length of thrombocytes as 4μ for Lacerta muralis, 4.5μ for Lacerta agilis and 5.5 to 8μ for Emys orbicularis. Bernstein (3, p. 329) records the thrombocyte size of Testudo geometrica as 8 X 6μ.

Ghost, or degenerating cells, were present in all smears. Measurements of these are without significance.

Cell Concentrations

Examination of Table III discloses that the average cell concentration per cubic millimeter of blood is higher in the females of each group than in the males. The average cell concentration is also higher for the summer lizards of both sexes than for the corresponding winter lizards.

The leucocyte concentration was computed from the total cell concentration for each lizard and the relative leucocyte percentage determined from the differential counts (8, p. 122). An obvious source of error inherent in this method is the fact that an even distribution of red and white cells is assumed. An error might be introduced in this manner, but the computed white cell concentration should still closely approach the actual concentration.

The composite average red cell concentration for the lizards examined was 1,516,000 cells per mm^3. The average leucocyte concentration for the entire group of lizards was 72,800 cells per mm^3. These concentrations compare as follows to those reported by other investigators for various reptiles. Alder and Huber (1, p. 19) reported 954,000 red blood cells and 19,000 white blood cells per mm^3 for *Lacerta agilis*; 1,516,000 red blood cells and 7,000 white blood cells per mm^3 for *Anguis fragilis*; and 1,447,000 red blood cells and 8,200 white blood cells per mm^3 for *Lacerta muralis*. Bernstein (3, p. 330) reported 642,000 red blood cells and 45,487 white blood cells per mm^3 for *Testudo geometrica*. Rabalais (18, p. 147) reported 1,174,000 red blood cells and 220,000 white blood cells per mm^3 for *Natrix clarkii*, and 1,185,000 red blood cells and 89,200 white blood cells per mm^3 for *Anolis carolinensis*.

Differential Counts

Data in Table III indicate that the average percentage of white blood cells is significantly greater in the winter lizards than in summer lizards. Also, the percentage of degenerating, or ghost, cells in the winter lizards is only about one-half that found in the summer lizards. Both these conditions are to be expected. The lizards have an average of nearly twice as many blood cells per mm^3 in the summer as they have in the winter. This is related to the fact that the animal is extremely active during the summer, but pseudo-hibernates during the winter. It seems reasonable to expect that an animal would have more blood cells, per unit volume, during the season when its physiological requirements reach a peak, than while it is pseudo-hibernating. A greater percentage of leucocytes would be expected during the active, rather than during the inactive season. When blood cells are developing in greater numbers, there is a possibility of an increase in the percentage of degenerating blood cells.

Examination of Table IV makes it apparent that more hemopoiesis occurs in the summer lizards than in the winter lizards. This is indicated by the fact that both erythroblasts and hemoblasts are more than twice as numerous in summer than in winter.

Lymphocytes are present in a much greater average percentage in summer than in winter. The lymphocytes are very likely differentiating into other cell types at an increased rate. This would explain the increase in their percentage in summer lizards, which is nearly twice that of winter lizards.

The average relative percentages of both basophils and heterophils in the two seasonal groups are nearly equal.

A much greater percentage of spindle cells was found in winter lizards than in summer lizards. The significance which can be attached to this fact is difficult to assess.

The relative percentage of leucocytes for other reptiles has been tabulated by various authors. Among these have been Alder and Huber (1, p. 19) who reported 72 percent combined hemocytoblasts and thrombocytes, 1 percent myelocytes, 6 percent neutrophils, 10 percent eosinophils and 11 percent basophils for <u>Lacerta agilis</u>. For <u>Tarentola mauritanica</u> they reported 73 percent combined hemocytoblasts and thrombocytes, 1 percent myelocytes, 1 percent neutrophils, 7 percent eosinophils and 18 percent basophils. Bernstein (3, p. 328) reports 0.3 percent neutrophils, 10.8 percent eosinophils, 8.0 percent basophils, 56.1 percent lymphocytes, 9.4 percent monocytes, 12.8 percent thrombocytes and 2.6 percent macrocytes for <u>Testudo geometrica</u>.

These percentages cannot be readily compared with the leucocyte distributions found for <u>Gerrhonotus coeruleus</u>, since seasonal differences are not indicated and there are actual differences in cell types present.

SUMMARY AND CONCLUSIONS

1. The blood cells present in <u>Gerrhonotus</u> <u>coeruleus</u> (Baird and Girard) are erythrocytes, erythroblasts, hemoblasts, lymphocytes, basophils, heterophils, spindle cells and ghost cells.
2. Blood cell sizes apparently are little affected by the sex of the lizard.
3. The average blood cell concentration per mm^3 in summer (active) lizards is nearly twice that in winter (pseudo-hibernating) lizards (2,165,000 and 1,113,000). Blood cell concentrations are significantly larger in females of each seasonal group than in males.
4. The percentage of red blood cells averages slightly greater in summer lizards than in winter lizards (94.8 and 93.0). White blood cell average percentages are approximately twice as high in winter as in summer lizards (6.4 and 3.7); the reverse is true of ghost cells (0.7 and 1.5).
5. The average relative percentages of erythroblasts, hemoblasts, lymphocytes and basophils are larger in summer lizards than in winter lizards. Erythrocyte, heterophil and spindle cell percentages are larger in winter lizards than in summer lizards.

6. There is virtually no difference between the average cell size of either erythrocytes or erythroblasts in summer and winter lizards. The hemoblast and basophil average sizes are both slightly larger in summer than in winter. Heterophil and spindle cell average sizes are somewhat larger in winter lizards than in summer lizards.

PLATES AND TABLES

PLATE I

Four Figures

d-er = degenerating erythrocyte

er = erythrocyte

erb = erythroblast

hem = hemoblast

ly = lymphocyte

PLATE I

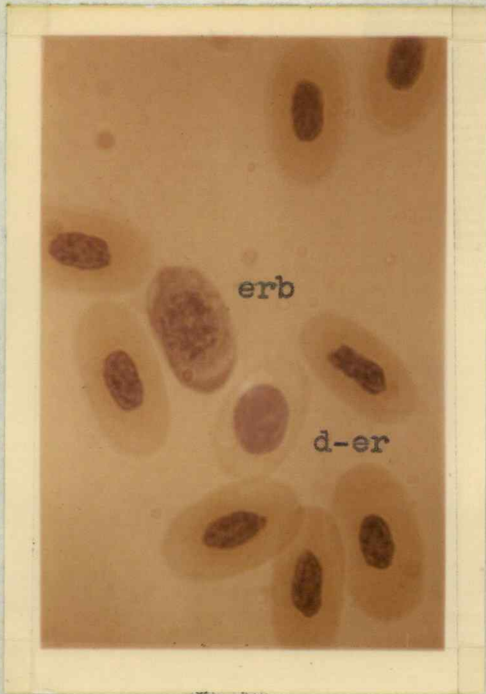
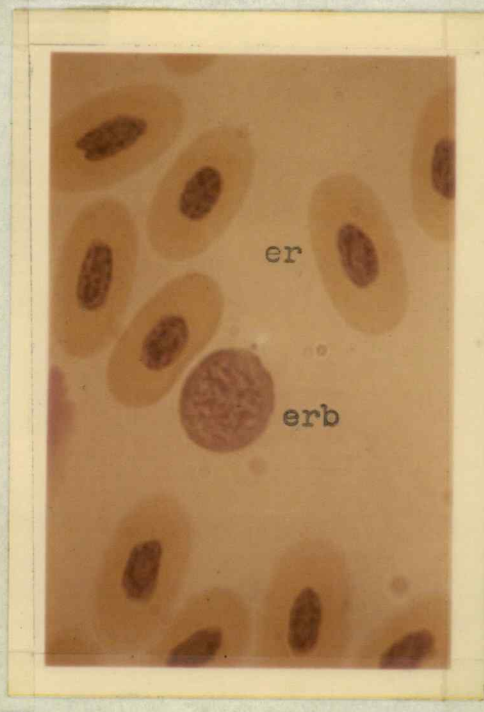


Figure 1



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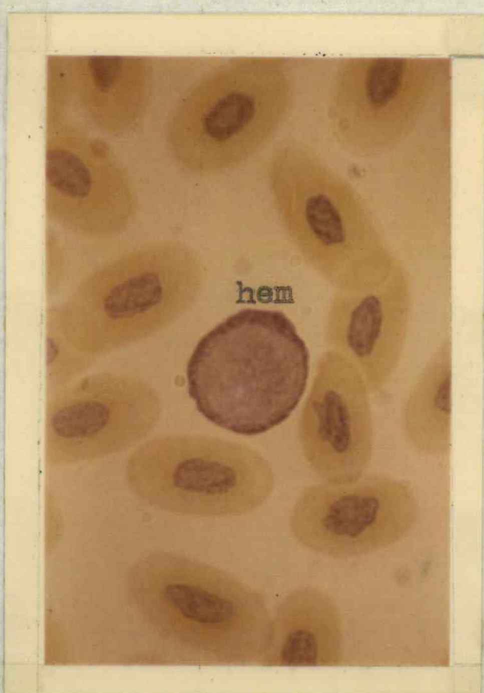


Figure 3

Figure 4

PLATE II

Three Figures

b = basophil

g = ghost cell

het = heterophil

i-er = immature erythrocyte

s = spindle cell

PLATE II

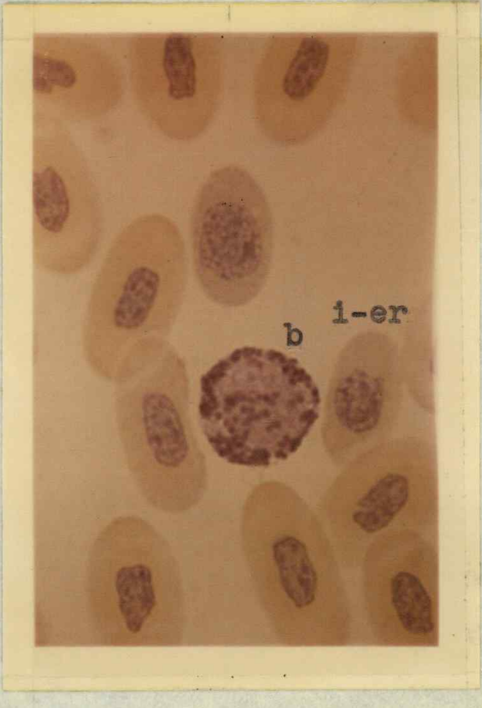


Figure 1

Figure 2

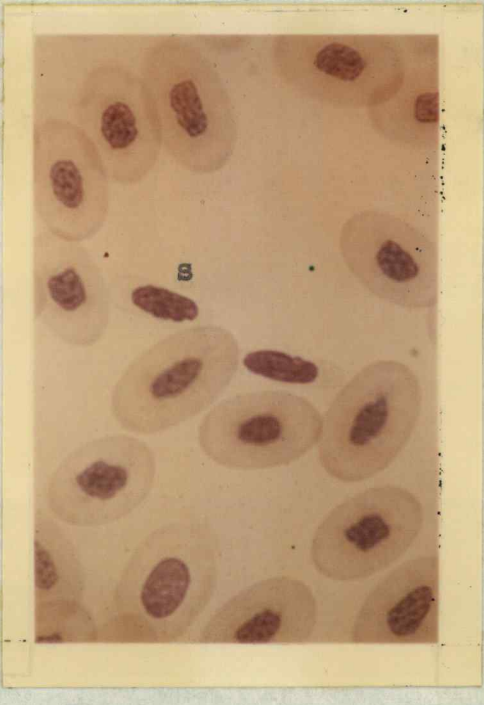


Figure 3

PLATE III

Six Figures

e-erb = early erythroblast

erpl = erythroplastid

i-erb = intermediate erythroblast

m-erb = erythroblast in mitosis

PLATE III

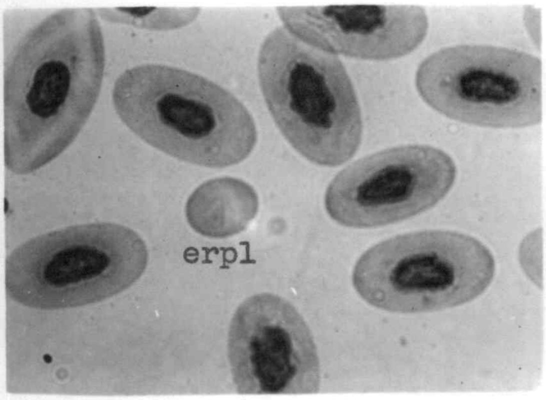
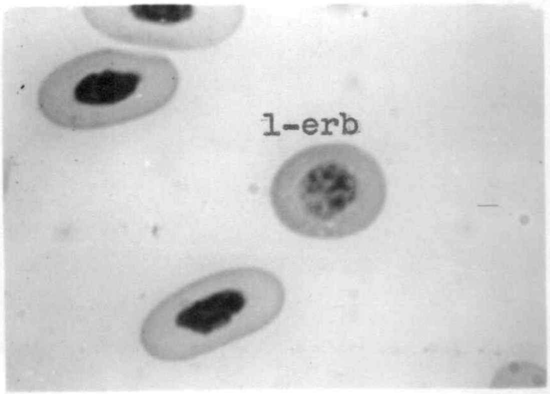


Figure 1



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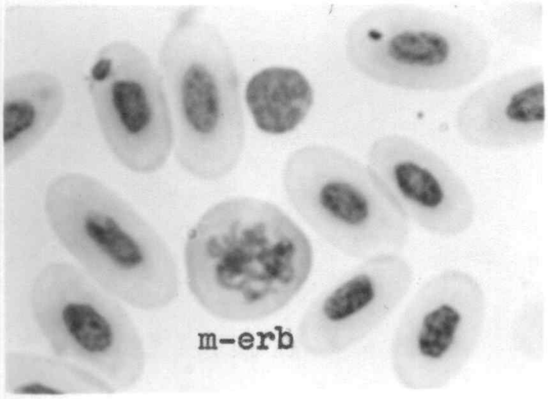

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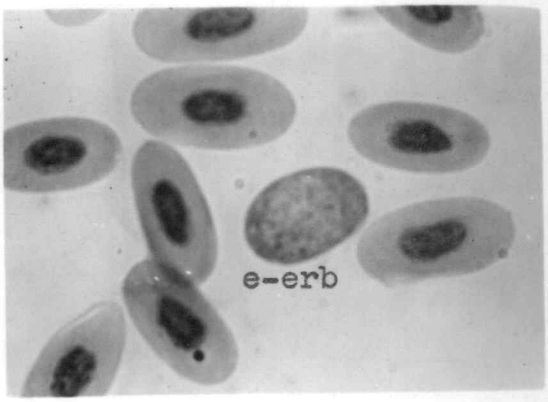

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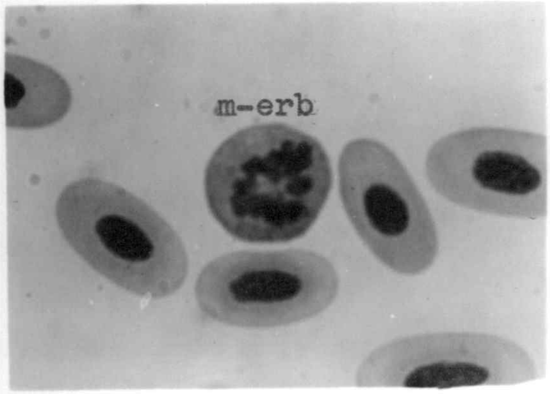


Figure 5

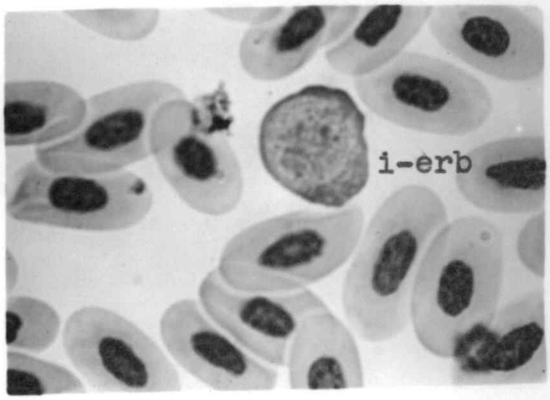


Figure 6

PLATE IV

Six Figures

b = basophil

hem = hemoblast

l-ly = large lymphocyte

m-ly = medium lymphocyte

s-ly = small lymphocyte

PLATE IV

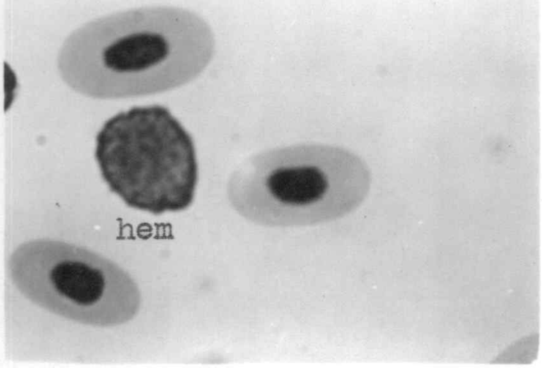
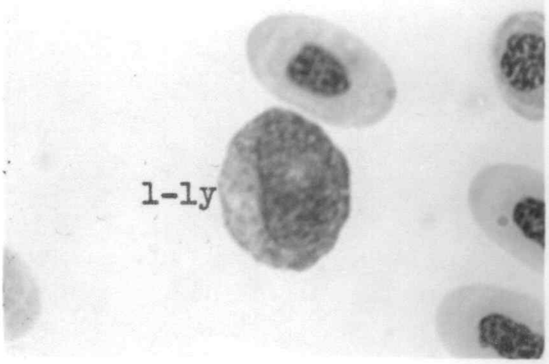


Figure 1



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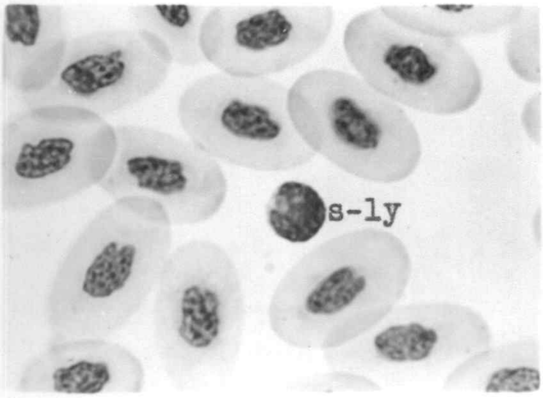

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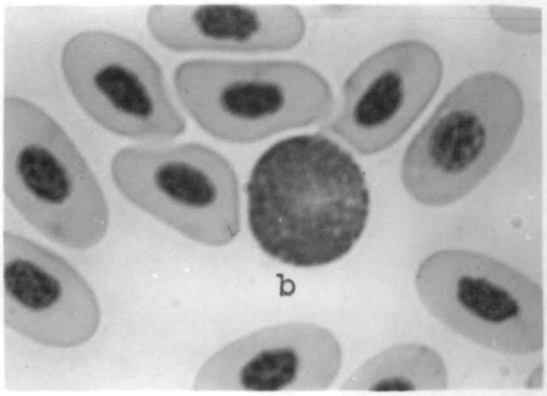

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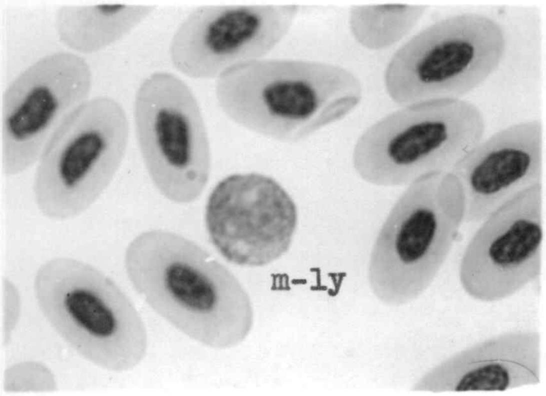

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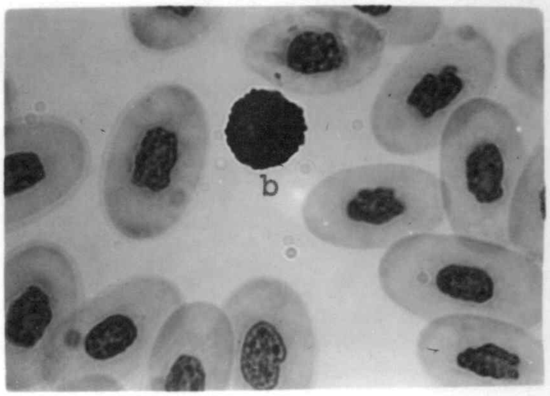


Figure 6

PLATE V

Six Figures

b = basophil

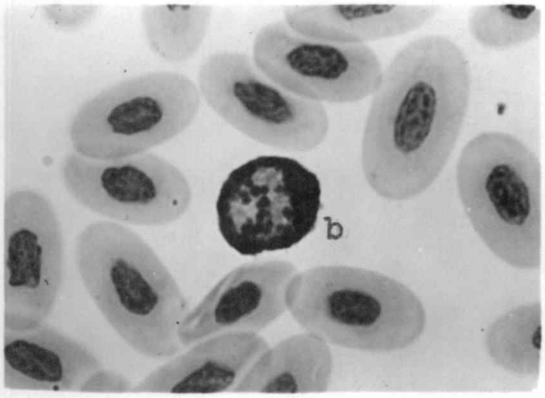
g = ghost

het = heterophil

r-het = ruptured heterophil (shows both elongated and nearly spherical granules)

s = spindle cell

PLATE V



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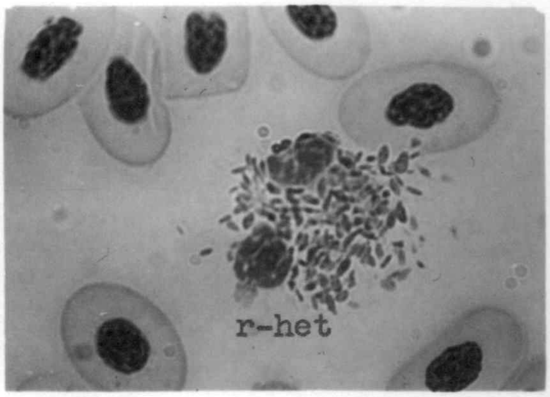

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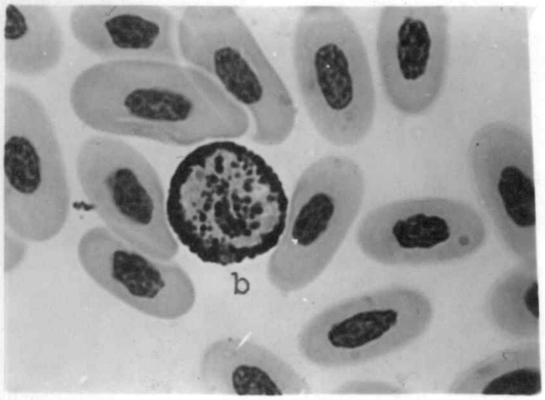

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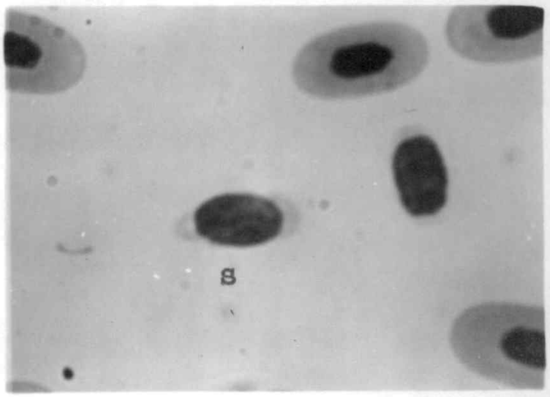

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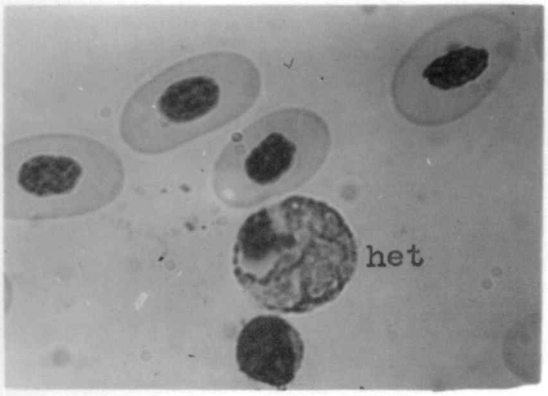

Figure 5

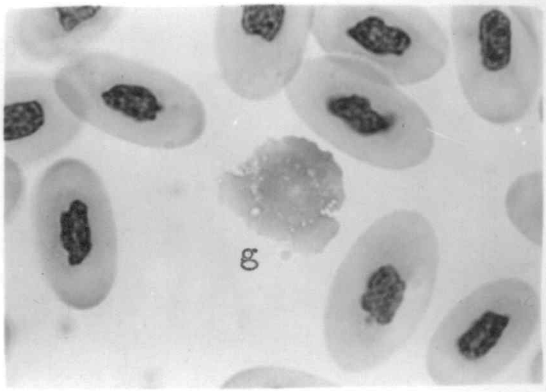

Figure 6

Table I. AVERAGE SIZE OF CELL AND NUCLEUS, IN MICRA, FOR BLOOD CELLS OF GERRHONOTUS COERULEUS[1]

Summer Lizards

Lizard	Erythrocytes	Erythroblasts	Hemoblasts	Basophils	Heterophils	Spindle Cells
1 Cell	17.3 X 9.5	14.4 X 10.1	15.9 X 12.3	11.5 X 10.7	16.1 X 15.5	13.3 X 6.7
Nucleus	7.3 X 4.2	7.8 X 6.7	11.6 X 10.1	8.1 X 7.6	10.6 X 4.7	9.8 X 6.0
2	17.0 X 9.8	14.5 X 11.0	13.3 X 12.0	11.6 X 10.2	15.3 X 14.7	13.0 X 6.8
	6.8 X 4.0	9.0 X 8.1	11.3 X 10.2	7.1 X 6.8	8.0 X 4.9	8.8 X 5.7
3	16.8 X 10.0	14.0 X 11.7	13.4 X 12.8	12.1 X 10.9	15.8 X 15.3	10.8 X 6.0
	7.4 X 4.2	9.0 X 8.2	11.9 X 10.6	8.7 X 7.8	10.4 X 4.8	7.3 X 5.4
4	18.5 X 9.5	14.0 X 10.9	13.9 X 12.1	11.5 X 10.5	14.7 X 13.6	9.5 X 5.4
	7.3 X 4.1	7.0 X 6.3	11.9 X 10.9	6.4 X 5.9	8.6 X 3.6	6.4 X 4.8
5	17.1 X 9.8	12.8 X 10.9	17.6 X 16.5	11.2 X 10.5	16.1 X 15.5	9.7 X 5.7
	7.0 X 4.3	9.1 X 8.1	15.5 X 14.7	7.3 X 6.8	9.6 X 4.5	6.6 X 5.1
6	17.1 X 10.1	14.4 X 12.8	11.9 X 10.9	11.8 X 10.8	15.1 X 14.5	10.8 X 5.9
	6.5 X 3.9	9.2 X 8.6	10.2 X 9.5	7.7 X 7.2	9.0 X 4.3	7.3 X 5.3
7	18.1 X 10.6	14.0 X 11.6	11.4 X 10.5	11.6 X 10.5	16.4 X 15.8	12.1 X 5.9
	7.1 X 4.2	9.1 X 7.7	10.1 X 9.6	8.3 X 7.5	9.9 X 4.0	8.2 X 5.3

Table I. (continued)

Lizard	Erythrocytes	Erythroblasts	Hemoblasts	Basophils	Heterophils	Spindle Cells
8	17.4 X 10.5 7.1 X 4.3	13.1 X 12.2 8.9 X 8.3	13.2 X 11.7 11.1 X 10.4	12.6 X 11.7 8.5 X 8.0	17.2 X 16.8 9.2 X 4.6	11.5 X 5.7 7.8 X 5.1
9	17.9 X 10.5 6.6 X 3.7	13.5 X 11.2 8.5 X 7.2	14.6 X 13.1 12.5 X 11.2	10.1 X 9.4 6.7 X 5.9	13.9 X 13.3 8.6 X 4.3	9.0 X 5.4 6.1 X 4.8
Average	17.5 X 10.0 7.0 X 4.1	13.9 X 11.4 8.6 X 7.7	13.9 X 12.4 11.8 X 10.8	11.6 X 10.6 7.6 X 7.1	15.6 X 15.0 9.3 X 4.4	11.1 X 5.9 7.6 X 5.3

[1] No averages for lymphocytes were computed due to the disparity in the size between individual cells. The extremes in lymphocyte dimensions are listed on page 10 of the text.

Table II. AVERAGE SIZE OF CELL AND NUCLEUS, IN MICRA, FOR BLOOD CELLS OF GERRHONOTUS COERULEUS[1]

Winter Lizards

Lizard	Erythrocytes	Erythroblasts	Hemoblasts	Basophils	Heterophils	Spindle Cells
1 Cell	17.0 X 10.1	14.9 X 13.0	11.2 X 10.7	11.9 X 11.2	18.1 X 16.9	14.7 X 5.6
Nucleus	7.1 X 4.1	10.1 X 9.4	9.8 X 9.2	8.5 X 8.2	9.3 X 6.3	9.3 X 5.1
2	17.7 X 11.1	13.9 X 11.1	13.4 X 12.8	9.7 X 8.6	14.7 X 14.1	11.1 X 5.1
	6.6 X 3.7	7.6 X 7.0	11.6 X 10.7	6.4 X 6.1	10.1 X 3.8	7.0 X 4.6
3	18.3 X 10.8	13.7 X 12.1	12.1 X 11.6	10.1 X 9.8	16.6 X 15.7	14.1 X 5.5
	7.0 X 4.3	9.0 X 8.5	10.7 X 9.8	6.8 X 6.4	10.8 X 5.3	8.9 X 5.0
4	17.2 X 10.9	13.3 X 12.3	11.9 X 11.5	11.0 X 10.2	18.0 X 17.2	15.5 X 5.3
	7.3 X 4.2	9.0 X 8.5	10.4 X 9.8	8.0 X 7.4	10.5 X 4.3	9.8 X 4.8
5	17.5 X 10.4	12.7 X 11.4	12.3 X 11.7	10.9 X 9.9	17.7 X 16.9	11.7 X 5.4
	7.3 X 4.1	8.5 X 8.0	10.5 X 10.1	7.1 X 6.9	9.7 X 4.3	7.4 X 4.9
6	17.3 X 10.7	12.4 X 11.2	11.3 X 10.9	10.9 X 10.1	17.6 X 16.7	14.1 X 5.3
	6.9 X 4.2	9.1 X 8.3	9.9 X 9.3	7.2 X 6.8	10.5 X 4.3	8.9 X 4.8
7	17.5 X 10.1	13.4 X 11.2	11.9 X 11.5	11.4 X 10.4	17.4 X 16.7	15.8 X 5.0
	6.6 X 3.7	8.3 X 7.5	10.5 X 9.8	7.6 X 7.3	9.5 X 3.6	10.0 X 4.5

Table II. (continued)

Lizard	Erythrocytes	Erythroblasts	Hemoblasts	Basophils	Heterophils	Spindle Cells
8	17.7 X 10.7 7.1 X 4.1	13.6 X 12.3 8.9 X 8.4	12.2 X 11.7 10.7 X 10.2	11.4 X 10.4 7.0 X 6.5	17.8 X 16.9 11.0 X 4.5	14.7 X 4.5 9.3 X 4.1
9	17.6 X 10.5 6.5 X 4.1	13.2 X 11.5 9.9 X 9.0	12.5 X 12.0 11.1 X 10.4	11.0 X 10.4 7.2 X 6.8	16.8 X 16.1 9.2 X 4.4	13.6 X 5.1 8.6 X 4.6
Averages	17.5 X 10.6 6.9 X 4.1	13.5 X 11.8 9.9 X 9.2	12.1 X 11.6 10.6 X 9.9	10.9 X 10.1 7.3 X 6.9	17.2 X 16.4 10.1 X 4.5	13.9 X 5.2 8.8 X 4.7

[1] No averages for lymphocytes were computed due to the disparity in the size between individual cells. The extremes in lymphocyte dimensions are listed on page 10 of the text.

Table III. CONCENTRATION OF BLOOD CELLS IN GERRHONOTUS COERULEUS

Lizard	Sex	Length in Inches	Blood Cells Per mm^3	Computed Leucocytes per mm^3	Percent Red Blood Cells	Percent White Blood Cells	Percent Ghost Cells
				Summer Lizards			
1	M	2-3/4	1,849,000	39,000	95.9	2.1	2.0
2	M	2-5/8	1,772,000	97,000	43.5	5.5	1.1
3	F	3-1/8	2,021,000	77,000	95.0	3.8	1.2
4	F	3-1/4	2,147,000	52,000	95.3	2.4	2.3
5	F	3-1/4	2,270,000	48,000	96.5	2.1	1.4
6	M	3-1/8	1,861,000	114,000	92.7	6.1	1.2
7	M	3-1/8	1,996,000	110,000	94.0	5.5	0.5
8	F	3-5/8	2,428,000	75,000	95.3	3.1	1.6
9	F	3-1/2	2,243,000	63,000	95.3	2.8	1.9
				Winter Lizards			
1	F	3-3/8	1,169,000	113,000	90.0	9.7	0.3
2	M	3-1/8	1,147,000	57,000	93.7	5.0	1.4
3	F	3-1/2	1,235,000	84,000	92.5	6.8	0.7
4	M	3-1/8	1,064,000	71,000	92.1	6.7	1.2
5	F	3-1/4	1,253,000	54,000	95.1	4.3	0.6
6	M	3-1/4	1,024,000	65,000	93.4	6.3	0.3
7	F	3-1/8	1,018,000	61,000	93.4	6.0	0.6
8	M	2-5/8	989,000	70,000	92.6	7.1	0.3
9	M	2-1/4	1,118,000	60,000	93.9	5.4	0.7
Average	Summer Lizards		2,065,000		94.8	3.7	1.5
	Winter Lizards		1,113,000		93.0	6.4	0.7

Table IV. RELATIVE PERCENTAGE OF INDIVIDUAL BLOOD CELL TYPES IN *GERRHONOTUS COERULEUS*[1]

Lizard	Red Blood Cell Series: Erythrocytes	Red Blood Cell Series: Erythroblasts	White Blood Cell Series: Hemoblasts	White Blood Cell Series: Lymphocytes	White Blood Cell Series: Basophils	White Blood Cell Series: Heterophils	White Blood Cell Series: Spindle Cells
Summer Lizards							
1	97.6	2.4	5.3	35.5	12.6	2.9	44.5
2	95.0	5.0	3.6	54.3	7.1	1.4	33.6
3	94.6	5.4	4.7	42.3	6.1	4.4	42.5
4	95.5	4.5	0.5	44.9	3.4	13.0	38.2
5	94.5	5.5	2.0	51.0	10.5	5.3	31.2
6	96.5	3.5	0.0	27.7	4.2	2.5	65.6
7	94.8	5.2	0.0	14.4	4.5	4.7	76.4
8	95.6	4.4	1.4	20.2	6.5	5.9	66.0
9	94.2	5.8	1.9	30.6	3.7	5.6	58.3
Winter Lizards							
1	97.3	2.7	0.4	17.5	1.3	4.9	75.9
2	98.4	1.6	0.8	10.2	5.9	8.6	74.6
3	97.8	2.2	1.4	8.4	6.3	8.0	75.9
4	98.2	1.8	1.2	17.6	4.5	3.2	73.5
5	99.1	0.9	1.2	13.3	5.4	9.3	70.9
6	98.4	1.6	1.2	14.6	3.8	2.6	77.9
7	98.9	1.1	1.0	12.3	6.6	5.4	74.7
8	97.8	2.2	1.5	10.7	7.7	10.0	70.1
9	99.1	0.9	0.0	15.3	7.6	14.0	63.1
Averages							
Summer	95.4	4.6	2.2	35.7	6.5	5.1	50.7
Winter	98.3	1.7	1.0	13.3	5.5	7.3	73.0

[1] Hemoblasts arbitrarily included in white blood cell series.

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