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Morphology of Blood Cells of *Agama Stellio* (Linnaeus, 1758)

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Abstract:

Ten were chosen *Agama stellio* healthy adults, five males and five females, for this study. The morphological characteristics were examined because they are of peripheral blood cells. Been identified seven different types of blood cells, such as red blood cells, thrombocytes, Kperalmufoyat of, monocytes, heterophil, basophils, small lymphocytes. Lengths were measured and declined to these cells, and also to measure the lengths of red cell nuclei of *Agama stellio*. The red blood cells for females was (± 2.71 17.69 micron length x width 8.34 ± 2.31 microns). And for males (17.26 ± 2.61 length x width of 8.02 ± 2.02 micrometer microns). Httropeals the granules contain The spherical features with homogeneous content., Eosinophils of similar size to neutrophils or slightly larger. The subunit contains an average amount of light bluegray, cytoplasm darling smooth, oval nucleus. And A Thrombocytes were oval and often showed vacuolation.

Keywords: Blood cells, (*Agama*)*stellio*(Linnaeus,1758).

Introduction

Stellagama is a monotypic genus of agamid lizards containing the single species *Stellagama stellio* (Muro, et al.1998.; Jacobson E.R, Origi F. 2002). Common names for the species include *Stellion*, hardun, star lizard, painted dragon, starred *Agama*, sling-tailed agama and roughtail rock *Agama*. It is found in Greece, Southwest Asia, and Northeast Africa. Like many *Agamids*, *stellions* can change color to express their moods. They bask on stone walls, rocks, and trees. They are usually found in rocky habitats, and are quite shy, being very ready to dive into cracks to hide from potential predators. Most studies on hematology in saurian species dealt mainly with blood cell counts and cell sizes. (Hartman and Lesler 1964; Hutcheson and szarski 1965; szarski and czopek 1966; Duguay 1970; Mermer 1999; sevinçet al. 2000; sevinçet & uęurtaş 2001; atatüret al. 2001; tosunoęLuet al.2004; sevinçet al. 2004; sacchi et al.2007; ponsenet al. 2008; troiano et al.2008). other hematological parameters, such as hematocrit value, total hemoglobin concentration, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration, were rarely

studied (Harret al. 2001; cuaDraDoet al.2002; ponsenet al.2008; troiano et al.2008). Erythrocytes are morphologically similar among the various lizard species. However, it was reported that red blood cell count and size displayed significant individual and interspecies variation, and that this variation was also related to body weight, Data for the *agama stellio* have included red and white blood cell counts (.Ryerson DL.1943). erythrocyte size measurements (Hartman FA, et al. 1968). hematocrit, specific gravity, blood volume, plasma volume, and total body water Thorson (TB.1968). percent protein (Friar W. 1964). total protein, sialic acid (Seal US.1964) and calcium, sodium, magnesium, and cholesterol (Jackson OF.1991). However, little or no previous study has presented values for a widerange of blood variables for the *Agama stellio*. Clarification of the morphological characteristics and classification of blood cells, especially WBCs, from the *Agama stellio* is important because this species information could be used to assist those attempting to interpret WBC counts for clinical and research specimens in the wild.

Materials and Methods animals

Agama stellio collected adult males and females from different locations in the desert area, "Borg", 50 km west of Alexandria, Egypt. Ajami was arrested in the active season (July 2012). Ten animals (5 male and 5 female) during the summer and kept in large terrarium at a depth of 30 cm sand in the laboratory. The animals were decapitated with a sharp blade, blood was collected in a tube heparinized. (Fig.1)



Fig.(1). Photograph of the adult agama stellio (Adel A. Ibrahim and Moustafa H. El-Naggar. 2013)

Blood smears stained with Wright's stain were used in measuring the blood cells (erythrocytes, leukocytes and thrombocytes). Cell measurements were performed under a microscope with an ocular micrometer. erythrocytes were randomly chosen on each blood smear; cell lengths (eL) and widths (eW), together with the lengths (nL) and widths (nW) of their nuclei, were measured (μm). The measurements included maximal length (L) and maximal width (W). Sizes of cells were calculated according to the formula $(L \times W \times \mu) / 4$ (Ugurtas SH, 2003); Metin K, et. AI. 2006).

Statistical Analysis

Numerical Data are presented as mean \pm standard deviation (S.D.). Data were analyzed for statistical significance using student t test. P value of less than 0.05 indicates significance. Confidence intervals, at 95% level, were also calculated. All statistical analyses were accomplished with the statistical package for the social sciences (SPSS 9.0, SPSS Inc. Corp., Chicago, IL, USA) computer package.

RESULTS

Physical Parameters

Red Blood Cells Morphology

The erythrocytes of *Agama stellio* (Fig. 2a) Mature erythrocytes were ellipsoidal. The cytoplasm of mature erythrocytes appeared

light and dark pink. The nuclei of mature erythrocytes are chromophilic. They contain vacuoles (Fig. 2b). Erythrocyte measurements for females were (length $17.69 \pm 2.71 \mu\text{m} \times$ width $8.34 \pm 2.31 \mu\text{m}$) and for males were (length $17.26 \pm 2.61 \mu\text{m} \times$ width $8.02 \pm 2.02 \mu\text{m}$)

The nucleus was centrally. The measurements of the nucleus for were ($4.64 \pm 0.60 \mu\text{m} \times 2.93 \pm 0.67 \mu\text{m}$) and for females were ($4.81 \pm 0.61 \mu\text{m} \times 3.28 \pm 0.67 \mu\text{m}$) (Fig.3). Size means of the erythrocytes for was females ($136.60 \pm 49.52 \mu^2$) (Fig.3). ($134.94 \pm 43.88 \mu^2$) and for males ($136.60 \pm 49.52 \mu^2$) (Fig.3).

White Blood Cells Morphology Granulocytes

Granulocytes include the leukocytes that contain specific granules that identify the cell lineage. These cells include heterophils, eosinophils, and basophils

Heterophils—

The heterophil (Fig. 2c) reddish-orange granules in cytoplasm. These granules were spindle-shaped (with sharp poles). However, the shape was not always clearly visible, especially when the cytoplasm was filled with them. The eccentric placed nucleus of heterophil was round to oval pale blue and darker toward the centre

Heterophil measurements in males were (length $14.02 \pm 2.21 \mu\text{m}$ and width $11.98 \pm 2.09 \mu\text{m}$) and in females were (length $13.97 \pm 2.01 \mu\text{m}$ and width $11.35 \pm 2.03 \mu\text{m}$) (Fig. 3). Heterophils size means of the male was ($167.25 \pm 53.13 \mu^2$) and in the female was ($151.58 \pm 43.77 \mu^2$) (Fig.4).

Eosinophils

The eosinophil (Fig. 2d) were round cells and less numerous. Their granules were darker, more red and round. Nucleus was eccentric placed, uniform in colour, with clumped Chromatin. Its measurements in males were (length $12.97 \pm 1.33 \mu\text{m}$ and width $9.21 \pm 1.49 \mu\text{m}$) and in females were (length $12.683 \pm 1.59 \mu\text{m}$ and width $8.57 \pm 1.09 \mu\text{m}$) (Fig.3). Eosinophil size means of the male was ($117.37 \pm 27.75 \mu^2$) and in female was ($112.87 \pm 30.23 \mu^2$) (Fig. 4).

Basophils-

The basophil (Fig. 2e) was filled with round granules, which are large, prominent over

periphery. Colour varied from deep purple to deep blue or black. The nucleus was almost invisible, only chromatin. Its measurements in males were (length $10.67 \pm 1.16 \mu\text{m}$ and width $10.40 \pm 1.13 \mu\text{m}$) and in females were (length $11.04 \pm 1.29 \mu\text{m}$ and width $10.75 \pm 1.33 \mu\text{m}$) (Fig.3). Basophil size means of the male was ($109.42 \pm 18.97 \mu^2$) and in female was ($116.52 \pm 23.20 \mu^2$) (Fig. 4).

Mononuclear Cells- The mononuclear cells include lymphocytes and monocytes. These cells generally lack significant lobulation of the nucleus and do not contain an abundance of cytoplasmic granules.

Lymphocytes - The lymphocytes (Fig. 2f) They had compact dark nucleus and thin cytoplasm fringe of blue or violet colour. Its measurements in males were (length $7.64 \pm 2.03 \mu\text{m}$ and width $6.19 \pm 1.63 \mu\text{m}$) and in females were (length $8.58 \pm 2.01 \mu\text{m}$ and width $7.20 \pm 2.15 \mu\text{m}$) (Fig.3). Lymphocytes size means of the male was ($52.68 \pm 17.52 \mu^2$) and in female was ($67.57 \pm 22.52 \mu^2$) (Fig.4).

Monocytes - The monocyte (Fig. 2g). were cells with large quadratic nuclei with pale and fine chromatin. These cells had square shape, their cytoplasm was blue-gray, spacious, with or without vacuoles.. Monocytes in males were (length $13.15 \pm 2.21 \mu\text{m}$ and width $8.76 \pm 1.73 \mu\text{m}$) and in females were (length $13.48 \pm 2.51 \mu\text{m}$ and width $9.407 \pm 1.61 \mu\text{m}$) (Fig.3). Size means of the monocyte in male was ($112.85 \pm 43.87 \mu^2$) and in female was ($120.27 \pm 54.51 \mu^2$) (Fig.4).

Thrombocytes- The thrombocytes (Fig. 2h) The nucleus of was round to oval and dark. The cytoplasm of one form of thrombocytes was find sitting on the nucleus, or instead of that the membrane loosely frilled around the nucleus. The other form was oval with a good visible membrane and transparent cytoplasm. The thrombocytes in males were (length $4.67 \pm 0.80 \mu\text{m}$ x width $3.07 \pm 0.83 \mu\text{m}$) and in females were (length $4.77 \pm 0.88 \mu\text{m}$ and width $3.10 \pm 0.90 \mu\text{m}$) (Fig.3). Thrombocyte size means of the male was ($17.23 \pm 5.92 \mu^2$) and in female was ($17.75 \pm 6.38 \mu^2$) (Fig. 4).

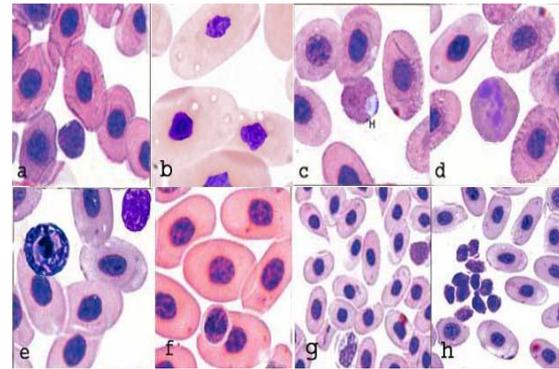


Fig. (2). Light micrograph of Wright's stained blood smears from the *Agama stellio* showing: (a) Erythrocytes, (b) Erythrocytes with prominent vacuoles, (c) Heterophil, (d) Eosinophil, (e) Basophil, (f) Lymphocyte, (g) Monocyte, (h) Clumps of thrombocytes. (Wright stain $\times 1000$).

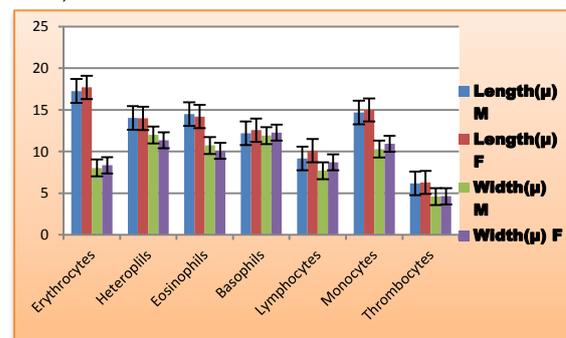
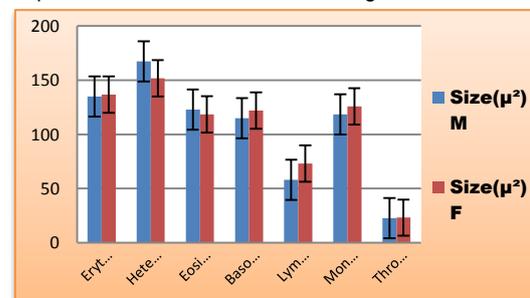


Fig (3) cell lengths (eL) and widths (eW), together with the lengths (nL) and widths (nW) of their nuclei, in males and in females. were measured (μm). *P value of less than 0.05 indicates a significant difference between the compared means of male and female *Agama*.



Fig(4) The measurements included maximal length (L) and maximal width (W), males and in females. were measured (μ^2). *P value of less than 0.05 indicates a significant difference between the compared means of male and female *Agama*.

DISCUSSION

This study attempt was to the morphological characteristics of the blood cells. The data presented will provide the veterinary clinician with specific hematological data which will allow for a more comprehensive medical assessment in *Agama stellio* clinical cases. Picea have a maximum total length of 25 to 30 cm. They have a rough scale structure. The ground color is dark black/blue with intensely colored dark yellow to orange spots which leads to bands on the tail (Avital E. 1981).

The head is almost totally black in males but can vary from grey to dark grey in females. The males have a grayish belly and throat Werner (1998, 1992). (The picture on the right is a male)(Fig. 5).



(Fig. 5). *Agama stellio* .males and females, (The picture on the right is a male). (Alel A. Ibrahim and Moustafa H. El-Nagar. 2013).

Hematology and bloodbiochemical parameters are essential to assess the health and physiological status of stoic animals like the *agama stellio* (Khalifa, S.A. 2003).several authors (saintgiron 1970; sevinçet al. 2000; sevinç and uğurtaş2001; atatüret al. 2001; ponsenet al. 2008), who studied the blood of lizards, reported species-specific variations concerning both erythrocyte and leukocyte counts. sevinçet al. (2004) and MerMer (1999) reported no sexual dimorphism in blood cell counts in the gecko and scincid species studied. our results did not show any sexual differences in terms of erythrocyte counts.erythrocytes play an important role in oxygen and carbon dioxide transport. the erythrocytes' surface-area-to-volume ratio is a determining factor in their capacity of oxygen and carbon dioxide exchange in the tissues in that a small erythrocyte is relatively more efficient in gas exchange than a larger one (HartMan LessLer 1964; sevinçet al. 2000). our results are not in agreement with paL et al. 2008. according to our observations szarski czopek (1966) found no differences in the total hemoglobin between male and female *Agama*. However, the results of the present study showed that total hemoglobin, Mean cell Hemoglobin and Mean cell Hemoglobin

concentration were different between males and females.

II. Erythrocytes

The erythrocytes or red blood corpuscles of the circulating blood are Nucleated, oval cells, rounded at their. Their nuclei are also oval, more or less regular, and centrally located; their long axes lie parallel to those of the cells. In blood smears stained by the classic May-Grünwald-Giemsa technique, the yellowish cytoplasm most often appears translucent and homogeneous. The nuclei of mature erythro- *In the lizard *Acanthodactylus erythrums* the long axes of more than 50% of the nuclei deviate from those of the cells in the only specimen of this form that I have studied.

2. MORPHOLOGY OF THE CIRCULATING BLOOD CELLS 75 cytes are chromophilic. The masses of chromatin are more or less visible, depending on the age of the cells. The most critical staining permits the demonstration of granules within the cytoplasm (Hirschler, 1928; Ryerson, 1949) and of the Golgi apparatus (Bhattacharya and Brambell, 1924).

The circulating blood contains immature cells of the erythrocytic series (basophilic and polychromatophilic normoblasts) characterized by a rounded form, blue cytoplasm, and a large nucleus which is less chromophilic than that of a mature erythrocyte. These cells are especially common in young or moulting animals (Saint Girons, 1961) or ones heavily infected by hemoparasites (Pienaar, 1962). The senile forms of the red blood cells are larger than the normal erythrocytes. Their cytoplasm stains weakly, and their nuclei are pycnotic and often irregularly shaped. In the final stage, the cytoplasm disappears, and only the nucleus is visible in a smear. These different stages have been studied in detail in one turtle (Jordan and Flippen, 1913), one lizard (Pienaar, 1962), and two snakes (Slonimski, 1934). the erythrocytes and their nuclei become more circular (Wood, 1935). In lizards from Mexico and Florida, the presence of *Plasmodium* spp. within the corpuscles affected the dimensions of the cells, causing a more or less marked increase in their size (Thomson and Huff, 1944 and Bergman, 1957; Reichenbach-Klinke, 1963).

III. Eosinophilic Granulocytes

The eosinophilic granulocytes of all reptiles are large, generally rounded cells in which the variably shaped nuclei lie near the periphery. The nuclei stain rather weakly with the May-Grunwald-Giemsa technique. The cytoplasm contains granules that are usually highly chromophilic and appear a more or less brilliant yellow-brown. Loewenthal (1931) reported only a weak staining reaction of the granules in *Anguis fragilis*. Two types of eosinophils may be distinguished on the basis of the shapes of the intracytoplasmic granules which may be cylindrical or, more commonly, spherical. These two types have been reported in all the orders of reptiles: Testudines (Loewenthal, 1930; Ryerson, 1943), Rhynchocephalia (Komocki, 1936), Squamata (Pena Roche, 1939; Pienaar, 1962; Loewenthal, 1928, *Vipera aspis*), and Crocodylia (Slonimski, 1935; Ryerson, 1943). Bernstein (1938) differentiated three types of eosinophils in *Psammobates (Testudo auct.) geometricus* based on the affinity of the cytoplasmic granules for eosin. A recent study presents histochemical and ultrastructural data on these cells in *Lacerta agilis* and *Emys orbicularis* (Kelenyi and Nemeth, 1969).

The size of these cells is very variable, not only between species, but even in one animal and in a single blood smear. Because of this variation, the data presented which gives the maximum, mean, and minimum lengths of the greatest diameter, can only be taken as indications, valuable only in that a certain number of species belonging to different orders may be compared. Surprisingly the average sizes are nearly similar in all cases. The eosinophilic granulocytes of *Sphenodon punctatus*, unlike its erythrocytes, are among the smaller ones. Lizards have small eosinophils, snakes the largest ones, and turtles and crocodylians are intermediate in this regard. The intracytoplasmic granules vary in size, reaching two to three micra in their largest diameter. The density of the granulation may obscure the position and form of the nucleus. The latter is often multilobate, but the number of lobes does not appear to be very high.

IV. Basophilic Granulocytes The basophilic granulocytes are very easy to recognize. In all the species studied, they are small, circular or nearly circular corpuscles.

2 Morphology of the circulating blood cells

plasm is usually filled with extremely chromophilic granules. In a blood smear, they resemble mulberries. The granulation is so dense that the weakly staining nucleus may only rarely be seen (Michels, 1923). In some species, including *Lacerta viridis* and *Typhlops punctatus*, the basophils are slightly oval.

The interspecific variation in the size of the basophilic granulocytes is less than that of the eosinophils. They are smallest in the lizards, especially *Lacerta*, larger in snakes, still larger in turtles and crocodylians, and largest in *Sphenodon punctatus*. Michels (1923) Largest Diameters (in micra) of Basophilic Granulocytes in some Representatives of the Different Orders of Reptiles (after Saint Girons and Duguy, 1963, with supplements V. Azurophilic Granulocytes

The presence of azurophilic granulocytes in reptilian blood was first reported by Pappenheim (1909). Although they are, in most cases, difficult to differentiate by the May-Grunwald-Giemsa technique, they may be characterized by the possession of granules that stain with pure azure dyes and with the pyronin of methylgreen-pyronin mixtures (Pienaar, 1962). Hematologists have attributed them to different leucocytic series, most often to the monocytic series (Zylberszac, 1937; Ryerson, 1949) or the neutrophilic granulocytic series (Loewenthal, 1930; Slonimski, 1934; Bernstein, 1938). These cells have relatively small, irregular, eccentric nuclei. They vary greatly in size. Azurophilic granulocytes usually contain lipid bodies in the cytoplasm, but those may be absent, as in *Cordylus* (Pienaar, 1962). Senile cells contain many vacuoles.

VI. Neutrophilic Granulocytes

The neutrophilic granulocytes are circular or oval cells that frequently form groups of two or three cells beside each other in blood smears (Pienaar, 1962). Their name refers to their staining reactions only; they are probably not homologous to the neutrophils of mammals. The small, irregular nucleus lies near the periphery of the cell. The neutrophils may be regarded as morphological variants of the azurophilic granulocytes with which they have often been confused. They have also been described as heterophils; the fact that this last name has also been applied to the eosinophilic granulocytes with cylindrical granules only

adds to the nomenclature confusion (Ryerson, 1949).

VII. Lymphocytes

Lymphocytes form the most important class of leucocytes if, as many believe, they retain all of their potential to differentiate into the other blood cells while they are circulating in the blood. They show a large range in size, and the distinction between "large" and "small" lymphocytes is most often arbitrary. In *Cordylus vittifer*, Pienaar (1962) divided this category into large lymphocytes in which the diameter is at least 14.5 μ and small lymphocytes with diameters of 5.5 to 10 μ . Lymphocytes are characterized by transparent, moderately to weakly basophilic nuclei. The nuclei are circular and centrally located in small lymphocytes, but more irregular in large ones. Chromophobic vacuoles and some azurophilic granules occur in the cytoplasm. In *Ranapiens* there is a direct genetic relation between lymphocytes and monocytes (Jordan, 1925); all gradations occur, in morphology, physiology, and staining properties, between small lymphocytes and large monocytes.

The situation is probably the same in reptiles (Pienaar, 1962).

VIII. Plasma Cells

The plasma cells, which are usually rare in the circulating blood, may arise from medium-sized or large lymphocytes. They are characterized by an eccentric nucleus, opaque basophilic cytoplasm, and a perinuclear halo of hyaloplasm.

IX. Monocytes

Monocytes have been confused with several other types of cells and may not always be distinct (Fig. 12). In *Cordylus* Pienaar (1962) considered them as a type of azurophilic granulocyte although, in his table of terms, he treats them as a distinct series. They are generally rare and have markedly irregular shapes. Their nuclei vary in shape, but are frequently polymorphous.

X. Thrombocytes

The thrombocytes are small, oval cells characterized by elongate, centrally located, highly chromophilic nuclei. The nucleocytoplasmic ratio is especially high, and the cytoplasm appears as a narrow border around the nucleus. The cytoplasm is almost colorless (faintly acidophilic) and hence difficult to see in a blood smear. It frequently contains some azurophilic granules. Pienaar (1962) described a large acidophilic granule at

one pole of the nucleus in thrombocytes of the turtle *Pelomedusa subrufa*. Thrombocytes are fragile cells, and in blood smears their very viscous cytoplasm is often lost. The nuclei are then clumped in groups which may contain ten or more elements.

XI. Parasites in the Blood of Reptiles

A. GENERAL

Although a detailed treatment of the parasites in the blood is outside the scope of this chapter, a brief mention of them may be appropriate here since they can be seen in blood smears and may affect the morphology of the different blood cells. The literature on the parasites in reptilian blood is very extensive, with papers treating hemogregarines being especially numerous. So as not to overload the text, I have cited only the most important or especially interesting works. The recent papers of Pienaar (1962) and Reichenbach-Klinke (1963) contain extensive bibliographies. (A detailed treatment of the various reptilian parasites will furthermore be included in the volume on Ecology). Parasites of the blood may be divided into two classes depending on whether they infect the blood without attacking the corpuscles or whether they become established within the corpuscles.

Acknowledgements

This study is the first attempt to characterize the health status of the *Agama stellio*. In general, our findings suggest that these agamas are in good health. However, some of our findings suggest that these keystone species should continue to be monitored to protect them into the future.

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