The Morphology and Size of Blood Cells of *Lacerta rudis bithynica*

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Abstract.- In this study, the morphology of the blood cells of *Lacerta rudis bithynica* is described using Wright's technique. The sizes of erythrocytes and their nuclei, leukocytes (monocytes, lymphocytes, basophils, neutrophils, and eosinophils), and thrombocytes of *L. rudis bithynica* were measured using an ocular micrometer at a magnification of 1600X with an oil immersion objective. The results of this study are compared with previous work on other reptile species.

Key words.- Lacertidae, *Lacerta rudis bithynica*, erythrocyte, leukocyte, thrombocyte, measurement, morphology.

Introduction

The first studies on the blood of reptiles described the cellular structures, often comparing them with those of other vertebrates. Literature on the haematology of reptilian blood is based on few studies and is usually concerned with European species (Saint Girons, 1970).

Recent studies have concentrated on single species (*Tiliqua* sp., Cannon et al., 1988; *Cyrtopodion scabrum*, Canfield and Shea, 1996). Various authors have described different circulating blood cells of different reptile species (Taylor and Kaplan, 1961; Heady and Rogers ,1962; Hartman and Lessler, 1964; Szarski and Czopek, 1966; Duguy, 1970; Saint Girons, 1970, Cannon et al., 1988, Canfield and Shea, 1996). Other authors have studied seasonal (Hutton, 1960; Cline and Waldman, 1962; Haggag and et. al, 1966) or sexual (Altland and Thompson, 1962) variation in the number of blood cells of different reptile species. In addition, authors have studied the number of blood cells of different reptile species (Charipper and Davis, 1932; Baker and Cline, 1932; Hutton, 1961; Altland and Thompson, 1962; Hutchinson and Szarski, 1965; Engbretson and Hutchinson, 1976). Finally, authors have studied haemoglobin and hematocrit content of blood and hematopoiesis of different reptile species (Altland and Thompson, 1958; Hutton, 1961; Goin and Crawford, 1965; Engbretson and Hutchinson, 1976; Newlin and Ballinger, 1976).

In Turkey, haematological studies have been conducted on humans and economically important animals. However, there are no haematological studies on the Turkish reptiles.

In this study, our aim is to describe and measure blood cells (erythrocyte, leukocyte, thrombocyte) of *Lacerta rudis bithynica* (Squamata: Lacertidae). This study is the first of its kind on a Turkish species.

Material and Methods

In this study, 31 individuals (17 male) of *Lacerta rudis bithynica* (Squamata: Lacertidae) were examined. The study was carried out between June and August 1998. The specimens were collected from Uludağğ (Bursa) at an altitude of 1745 m. Blood was obtained by cardiac puncture of the lizards (Canfield and Shea, 1988). Immediately after the blood was obtained in heparinized capillary tubes, the blood smears were prepared. Three to five blood smears were prepared per individual. The smears were air-dried and stored until stained with Wright's stain (Hartman and Lessler, 1964). Twelve drops of Wright's stain were dropped on the slides and allowed to remain on the slide one and a half minutes before rinsing with a phosphate buffer (pH=6.5). The slides were allowed to stand for ten minutes at room temperature and were then washed with distilled water and allowed to dry.

On each slide fifty mature erythrocytes and their nuclei, ten thrombocytes, and ten leukocytes (monocyte, lymphocyte, eosinophil, basophil and neutrophil) were measured by means of an ocular micrometer at a magnification of 1600 x with an oil immersion objective. Cell sizes were calculated from the measurements.

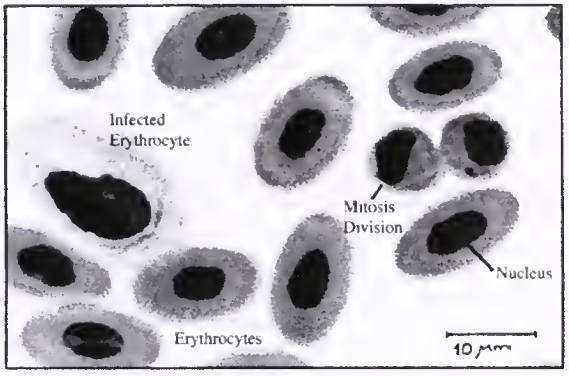

Figure 1. Erythrocytes, an infected erythrocyte and a mitosis dvision.

Results

I. Erythrocytes

Erythrocytes are nucleated, oval cells. Their nuclei are also oval, more or less regular and centrally located (Fig. 1). The cytoplasm of mature erythrocyte appeared light and dark pink and homogeneous under Wright's stain. The nuclei of mature erythrocytes are chromophilic. In some blood smears, immature erythrocytes are seen. They are characterised by a rounded form, blue cytoplasm and a large nucleus. Mitotic figures are also present and in some smears, intracorpuscular parasites are seen (Fig. 1). Parasites alter the shape and size of erythrocytes remarkably. When intracorpuscular parasites are seen, immature erythrocytes and mitotic figures are abundant (Fig 1). Intracorpuscular parasites alter the shape and size of infected erythrocytes. The shape and size of other erythrocytes that are not infected by intracorpuscular parasites are normal.

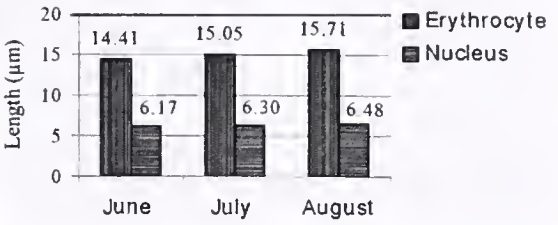

Figure 2. Erythrocyte and nucleus lengths of *Lacerta rudis bithynica* over three months.

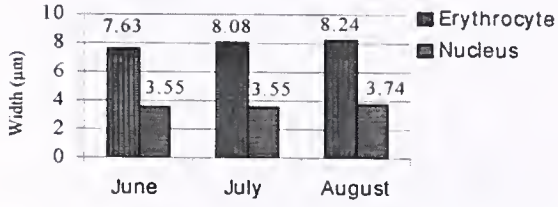

Figure 3. Erythrocyte and nucleus widths of *Lacerta rudis bithynica* over three months.

In June mean length of mature erythrocytes was 14.41 μm (±0.77 standard deviations, with a range of 12.20–16.47 μm). In July, the mean length of mature erythrocytes was 15.05 μm (±0.79, 12.81–17.08 μm). In August, the mean length of mature erythrocytes was 15.71 μm (±0.79, 12.81–18.30 μm). Other measurements are given in Tables 1, 2 and 3. There are no significant differences in erythrocyte and nucleus sizes between females and males. Based on Tables 1, 2, and 3 and Figs. 2 and 3, it appears that there were little monthly variations in erythrocyte and nucleus sizes among June, July and August.

II. Leukocytes

1. Eosinophils. In blood smears stained by Wright technique, eosinophils are circular, and the cytoplasm

Table 1. Erythrocyte dimensions of *Lacerta rudis bithynica* with the standard deviations in June. EL: Erythrocyte length; EW: Erythrocyte width; ES: Erythrocyte size;NL: Nucleus length; NW: Nucleus width; NS: Nucleus size.

	EL (μm)	EW (μm)	EL/EW	ES (μm²)	NS/ES
Maximum	16.47 ± 0.77	9.15 ± 0.48	2.27 ± 0.13	110.41 ± 8.00	0.30 ± 0.02
Minimum	12.20 ± 0.77	6.71 ± 0.48	1.42 ± 0.13	64.26 ± 8.00	0.13 ± 0.02
Mean	14.41 ± 0.77	7.63 ± 0.48	1.89 ± 0.13	86.46 ± 8.00	0.20 ± 0.02
	NL (μm)	**NW (μm)**	**NL/NW**	**NS (μm²)**	
Maximum	7.32 ± 0.40	4.88 ± 0.34	2.20 ± 0.19	24.53 ± 2.19	
Minimum	4.88 ± 0.40	3.05 ± 0.34	1.25 ± 0.19	11.68 ± 2.19	
Mean	6.17 ± 0.40	3.55 ± 0.34	1.75 ± 0.19	17.25 ± 2.19	

Table 2. Erythrocyte dimensions of *Lacerta rudis bithynica* together with the standard deviations in July. EL: Erythrocyte length; EW: Erythrocyte width; ES: Erythrocyte size; NL: Nucleus length; NW: Nucleus width; NS: Nucleus size.

	EL (μm)	EW (μm)	EL/EW	ES (μm^2)	NS/ES
Maximum	17.08 ± 0.79	9.15 ± 0.49	2.16 ± 0.12	122.68 ± 8.95	0.28 ± 0.02
Minimum	12.81 ± 0.79	6.71 ± 0.49	1.53 ± 0.12	67.47 ± 8.95	0.13 ± 0.02
Mean	15.05 ± 0.79	8.08 ± 0.49	1.86 ± 0.12	95.62 ± 8.95	0.18 ± 0.02
	NL (μm)	**NW (μm)**	**NL/NW**	**NS (μm^2)**	
Maximum	7.30 ± 0.70	4.27 ± 0.32	2.40 ± 0.17	24.53 ±2.38	
Minimum	5.40 ± 0.70	3.05 ± 0.32	1.33 ± 0.17	13.14 ± 2.38	
Mean	6.30 ± 0.70	3.55 ± 0.32	1.79 ± 0.17	17.67 ± 2.38	

is stained light red. Eosinophils contain circular to elongate cytoplasmic granules stained brilliant red (Fig 4). Eosinophils are different from neutrophils in that, eosinophils' granules are stained bright red and neutrophils' granules were stained dim red.

In June, the mean diameter of eosinophils was 12.82 μm, (±1.71 standard deviations, with a range of 9.93–15.25 μm). In July, the mean diameter was 13.29 μm (±1.25, 10.98-15.25 μm). In August, the mean diameter was 13.80 μm (±1.37, 12.20-15.25 μm).

There were no significant differences in eosinophil diameters between females and males. Based on Tables 4, 5, and 6 and Fig. 8, it appears that there was little monthly variation in the diameter of eosinophils during the three months.

2. Basophils. Basophils are easily recognised. They are small and circular cells. Nuclei stained blue by Wright technique are commonly obscured by chromophilic circular granules. These cytoplasmic granules are large and stained dark purple. In the blood smears, they resemble mulberries (Fig. 5). The granules are so dense that nucleus stained dim blue is rarely seen.

In June, the mean diameter of basophils was 8.55 μm, (±0.61 standard deviations, with a range of 7.32–9.15 μm). In July, the mean diameter was 9.02 μm (±0.24, 8.54–9.15 μm). In August, the mean diameter was 9.00 μm (±0.45, 7.93 –10.37 μm).

There were no significant differences in basophil diameters between females and males. Based on Tables 4, 5, and 6 and Fig. 8, it appears that there was little monthly variation in the diameter of basophils during the three months.

3. Neutrophils. Neutrophils are circular cells like eosinophils (Fig. 6). These cells are also called as heterophils. They have cytoplasmic granules. The granules are circular and stained dim red. Cytoplasm is stained light red.

In June, the mean diameter of neutrophils was 10.15 μm, (±1.19 standard deviations, with a range of 9.15-13.42 μm). In July, the mean diameter was 10.49

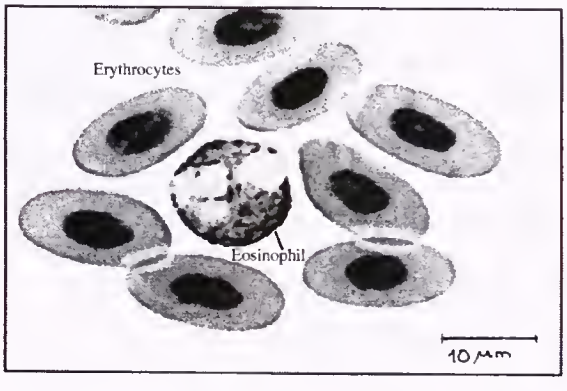

Figure 4. Erythrocytes and an eosinophil.

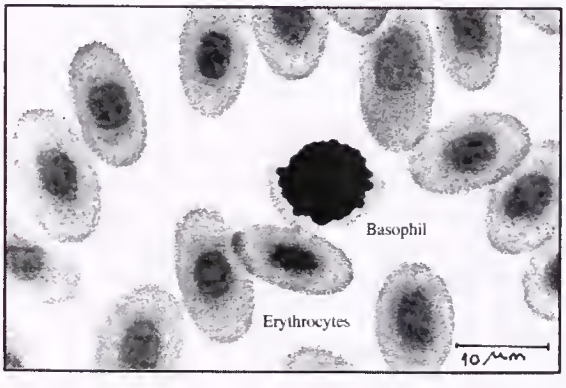

Figure 5. Erythrocytes and a basophil.

Table 3. Erythrocyte dimensions of *Lacerta rudis bithynica* together with the standard deviations in August. EL: Erythrocyte length; EW: Erythrocyte width; ES: Erythrocyte size; NL: Nucleus length; NW: Nucleus width; NS: Nucleus size.

	EL (µm)	EW (µm)	EL/EW	ES (µm^2)	NS/ES
Max	18.30 ± 0.76	9.76 ± 0.48	2.33 ± 0.12	140.20 ± 8.96	0.30 ± 0.02
Min	12.81 ± 0.76	6.71 ± 0.48	1.57 ± 0.12	73.60 ± 8.96	0.12 ± 0.02
Mean	15.71 ± 0.76	8.24 ± 0.48	1.91 ± 0.12	101.72 ± 8.96	0.18 ± 0.02
	NL (µm)	NW (µm)	NL/NW	NS (µm^2)	
Max	7.93 ± 0.51	4.27 ± 0.31	2.40 ± 0.20	24.53 ± 2.20	
Min	4.88 ± 0.51	3.05 ± 0.31	1.28 ± 0.20	11.68 ± 2.20	
Mean	6.48 ± 0.51	3.74 ± 0.31	1.74 ± 0.20	19.05 ± 2.20	

Table 4. Leukocyte and thrombocyte measurements of *L. rudis bithynica* with the standard deviations in June.

	Lymphocyte (µm)	Monocyte (µm)	Neutrophil (µm)	Basophil (µm)	Eosinophil (µm)	Thrombocyte Length (µm)	Thrombocyte Width (µm)
Max	8.54 ± 0.81	12.81 ± 1.23	13.42 ± 1.19	9.15 ± 0.61	15.25 ± 1.71	7.32 ± 0.49	4.88 ± 0.54
Min	4.27 ± 0.81	9.32 ± 1.23	9.15 ± 1.19	7.32 ± 0.61	9.93 ± 1.71	4.88 ± 0.49	3.05 ± 0.54
Mean	6.12 ± 0.81	11.10 ± 1.23	10,15 ± 1.19	8.55 ± 0.61	12.82 ± 1.71	6.12 ± 0.49	3.72 ± 0.54

Table 5. Leukocyte and thrombocyte measurements of *L. rudis bithynica* with the standard deviations in July.

	Lymphocyte (µm)	Monocyte (µm)	Neutrophil (µm)	Basophil (µm)	Eosinophil (µm)	Thrombocyte Length (µm)	Thrombocyte Width (µm)
Max	7.32 ± 0.41	15.25 ± 1.5.0	12.20 ± 0.97	9.15 ± 0.24	15.25 ± 1.25	7.32 ± 0.52	4.88 ± 0.42
Min	6.10 ± 0.41	9.15 ± 1.50	8.54 ± 0.97	8.54 ± 0.24	10.98 ± 1.25	6.10 ± 0.52	3.66 ± 0.42
Mean	6.62 ± 0.41	11.46 ± 1.50	10.49 ± 0.97	9.02 ± 0.24	13.29 ± 1.25	6.62 ± 0.52	4.08 ± 0.42

Table 6. Leukocyte and thrombocyte measurements of *L. rudis bithynica* with the standard derivations in August.

	Lymphocyte (µm)	Monocyte (µm)	Neutrophil (µm)	Basophil (µm)	Eosinophil (µm)	Thrombocyte Length (µm)	Thrombocyte Width (µm)
Max	9.15 ± 0.58	15.25 ± 1.29	12.20 ± 0.97	10.37 ± 0.45	14.03 ± 1.37	7.32 ± 0.35	5.49 ± 0.49
Min	6.10 ± 0.58	9.15 ± 1.29	8.54 ± 0.97	7.93 ± 0.45	12.20 ± 1.37	5.49 ± 0.35	3.05 ± 0.49
Mean	6.53 ± 0.58	11.21 ± 1.29	10.77 ± 0.97	9.00 ± 0.45	13.80 ± 1.37	6.33 ± 0.35	4.22 ± 0.49

µm (±0.97, 8.54-12.20 µm). In August, the mean diameter was 10.77 µm (±0.97, 8.54-12.20 µm).

There were no significant differences in neutrophil diameters between females and males. Based on Tables 4, 5, 6 and Fig.8, it appears that there was little monthly variation in diameter of neutrophils during the three months.

4. Monocytes . Monocytes are round cells with round nuclei. The cytoplasm is stained blue and the nucleus is stained purple by Wright's technique. The monocyte's cytoplasm is more abundant than lymphocyte's cytoplasm. Nuclei vary in shape (Fig. 7). Nuclei may be nodular, but they are not lobular like granulocytes. Sometimes nuclei are horseshoe-shaped.

In June, the mean diameter of monocytes was 11.10 µm, (±1.23 standard deviations, with a range of 9.32-12.81 µm). In July, the mean diameter was 11.46 µm (±1.50, 9.15-15.25 µm). In August, the mean diameter was 11.21 µm (±1.29, 9.15-15.25 µm).

There were no significant differences in monocyte diameters between females and males. Tables 4, 5, 6 and Fig. 8 show that there was little monthly variation in the diameter of monocytes during the three months.

5. Lymphocytes . Lymphocytes are round cells like monocytes, but smaller (Fig. 7). The nuclei contain many parts of the cell. The nucleus is stained purple, cytoplasm, rarely seen, is stained blue.

In June, the mean diameter of lymphocytes was 6.12 µm, (±0.81 standard deviations, with a range of 4.27-8.54 µm). In July, the mean diameter was 6.62 µm (±0.41, 6.10 – 7.32 µm). In August, the mean diameter was 6.53 µm (±0.58, 6.10-9.15 µm).

There were no significant differences in lymphocyte diameters between females and males. Based on Tables 4, 5, 6 and Fig. 8, it appears that there was little monthly variation in diameter of lymphocytes during the three months.

III. Thrombocytes

Thrombocytes are small cells like lymphocytes, but they are oval and smaller than lymphocytes (Fig. 9). Their nuclei are highly chromophilic and stained purple. Cytoplasm is rarely seen.

In June, the mean length of thrombocytes was 6.12 µm (±0.49 standard deviations, with a range of 4.88-7.32 µm). In July, the mean length of thrombocytes

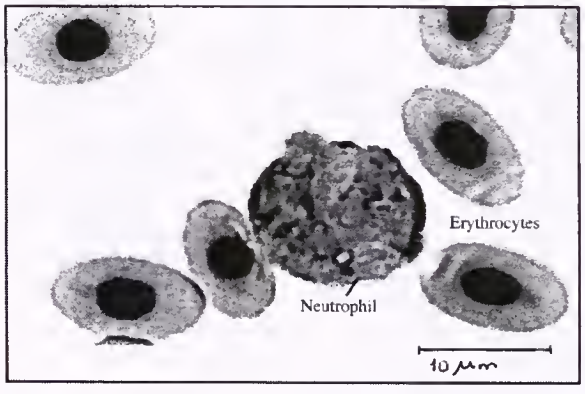

Figure 6. Erythrocytes and a neutrophil.

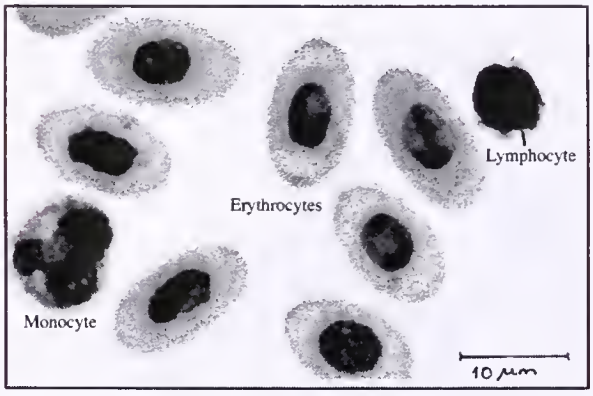

Figure 7. Erythrocytes, a monocyte and a lymphocyte.

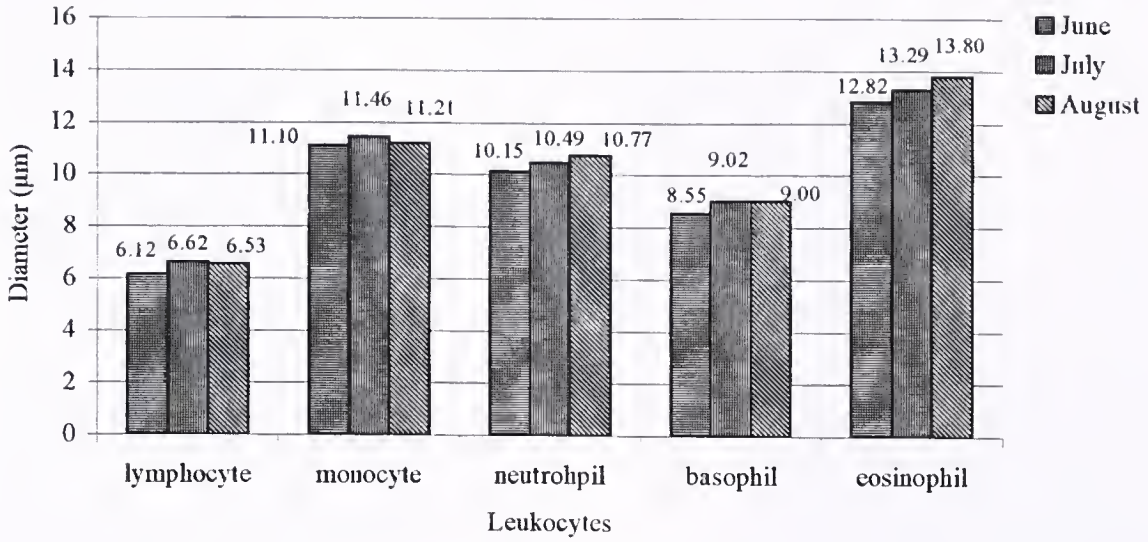

Figure 8. Leukocyte diameters of *Lacerta rudis bithynica* over three months.

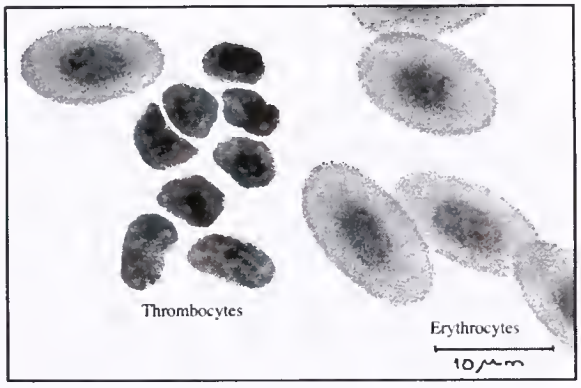

Figure 9. Erythrocytes and thrombocytes.

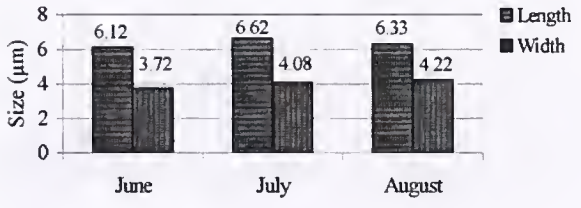

Figure 10. Thrombocyte sizes of *Lacerta rudis bithynica* over three months.

was 6.62 µm (±0.52, 6.10-7.32 µm). In August, the mean length of thrombocytes was 6.33 µm (0.35, 5.49-7.32 µm).

In June, the mean width of thrombocytes was 3.72 (±0.54 standard deviations, with a range of 3.05-4.88 µm). In July, the mean width of thrombocytes was 4.08 µm (±0.42, 3.66-4.88 µm). In August, the mean width of thrombocytes was 4.22 µm (0.49, 3.05-5.59 µm).

There were no significant differences in thrombocytes sizes between females and males. Based on Tables 4, 5, 6 and Fig. 10, it appears that there was little monthly variation in thrombocytes sizes during the three months.

Discussion

Investigations carried out by various authors (Hartman and Lessler, 1964; Szarski and Czopek, 1966; Saint Girons, 1970) reported that the sizes of the erythrocytes vary in members of the four orders of reptiles. Within the class Reptilia, the largest erythrocytes are seen in *Sphenedon punctatus*, turtles and crocodilians. The erythrocytes of lizards vary greatly in size depending on the family and sometimes even within one family (Saint Girons, 1970). The smallest erythrocytes are found in the lizard family Lacertidae (Saint Girons, 1970).

In the present study, erythrocyte morphology and the results of erythrocytes sizes (Table 1, 2 and 3) are agreement with the other results carried out by Hartman and Lessler (1964), Szarski and Czopek (1966), and Saint Girons (1970).

In one of the studies on the leukocytes of the rough tail Gecko *Cyrtopodion scabrum*, a bright-field and phase-contrast study Cannon et al. (1996),

reported that the neutrophils were not observed, but the other leukocytes were observed.

Another study on morphological observations on the erythrocytes, leukocytes and thrombocytes of blue tongue lizards by Canfield and Shea (1988) reported that all types of leukocytes were observed.

Saint Girons (1970) and Canfield and Shea (1988) divided granulocytes into neutrophils, basophils and eosinophils on the basis of light microscopy. However Cannon et al. (1996) divided granulocytes into basophils and eosinophils on the basis of bright-field and phase-contrast microscopy.

Heady and Rogers (1962) divided leukocytes into neutrophils, small acidophils, eosinophils, lymphocytes and monocytes on the basis of light microscopy in *Pseudemys elegans.* They, except for monocytes, gave the sizes of leukocytes and reported that eosinophils and neutrophils were numerous than the other leukocytes. Taylor and Kaplan (1961) also divided leukocytes into neutrophils, basophils, eosinophils, lymphocytes and monocytes on the basis of light microscopy in turtles.

In this study, it appears that on the basis of light microscopic findings there are three main types of granulocytes and two types of agranulocytes in *L. rudis bithynica* and also the size of all kinds of leukocytes are given in Table 4, 5 and 6.

In the present study, the descriptions and sizes (Table 4, 5 and 6) of thrombocytes are comparable to other descriptions. Canfields and Shea (1988) reported that thrombocyte morphology at the light microscopic level is influenced by the degree of aggregation and degranulation. Saint Girons (1970) reported that thrombocytes are small, oval cells characterised by elongate, centrally located highly chromophilic nuclei. The cytoplasm is almost colourless (faintly acidophilic) and hence difficult to see in a blood smears. Taylor and Kaplan (1961) reported the same findings in turtles.

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