

The blood cells of West Anatolian  
*Lissotriton vulgaris*  
(LINNAEUS, 1758)

A number of studies deal with blood cell counts and cell sizes in urodela (VERNBERG 1955; SZARSKI & CZOPEK 1966; JERRETT & MAYS 1973; KURAMOTO 1981; ATATÜR et al. 1998). Various environmental factors are known to effect erythrocyte size (VERNBERG 1955; DUGUY 1970), besides, the size of erythrocytes appears to be related to general metabolic activity.

The present work provides counts, sizes and photomicrographs of the blood cells of *L. vulgaris* (LINNAEUS, 1758) from an apparently healthy, uncontaminated population.

Thirty specimens of *L. vulgaris* (15 ♂♂, 15 ♀♀) were collected from the vicinity of Çanakkale (NW Turkey) at an altitude of 120 m during the species' breeding season (26th April, 2003). Blood samples of the live specimens were obtained in the laboratory within one day of their capture by means of ventricular puncture, via heparinized hematocrit capillaries. The fixation of the material was made according to OLGUN & BARAN (1993).

The blood cells were counted in a Neubauer hemocytometer. The standard

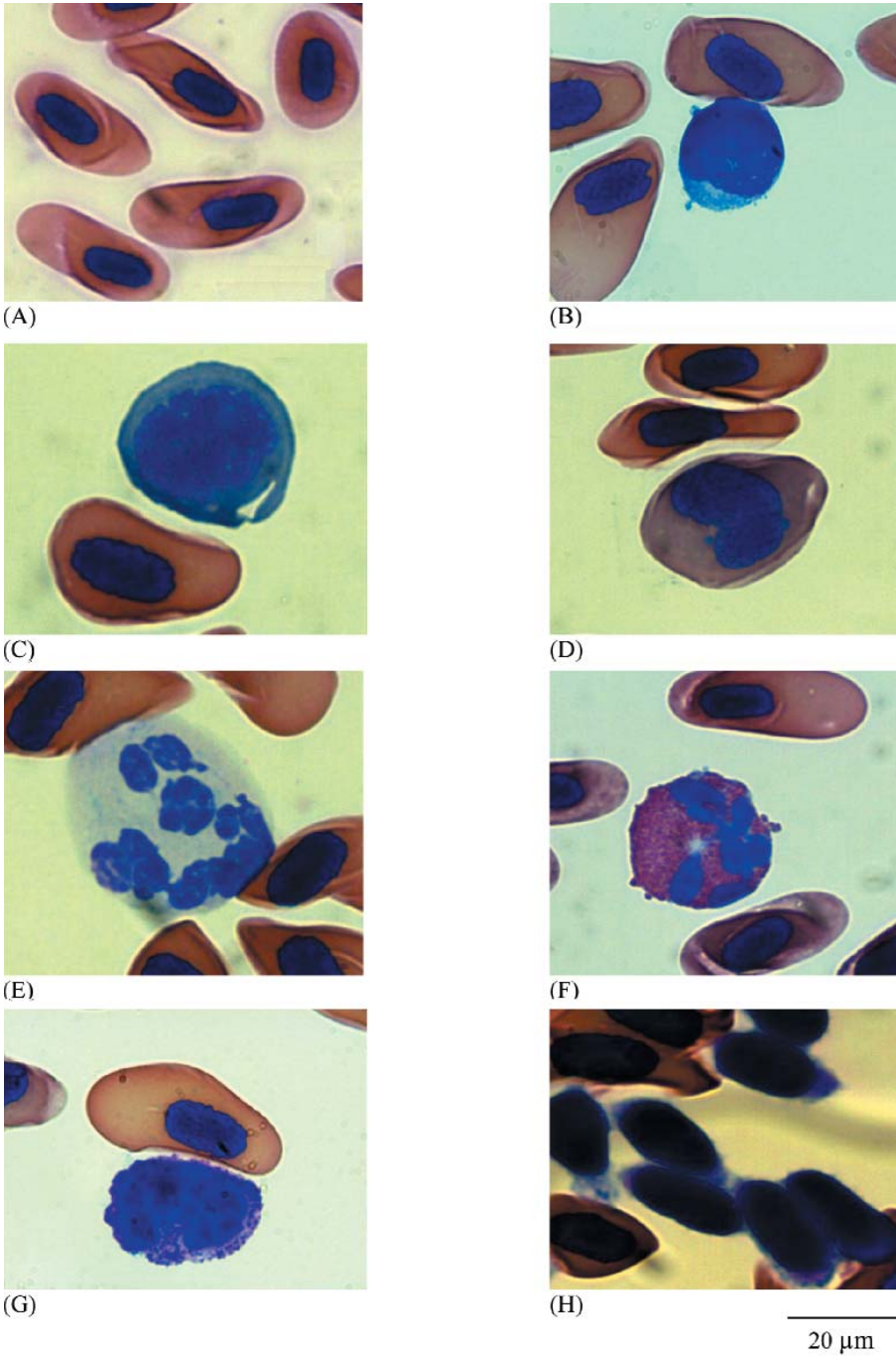


Fig. 1: The blood cells of *Lissotriton vulgaris* (LINNAEUS, 1758).  
 A - erythrocytes, B - small lymphocyte, C - large lymphocyte, D - monocyte, E - neutrophil leukocyte,  
 F - eosinophil leukocyte, G - basophil leukocyte, H - thrombocytes.

Table 1: The established counts [per mm<sup>3</sup>], measurements [in  $\mu\text{m}$ ] and sizes [in  $\mu\text{m}^2$ ] concerning the blood cells of West Anatolian *Lissotriton vulgaris* (LINNAEUS, 1758). N - Number of specimens; n - Number of measurements/ computings in each specimen; Ext - extreme values; SD and SE - standard deviation and standard error of the mean.

Blood Cells / Blutzellen	N	n	Ext	Mean/Mittel	SD	SE
Erythrocyte Count / Erythrozytenzahl	15	3	120000-220000	173670	27802.02	7178.45
Leukocyte Count / Leukozytenzahl	15	3	1360-2300	1948	278.39	71.88
Erythrocyte Length (EL) / Erythrozytenlänge	20	40	28.31-34.87	31.19	1.80	0.40
Erythrocyte Width (EW) / Erythrozytenbreite	20	40	15.21-20.00	17.18	1.23	0.27
Erythrocyte Size (ES) / Erythrozytenfläche	20	40	352.20-508.77	421.35	44.22	9.88
Ery Nucleus Length (NL) / Länge des Ery-Zellkerns	20	40	12.62-16.37	13.88	0.98	0.22
Ery Nucleus Width (NW) / Breite des Ery-Zellkerns	20	40	7.31-10.50	8.19	0.89	0.19
Ery Nucleus Size (NS) / Fläche des Ery-Zellkerns	20	40	73.77-134.79	90.12	15.81	3.53
Lymphocyte (large) diameter / Lymphocyt (groß) $\emptyset$	10	10	23.75-26.25	24.75	0.77	0.24
Lymphocyte (small) diameter / Lymphocyt (klein) $\emptyset$	10	10	16.25-19.25	18.22	0.90	0.28
Monocyte diameter / Monozyt $\emptyset$	10	20	25.62-30.00	27.42	1.40	0.44
Neutrophil diameter / Neutrophiler $\emptyset$	10	20	21.50-26.50	24.08	1.78	0.56
Eosinophil diameter / Eosinophiler $\emptyset$	10	20	21.62-25.75	23.40	1.38	0.43
Basophil diameter / Basophiler $\emptyset$	10	10	15.62-18.25	16.68	0.90	0.28
Thrombocyte Length (TL) / Thrombozytenlänge	10	20	16.62-18.62	17.78	0.69	0.22
Thrombocyte Width (TW) / Thrombozytenbreite	10	20	10.06-11.62	10.89	0.53	0.16

Hayem's solution was used as diluting solution for erythrocytes, while for the leukocytes, the method of JERRETT & MAYS (1973) (which is a slight modification of Blain's method - STURKIE 1954) was utilized; i.e., 1:1 mix of neutral red diluted to 1/5000 with 0.07% physiological saline and 12% formaline prepared with 0.07% physiological saline.

Blood smears stained with Wright's stain were used in blood cell measurements (erythrocytes, leukocytes and thrombocytes). Cell measurements were done under a microscope with an ocular micrometer. On each blood smear, 40 erythrocytes were randomly chosen; cell lengths (EL) and widths (EW), along with the lengths (NL) and widths (NW) of their nuclei were measured [ $\mu\text{m}$ ], then cell (ES) and nuclei area sizes (NS) were computed from the formulas  $EL \cdot EW \cdot \pi/4$  and  $NL \cdot NW \cdot \pi/4$ , respectively. Leukocyte diameter, thrombocyte length (TL) and throm-

bocyte width (TW) were also measured [ $\mu\text{m}$ ]. Cell photomicrographs were taken with an Olympus BX51 photomicroscope.

Because there were no significant differences between the blood cells counts and sizes of males and females, the data from both sexes were pooled. Blood cells counts and sizes are given in Table 1.

The erythrocytes of *L. vulgaris* were oval cells with similarly shaped and centrally placed nuclei like those of other amphibian species (Fig. 1 A).

Lymphocytes were the most common leukocytes (Table 2). These cells were of spherical shape; we observed both small and large lymphocytes in our blood smears (Figs. 1B, 1C). In general, using Wright's stain, their cytoplasm was stained a colourless blue, their nuclei violet-blue.

Monocytes place third among the most commonly seen leukocytes. These cells were easily distinguished by the shape

Table 2: Leukocyte formulas (%) of 10 specimens of West Anatolian *Lissotriton vulgaris* (LINNAEUS, 1758).

Leukocyte Cells	1	2	3	4	5	6	7	8	9	10	Total (%)
Lymphocyte	33	32	34	31	34	34	45	32	35	38	34.80
Monocyte	20	20	16	17	15	17	16	19	17	15	17.20
Neutrophil	23	25	27	25	26	25	18	25	23	24	24.10
Eosinophil	12	13	14	15	16	14	12	14	13	13	13.60
Basophil	12	10	9	12	9	10	9	10	12	10	10.30

Table 3: Synopsis of erythrocyte counts and sizes in amphibian species.

Species Art	Erythrocyte Count [per mm <sup>3</sup> ] Erythrozytenzahl [pro mm <sup>3</sup> ]	Erythrocyte Size [in µm <sup>2</sup> ] Erythrozytenfläche [in µm <sup>2</sup> ]
<i>Lissotriton vulgaris</i>	173670 (Present Study)	421.35 (Present Study)
<i>Lissotriton vulgaris</i>	198000 (SZARSKI & CZOPEK 1966)	-
<i>Triturus cristatus</i>	228000 (SZARSKI & CZOPEK 1966)	-
<i>Mesotriton alpestris</i>	207000 (SZARSKI & CZOPEK 1966)	-
<i>Neurergus strauchii</i>	127000 (ARIKAN et al. 2003)	463.82 (ARIKAN et al. 2003)
<i>Lissotriton vulgaris</i>	-	419.44 (ATATÜR et al. 1998)
<i>Triturus karelinii</i>	-	420.37 (ATATÜR et al. 1998)
<i>Ommatotriton vittatus</i>	-	367.05 (ATATÜR et al. 1998)
<i>Pelodytes ridibundus</i> (PALLAS, 1771)	326000 (ARIKAN 1989)	276.62 (ATATÜR et al. 1998)
<i>Bufo bufo</i> (LINNAEUS, 1758)	584000 (SZARSKI & CZOPEK 1966)	221.22 (ATATÜR et al. 1999)

of their nuclei (Fig. 1D). The kidney-shaped nuclei were half the size of the cells, or slightly larger. With Wright's stain, the cytoplasm was light purple, while the nucleus was stained dark blue. Monocytes were the most abundant leukocytes after lymphocytes and neutrophils (Table 2).

The cytoplasm of the spherical neutrophils (Fig. 1E) stained a light blue and their nuclei a dark purplish blue with Wright's stain. The cytoplasm contained very fine granules. The nuclei showed a multi-lobed or segmented structure. They were the most abundant leukocytes, after the lymphocytes (Table 2).

The eosinophils' cytoplasm stained a light blue and nuclei dark blue. Large, roundish, bright reddish granules characterize their cytoplasm (Fig. 1F). Nuclei were usually bilobed. Eosinophils were more scarce than the neutrophils (Table 2).

There were only few basophils observed. Stained with Wright's stain, their cytoplasm appeared light blue, dark purplish-to-bluish granules partly obscuring the dark blue nucleus (Fig. 1G).

The spindle shaped thrombocytes (Fig. 1H) had darkly stained large oval nuclei which left a small, irregular cytoplasmic area. They tended to clump together in blood smears.

ATATÜR et al. (1998), who studied different newt species, mentioned individual variations concerning erythrocyte counts and the lack of sexual dimorphism in blood morphological parameters. Similarly, the present study showed no sexual dimorphism in blood cell counts in a *L. vulgaris* sample collected during the breeding season.

In *L. vulgaris* the sizes of erythrocytes and their nuclei were found to be larger than those of *Triturus karelinii* (STRAUCH, 1870) and *Ommatotriton vittatus* (GRAY in JENYNS, 1835) previously given by ATATÜR et al. (1998) (Table 3).

Some authors (VERNBERG 1955; FOXON 1970; ATATÜR et al. 1998) reported that various environmental factors affect erythrocyte size. The variation in erythrocyte size among urodeles may arise from differences in activity levels as well as from variation in the habitat as was shown by ATATÜR et al. (1998).

The present study demonstrated that erythrocytes were subject to substantial variation in size and number in *L. vulgaris* (Table 1). Comparing our data with those from the literature revealed that erythrocyte counts were lower in *L. vulgaris* than e. g. in *T. karelinii* and *Mesotriton alpestris* (LAURENTI, 1768) but higher than in *Neurergus strauchii* (STEINDACHNER, 1887). Erythrocyte size of *L. vulgaris* was not significantly different from that of *T. karelinii* and *N. strauchii*, but bigger than in *O. vittatus* (Table 3).

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