A C T A T H E R I O L O G I C A

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Erythrocytes, Haemoglobin and Haematocrit in the Postnatal Development of the Root Vole

[With 3 Figs & 3 Tables]

The studies were carried out on 108 individuals of *Microtus oeconomus* (Pallas, 1776) deriving from the stock reared in laboratory conditions for a few years. The age of animals ranged from 1 to 61 days. The control group consisted of 32 adult individuals of the same species captured in the natural habitat and acclimatized for 3—4 months in the laboratory. The level of haemoglobin, number and diameter of erythrocytes, and haematocrit value were determined. In comparison with other *Microtidae* the root vole is characterized by a slightly lower level of haemoglobin and lower number of erythrocytes, but a higher haemoglobin content per cell. The postnatal anaemia appears as a decrease of haemoglobin concentration in the first days of life and a less conspicuous reduction of the haematocrit, whereas the number of erythrocytes does not change. Stabilization of such indices which are subjected to changes with age, as haemoglobin level, number of erythrocytes, haematocrit, volume of red cells, occurs around 20th day of life, while the diameter of erythrocytes — around 30th day of life.

I. INTRODUCTION

The course of formation of morphological features of peripheral blood cells in the postnatal development is known in three rodent species from the family of *Microtidae: Lagurus lagurus* (Müller, 1963), *Pitymys subterraneus* (Kostelecka-Myrcha, 1966b) and *Clethrionomys glareolus* (Kostelecka-Myrcha, 1967). Investigation of this process in the fourth species of this family — *Microtus oeconomus* — might provide interesting data. This species is similar to the other three both in the respect of systematics and physiology (Gębczyńska, 1970), but differs in geographic distribution and environmental conditions of life.

II. MATERIAL AND METHOD

Altogether 140 individuals of the root vole, *Microtus oeconomus* (Pallas, 1776) were examined. From this number 108 animals aged 1 to 61 days derived from the stock reared during a few years in the Mammals Research Institute, at Białowieża. A group of 32 individuals was captured in the Augustów Primeval Forest by the end of October 1967 and was treated as a control group after 3—4 months of acclimatization in the laboratory. The age of captured voles was estimated in approximation from the body weight and time of catch. Two third of these individuals were classified as 8 months old and one third as approximately 17 months old.

Blood samples were taken from the jugular vein under ether anaesthesia always between 9 and 11 a.m. The content of haemoglobin was determined in a Zeiss haemoglobin colorimeter, erythrocytes were counted in a Thoma chamber, and the volume of blood cells was determined by the microhaematocrit method. The diameter of red cells was estimated by means of filar micrometer eye piece in smears stained by the Pappenheim method. Measurements of 50 erythrocytes from one individual were assumed as sufficient to determine the mean value (Kostelecka-Myrcha, 1966a, 1966b, 1967). In selected age groups the curves of anisocytosis were drawn. The mean corpuscular haemoglobin (*MCH*), the volume and thickness of red cells and the mean corpuscular haemoglobin concentration (*MCHC*) were calculated.

Selected age groups of animals were characterized by mean values and the interval of confidence of morphological indices of the blood. The significance of differences between these mean values was examined by the *t*-test applied for the difference of means for two independent groups. The relationship between individual indices and the age of animals was characterized by regression lines running across the series of individual observations. The significance of deviation of these lines from the axis of voles age was determined by the *t*-test.

III. RESULTS

The data obtained for the group of over one-year-old animals acclimatized for 3—4 months in laboratory conditions were assumed as normal and haematological indices of other animals were compared with them. It was found that the number of 32 individuals in this group is sufficient to calculate mean values and that there are no significant differences between males and females in the studied blood properties. Also changes in morphological features of blood in the postnatal period are identical in males and females. Thus all further results were analysed without paying respect to sex of animals.

The content of haemoglobin in 100 ml of blood decreases during the first few days of life of voles reaching a minimum in the fifth day. A subsequent increase of haemoglobin contents leads to the establishment of this index around 23rd day of life on the level similar to the initial one (Fig. 1A, Table 1). This level does not change with age as indicated by the parallel course of the regression line in relation to the age axis, as well as by lack of significant differences ($0.4 > P > 0.3$) between the

Table 1.

Morphological indices of blood in selected age groups of *M. oeconomus*.
Mean values and confidence intervals.

	Age	N	Hb Conc. (g/100 ml)	*RBC* (mln)	*MCH* ($\gamma\gamma$)	Hematocrit (%)	*RBC* diameter (μ)	*RBC* vol. (μ^3)	*RBC* thickness (μ)	*MCHC* (%)
I	1 day	9	15.1 ± 0.6	4.18 ± 0.6	35.3 ± 4.7	40.0 ± 4.1	6.82 ± 0.35	99.46 ± 19.5	2.77 ± 0.99	38.6 ± 3.2
II	5 days	6	12.3 ± 3.0	4.37 ± 0.7	28.2 ± 4.7	33.9 ± 5.7	6.22 ± 0.23	76.59 ± 13.4	2.46 ± 0.35	36.6 ± 3.1
III	29—31 days	8	15.0 ± 1.2	8.89 ± 1.0	18.1 ± 3.1	44.1 ± 2.4	5.35 ± 0.10	48.76 ± 4.69	2.18 ± 0.28	33.4 ± 3.0
IV	8—17 months	32	14.9 ± 0.5	10.71 ± 0.96	14.3 ± 1.0	45.7 ± 1.9	5.08 ± 0.07	43.96 ± 2.97	2.16 ± 0.14	32.7 ± 0.7

level of haemoglobin in 29—31-day-old and over one-years-old animals (Table 2).

Changes in the haematocrit show a similar course but the difference between the value in the first and fifth day is on the borderline of significance ($0.1 > P > 0.05$) (Fig. 1B, Table 1). It seems that this relatively slight decrease of haematocrit accompanied by a stronger drop of haemoglobin level may be explained by lack of parallel alterations in the erythrocyte number (Table 1). The establishment of a constant level of haemotocrit occurs around 18th day of live, and deviation of regression line from the age axis, as well as the difference between the group of 29—31-day-old and over one-year-old animals are not significant ($0.2 > P > 0.1$) (Table 2).

During the first 10 days of life the number of erythrocytes remains on a constant level (deviation of the regression line is statistically not significant), while in the next 8 days a sharp increase is observed which

Table 2.

Comparison of values of blood indices in selected age groups of *M. oeconomus*. + statistically significant differences (at the level of $P = 0.05$ or $P = 0.01$ — the latest marked with an asterisk), — differences not significant.

Age groups	Hb Conc. (g/100 ml)	*RBC* (mln)	*MCH* ($\gamma\gamma$)	Hemato-crit (%)	*RBC* diame-ter (μ)	*RBC* vol (μ^3)	*RBC* thick-ness (μ)	*MCHC* (%)
I—II	+	—	+*	—	—	—	—	—
II—III	+	+	+	+*	+	+	—	—
I—III	—	+*	+	—	+	+	—	+
III—IV	—	+	+	—	+	—	—	—

reaches a fairly constant level of 9.5 million in 1 cu. mm of blood. The course of the regression line indicates the lack of relationship between the number of erythrocytes and age of animals between 19th and 61st day of life (Fig. 1C). This value, however, is significantly different ($0.05 > P > 0.02$) from the number of red cells in the group of voles of over one-year-old (Tables 1 & 2).

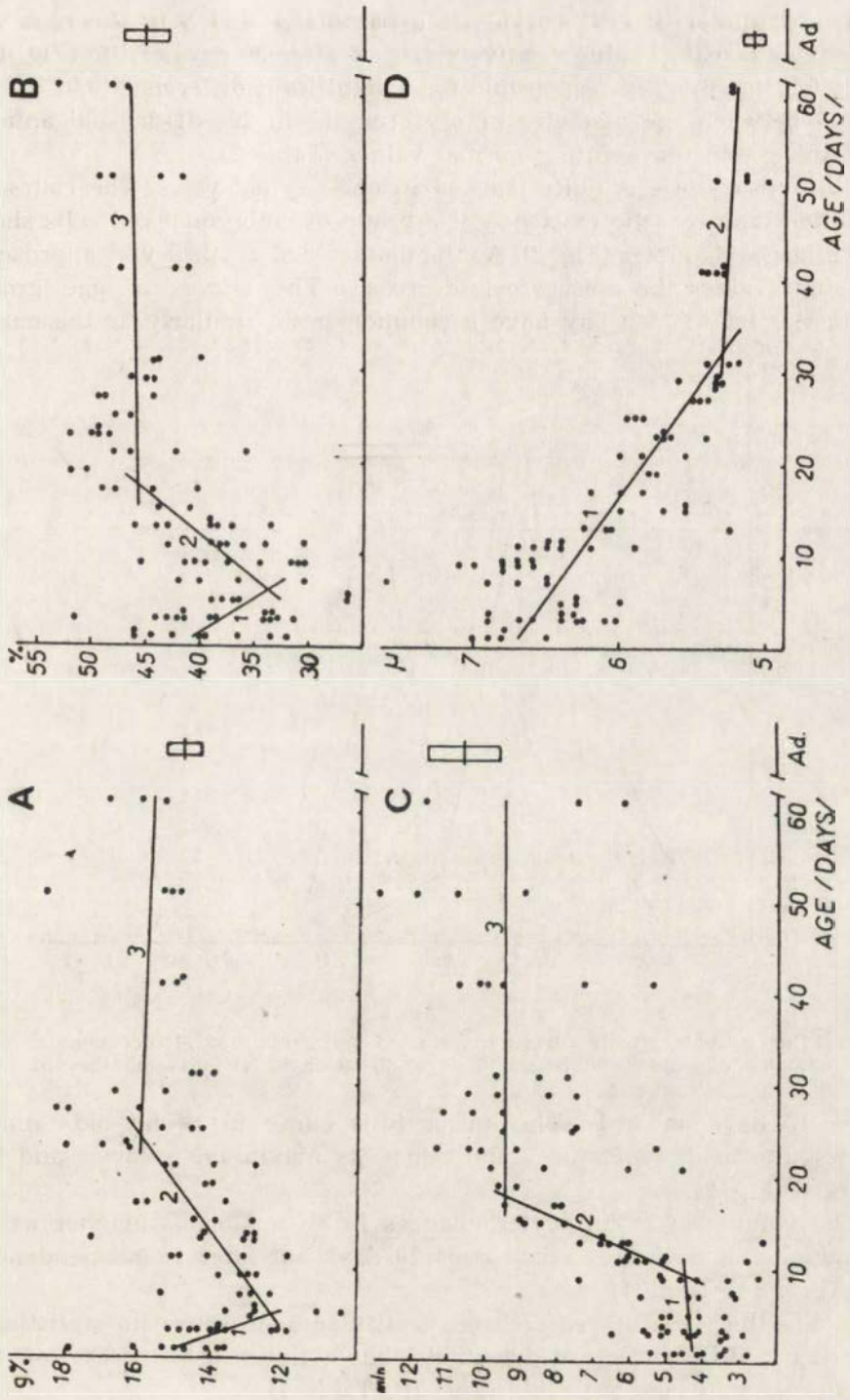
Fig. 1. Changes of average morphological indices of blood in *M. oeconomus* during postnatal development. For adult animals (Ad.) averages and confidence intervals are given.

A. Haemoglobin, g/100 ml: (1) $y_1 = 15.7 - 0.702x$; (2) $y_2 = 11.6 + 0.187x$; (3) $y_3 = 16.1 - 0.00686x$.

B. Haematocrit value: (1) $y_1 = 42.07 - 1.424x$; (2) $y_2 = 26.90 + 1.075x$; (3) $y_3 = 45.26 + 0.0303x$.

C. Number of erythrocytes in 1 cu.mm: (1) $y_1 = 4.28 + 0.0223x$; (2) $y_2 = -1.46 + 0.613x$; (3) $y_3 = 9.44 + 0.00297x$.

D. Diameter of erythrocytes: (1) $y_1 = 6.77 - 0.0464x$; (2) $y_2 = 5.58 - 0.00684x$.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The diameter of erythrocytes is a parameter which in the root vole reaches a constant value relatively late — after 30 days of life (Fig. 1D). This fact is probably responsible for a significant difference ($0.01 > P > 0.001$) between the diameter of erythrocytes in 29—31-day-old animals and the group representing normal values (Table 2).

The anisocytosis is quite marked in one-day-old voles. The course of this line indicates the existence of a group of embryonal red cells showing a larger diameter (Fig. 2). As the diameter of erythrocytes approaches constant values the anisocytosis decreases. The curves of age groups from the 1st to 11th day have a common peak, similarly as the curves

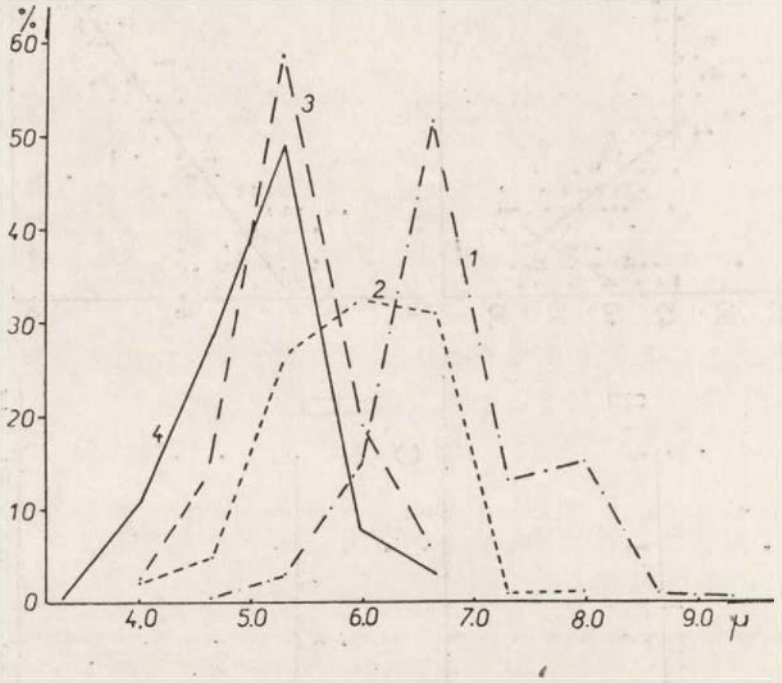

Fig. 2. Anisocytosis curves in selected age groups of *M. oeconomus*. (1) One day old animals, (2) 15 days, (3) 29 days, (4) 7—17 months old.

from 15 days onwards. The anisocytosis curve in 13-day-old animals shows intermediate features and hence its maximum is lower and less marked (Fig. 2).

The volume of erythrocytes changes in an analogous manner as the diameter and decreases up to over 20 days but later is independent of age (Fig. 3A, Table 1).

In the thickness of red cells in particular age groups no statistically significant differences are detectable, but a trend of decrease of this parameter with age is visible (Fig. 3B, Table 1).

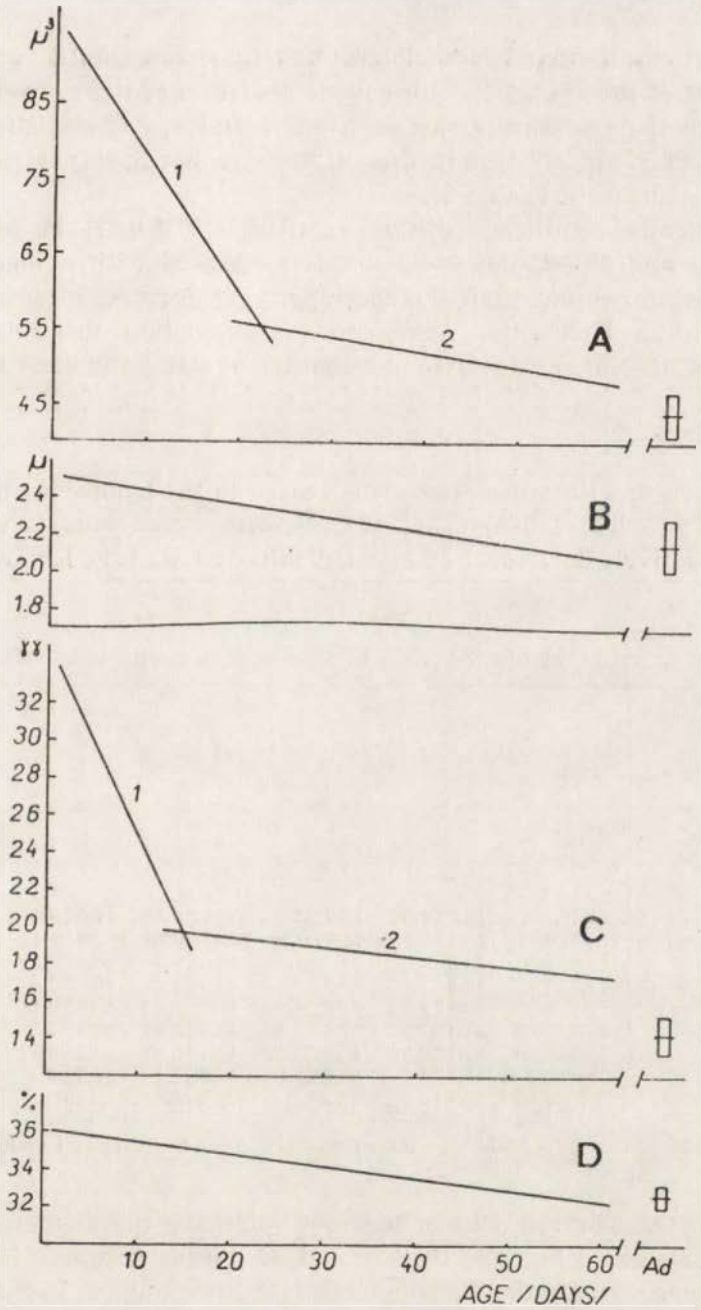

Fig. 3. Changes of morphological indices of blood in *M. oeconomus* during postnatal development.

A. Average erythrocyte volume: (1) $y_1 = 96.26 - 1.830x$; (2) $y_2 = 60.10 + 0.1997x$.

B. Average erythrocyte thickness: $y = 2.51 - 0.00727x$.

C. Mean corpuscular hemoglobin value (*MCH*): (1) $y_1 = 34.92 - 1.024x$; (2) $y_2 = 20.46 - 0.052x$.

D. Mean corpuscular hemoglobin concentration (*MCHC*): $y = 36.07 - 0.061x$.

The mean corpuscular haemoglobin (*MCH*) decreases until approximately 15 days of life (Fig. 3C). Subsequent deviation of the regression line representing the relationship between *MCH* and age is statistically not significant ($0.5 > P > 0.4$) but the differences between particular age groups are significant (Table 2).

A statistically significant difference ($0.05 > P > 0.02$) in *MCHC* in one-day-old and 29—31-day-old voles is associated with a bigger drop of haemoglobin content than the accompanying decrease of haematocrit value (Table 2). Hence the regression line depicting the relationship between *MCHC* and age deviates in a significant manner ($0.05 > P > 0.01$) (Fig. 3D).

IV. DISCUSSION

In comparison with other *Microtidae* reared in the laboratory (K o s t e l e c k a - M y r c h a, 1966a) (Table 3) *Microtus oeconomus* is characterized by relatively low level of haemoglobin and slightly lower number

Table 3.

Comparison of values of blood indices in *M. oeconomus* with other *Microtidae*.

Indices	*L. lagurus*[1]	*M. agrestis*[1]	*M. arvalis*[1]	*P. suąterraneus*[1]	*C. glareolus*[2]	*M. oeconomus*
Hb Conc. (g/100 ml)	14.8±0.5	16.8±0.3	16.0±0.9	16.0±0.4	17.2±0.3	14.9±0.5
RBC (mln)	11.62±0.34	11.34±0.43	11.94±0.70	13.05±0.40	12.55±0.47	10.71 ±0.96
MCH (γγ)	12.8±0.5	14.9±0.6	13.7±0.8	12.3±0.3	13.8±0.5	14.3±1.0
Hematocrit (%)	41.7±1.2	46.3±1.5	44.2±2.0	43.9±1.6	46.8±1.4	45.7±1.9
RBC diameter (μ)	5.08±0.07	5.04±0.05	5.10±0.06	4.87±0.08	5.10±0.11	5.08±0.07
RBC vol. (μ^3)	36.0±0.85	41.1±1.50	37.7±2.50	33.7±1.40	37.6±1.5	44.0±2.97
RBC thickness (μ)	1.79±0.06	2.07±0.09	1.50±0.23	1.81±0.07	1.85±0.07	2.16±0.14
MCHC (%)	35.8±0.5	36.3±1.1	36.6±1.1	36.6±1.1	36.7±0.9	32.7±0.7
N	42	30	27	30	32	32

[1]) Acc. to K o s t e l e c k a - M y r c h a, 1966a; [2]) Acc. to K o s t e l e c k a - M y r c h a, 1967.

of erythrocytes. The red cells of the root vole have the diameter similar to other *Microtidae* but are thicker and of larger volume. Hence the value of mean corpuscular haemoglobin is a little higher. Perhaps these slightly less favourable conditions of gaseous exchange are related to a rather uniform type of habitats of this species and to its lower expansiveness.

Changes in morphological indices of blood in the postnatal period of the root vole life are similar to those observed in the bank vole (K o s t e-

lecka-Myrcha, 1967). Anaemia appearing after birth in many mammals (Enzmann, 1934; Kalabukhov & Rodionov, 1934; Kunde, Green, Changnon & Clark, 1932), and particularly visible in *Clethrionomys glareolus* (Kostelecka-Myrcha, 1967), is manifested in *Microtus oeconomus* as a drop of haemoglobin contents during the first 5 days of life, and a slight decrease of haematocrit, but with no change in the erythrocyte number. A similar phenomenon of reduced amount of haemoglobin with simultaneous increase of the red cells number, observed in the early period of life of *Citellus pygmaeus*, was explained by Kalabukhov & Rodionov (1934) by disappearance of large embryonal blood cells.

Further intensive rise of haemoglobin content, erythrocyte number and haematocrit value ends in *Microtus oeconomus* already between 18th—23rd day of life, hence earlier than in *Clethrionomys glareolus* (Kostelecka-Myrcha, 1967). The haemoglobin level in adult voles is almost the same as in one-day-old animals, whereas in *Lagurus lagurus* (Müller, 1963) and *Mus musculus* (Kalabukhov & Rodionov, 1934) is proportionally higher, and in *Citellus pygmaeus* (Kalabukhov & Rodionov, 1934) and *Clethrionomys glareolus* (Kostelecka-Myrcha, 1967) is lower.

The decreased values of most haematological indices in animals over the age of one year is the phenomenon observed also in white mice (Ewing & Tauber, 1964) and pine voles (Kostelecka-Myrcha, 1966b).

The course of the anisocytosis curve in one-day-old voles differs slightly from analogous curves drawn for the pine vole (Kostelecka-Myrcha, 1966b) and for the bank vole (Kostelecka-Myrcha, 1967), but is very similar to the curve found for new-born calves (Iržak, 1964).

In conclusion it should be stated that *Microtus oeconomus*, in comparison with other *Microtidae* kept in laboratory conditions, is characterized by less favourable conditions for gaseous exchange due to slightly lower level of haemoglobin and lower number of erythrocytes. The red cells in this species are thicker and this fact is responsible for the higher *MCH*. The postnatal anaemia appears in *Microtus oeconomus* as a drop of haemoglobin content while the number of erythrocytes remains constant during the first days of life. Simultaneous decrease in the diameter and thickness of erythrocytes results in the reduction of the haematocrit value. Further intense increase in the number of erythrocytes, haemoglobin level and haematocrit leads to stabilization of these basic haematological indices around 20th day of life. The decrease of erythrocyte diameter ceases later, around 32nd day of life.

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ERYTROCYTY, HEMOGLOBINA I HEMATOKRYT W ROZWOJU POSTNATALNYM NORNIKA PÓŁNOCNEGO

Streszczenie

Przebadano 108 osobników *Microtus oeconomus*, pochodzących z kilkuletniej hodowli, w wieku od 1 do 61 dni oraz 32 osobniki złowione w terenie, traktowane po 3-miesięcznej aklimatyzacji w laboratorium jako grupa kontrolna. Oznaczono poziom hemoglobiny, liczbę i średnicę erytrocytów, hematokryt. Obliczono grubość i objętość krwinek, wewnątrzkomórkową koncentrację hemoglobiny (*MCHC*) i ilość hemoglobiny w jednym erytrocycie (*MCH*). Wykreślono proste regresji i krzywe anizocytozy.

Stwierdzono, że *Microtus oeconomus* w porównaniu z innymi *Microtidae* odznacza się nieco niższym poziomem hemoblobiny i mniejszą liczbą erytrocytów. Krwinki są natomiast grubsze i odznaczają się większą objętością, dzięki czemu większa jest zawartość hemoglobiny w krwince. Wykazano, że u nornika północnego występuje anemia pourodzeniowa, polegająca na obniżeniu się poziomu hemoglobiny w pierwszych dniach życia i mniej wyraźnym spadku hematokrytu, spowodowanym zmniejszaniem się średnicy erytrocytów. Liczba erytrocytów utrzymuje się natomiast w pierwszych dniach życia na niezmienionym poziomie. Ustalenie się wskaźników podlegających zmianom wiekowym następuje około dwudziestego (poziom hemoglobiny, liczba i objętość erytrocytów, hematokryt) lub trzydziestego (średnica erytrocytów) dnia życia. Zmiany wskaźników morfologicznych krwi w rozwoju postnatalnym samców i samic przebiegają jednakowo.