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Seasonal Variations of Some Haematological Values in Small Mammals Living in Natural Environment

Sezonowe zmiany niektórych wskaźników hematologicznych u małych ssaków, żyjących w naturalnym środowisku

[With 7 Figs. and 1 Table]

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I. INTRODUCTION

Few haematological data on *Sorex araneus* Linnaeus, 1758 and *Clethrionomys glareolus* (Schreber, 1780) have been reported (Kleinenberg, 1952; Koržujev & Koreckaja, 1962), but the counts were not complete and done on few animals. Only few reports on seasonal changes in blood of these or similar mammals have been met with (Dawson, 1956; News on, 1962; Sealander, 1962).

The erythropoietic system is influenced by metabolic activity to a high degree. It may be then assumed that due to variable external conditions and internal stimuli, operating during different seasons of a year, the particular haematological

indices should change accordingly during the year cycle. Not much is, unfortunately, known about such changes in wild animals living in their natural environment. The researches of Dudziński et al. (1962) on the rabbit, *Oryctolagus cuniculus* Linnaeus, 1758, kept in semi-natural conditions, and of Lucenko (1941) and Gorodeckij (1962) on reindeer, should be mentioned in this connection. Similar observations on cattle (Smith & Kilbourne, 1893, acc. to Riddle & Braucher, 1934) and on sheep (Bulatova, 1962) are pertinent to discussed matter, if the effects of human activity, changing animals' diet, their habitat and even climate they live in, may be neglected.

In the present research an attempt has been made to study some haematological values for small wild rodents *Clethrionomys glareolus* (Schreber, 1780) and an insectivorous *Sorex araneus* Linnaeus, 1758, living in their natural environment, relatively free of man's interference, in the forests of the National Park at Białowieża.

II. EXPERIMENTAL

1. Material and Methods

The method of catching the animals and the characteristic of the biotope, they had lived in, have been given in the papers of Dehnel (1949) and Borowski & Dehnel (1952). The animals, being caught at early morning hours, were taken without delay to a nearby laboratory and then bled by snipping the end of the tail. In some cases, blood from *S. araneus* was obtained directly from the heart. When parallel samples were taken from the tail, no significant differences could be noticed between samples obtained in different ways. Acid haematin method and Sahli haemoglobinometer were used for haemoglobin (*Hb*) determination. Enumeration of erythrocytes (*RBC*) and leucocytes (*WBC*) was done in Thoma haematocytometer, using Hayem's solution for *RBC* and the diluting fluid (2 p.c. acetic acid + gentian violet) for *WBC*. The sex and the age of the animals were then determined, the animals were killed and passed to other subdivisions of the laboratory for examinations in the anatomical, histological, microbiological and parasitological respects.

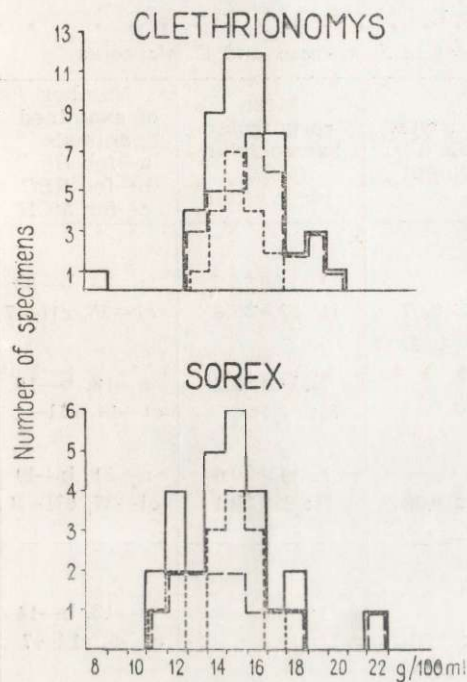
For haemoglobin determinations 51 specimens of *C. glareolus* and 27 of *S. araneus* were used; for blood counts — 49 specimens of *C. glareolus* and 25 of *S. araneus*.

2. Results

Results of determinations are given in Table 1.

a) Haemoglobin

Comparative data on the haemoglobin concentration in blood of wild animals are scarce (Wintrobe, 1933; 1952). It is generally accepted that haemoglobin concentration is in all mammals constant. It seems yet, that domesticated animals' blood is slightly poorer in haemoglobin than in wild animals. The values obtained for *C. glareolus* and *S. araneus* are almost identical and are in accord with this impression (Fig. 1, Table 1).



b) Erythrocytes count

The number of erythrocytes is variable in different animals. It is generally higher in small mammals; this tendency is deformed by some ecological factors, especially by the partial pressure of oxygen, being lower at higher altitudes. *RBC* in *C. glareolus* is similar to that of the rat or of the ferret. In *S. araneus*, on the other hand, the values of *RBC* are exceedingly high and variable (Table 1, Fig. 2).

Fig. 1. Distribution of haemoglobin levels in *S. araneus* and *C. glareolus*

c) The mean corpuscular haemoglobin (*MCH*)

Very little information is available with respect to this value in different mammals. This value may be yet related to efficiency of oxygen transport. *MCH* is normal in *C. glareolus* and extremely low in *S. araneus* (Table 1, Fig. 3). This matter will be discussed in "Discussion".

The *MCH* values for species could be further subdivided into two quite distinct groups (see Table 1, Fig. 3). This phenomenon, more marked in *S. araneus*, is yet quite apparent in *C. glareolus*, too. This heterogeneity of examined populations is due to the seasonal changes and will be discussed later.

d) The effect of age and sex

No statistically significant differences in *Hb*, *RBC*, and *MCH* values for different age groups were found. These values were, for a given season, similar for young adults and for the old ones, borne during the previous year.

The average values for both sexes were fairly close, when compared with the relatively broad range of normal values. Analysis of Fig. 1, 2

Table 1.
Mean values of haematological indices in *S. araneus* and *C. glareolus*

Animal	Haemoglobin (Hb) g/100 ml	Erythrocytes (RBC) $10^6/1 \text{ mm}^3$	Mean corpuscular haemoglobin (MCH) 10^{-12} g/cell	Number of examined animals a—for Hb b—for RBC c—for MCH
<i>C. glareolus</i>				
males, mean	16.2 ± 0.7	10.02 ± 0.57	I: 15.0 ± 0.5 II: 22.5 ± 2.8	a—33, b—33 cI—25, cII—7
min. & max.	13.0—20.0	6.44—12.23		
females, mean	15.4 ± 0.6	9.53 ± 0.77	I: 14.8 ± 1.2 II: 2.15	a—18, b—16 cI—14, cII—1
min. & max.	13.8—17.6	6.50—13.22		
♂ + ♀, mean	15.8 ± 0.6	9.77 ± 0.66	I: 14.9 ± 0.6 II: 22.3 ± 2.1	a—51, b—49 cI—37, cII—8
min. & max.	13.0—20.0	6.44—13.22		
<i>Sorex araneus</i>				
males, mean	15.1 ± 1.76	12.17 ± 1.98	I: 8.85 II: 16.4	a—13, b—14 cI—5, cII—7
min. & max.	11.6—22.0	8.24—20.68		
females, mean	14.8 ± 1.28	15.62 ± 3.48	I: 9.3 II: 17.3	a—14, b—14 cI—8, cII—2
min. & max.	11.0—18.0	7.23—25.10		
♂ + ♀, mean	15.0 ± 0.95	13.69 ± 1.81	I: 9.1 ± 0.7 II: 16.6 ± 1.1	a—27, b—25 cI—13, cII—9
min. & max.	11.0—22.0	7.23—25.10		

and 3 may, at the first sight, suggest the existence of slightly marked sexual dimorphism. This apparent dimorphism is yet caused by uneven distribution of examined specimens of both sexes during different seasons of the year. The analysis of the trend of examined values with season in both sexes separately, shows that differences between sexes for a given season are insignificant.

e) Effect of parasitic infestation, of body weight and length

The infestation by intestinal parasites was mild. The number of worms found in intestinal tract varied from 0 to over hundred specimens (*Cestoda* and *Nematoda*) per an animal. No correlation between kind or number of parasitic worms and haematological indices could be established.

There was no correlation between the values of haematological indices and the weight, the length of the animal's body or linear dimensions of liver, kidneys and spleen.

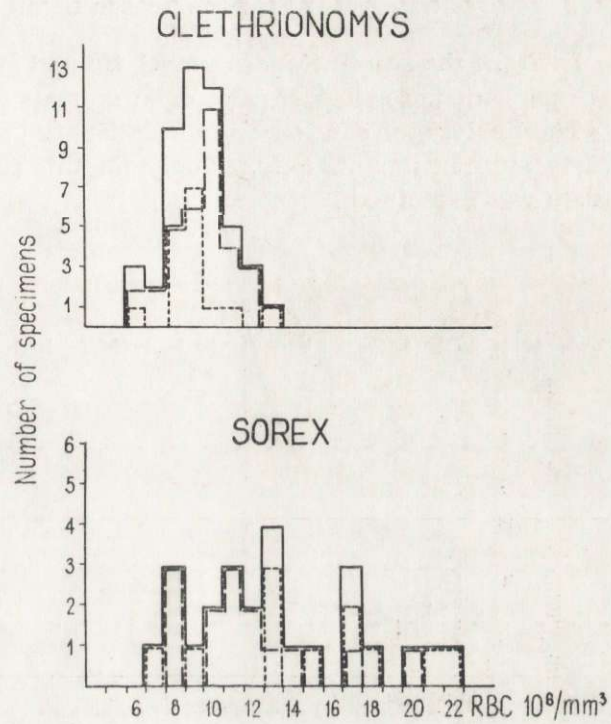


Fig. 2. Distribution of erythrocytes counts in *S. araneus* and *C. glareolus*.

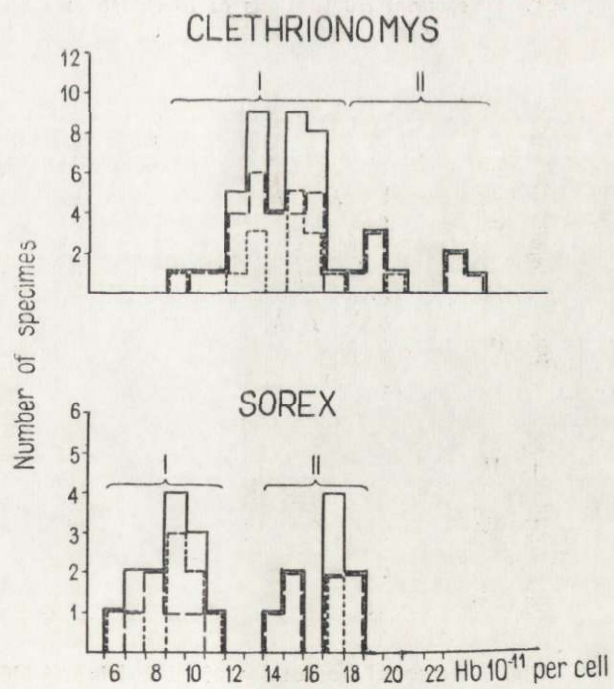


Fig. 3. Distribution of mean corpuscular haemoglobin concentrations in *S. araneus* and *C. glareolus*.

f) Effect of season of the year

In Fig. 4 the season trends of RBC, Hb and MCH values for *C. glareolus* are presented. Analogical data for *S. araneus* are shown in Fig. 5.

The analysis of data on *S. araneus* has to be restricted to summer and early autumn months only, as only for this period sufficient number of cases was examined.

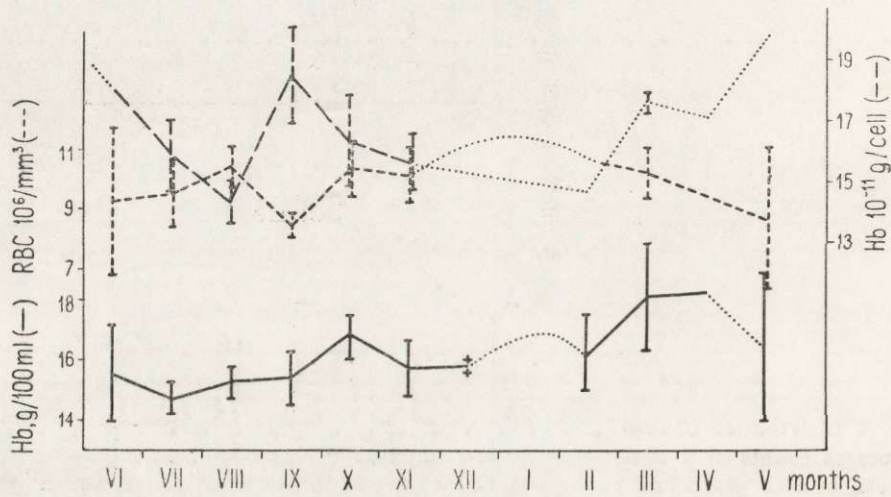


Fig. 4. Seasonal fluctuations of RBC, Hb and MCH values in *C. glareolus*.

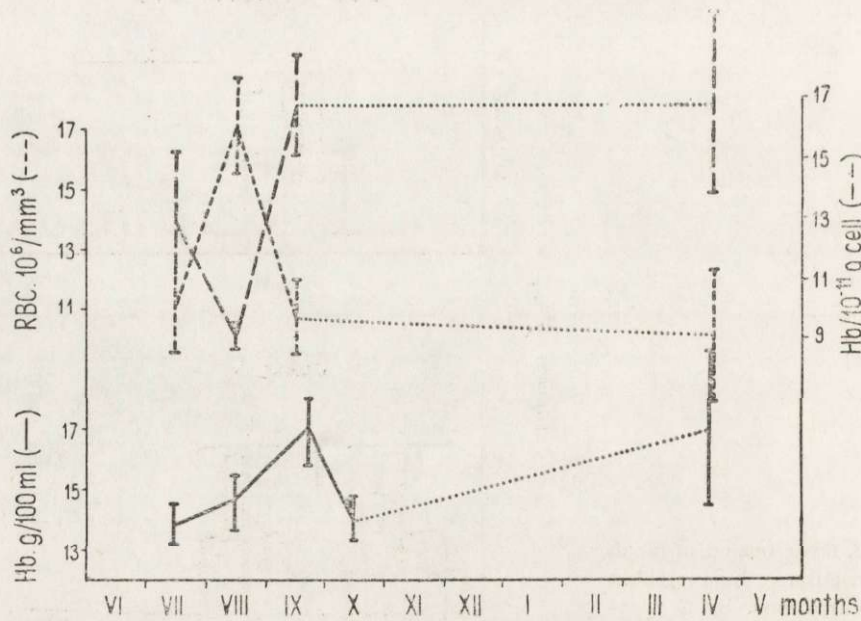


Fig. 5. Seasonal fluctuation of RBC, Hb and MCH values in *S. araneus*.

The *Hb* concentration was low at the beginning of the summer and rose sharply till the end of August, to drop again to the lowest level during September and October. Seasonal fluctuations in *RBC* counts were more pronounced, the peak coinciding with the end of the summer. Later, *RBC* value diminished and this decrease seems to persist till the spring.

In *C. glareolus* the seasonal fluctuations were less striking than in *S. araneus*. On the other hand, the much greater number of specimens examined enabled to cover almost the whole year cycle. There were two distinct peaks in haemoglobin concentration, at spring and autumn. Correspondingly, the minimal *Hb* values were observed in late summer. The autumn peak of haemoglobin concentration developed in *C. glareolus* a month later than in *S. araneus*.

The fluctuations in erythrocytes counts had a different trend from the one observed in haemoglobin concentration. There were also two peaks, but more pronounced and displaced. The first peak in *RBC* count was in

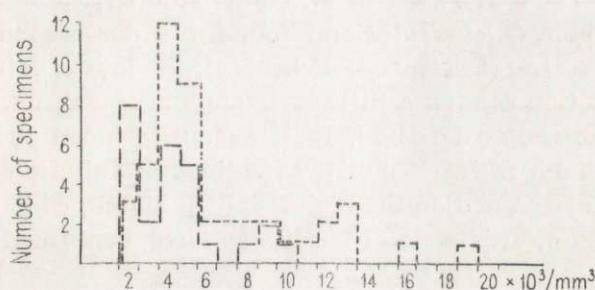


Fig. 6. Distribution of leucocytes counts in *S. araneus* and *C. glareolus*

late summer (August), the second one in late autumn (October—November). In *C. glareolus* seems to exist the third peak in *RBC* count during the spring (March), but too small number of specimens examined during this season made the exact location of it difficult. The lowest values of *RBC* counts were found in early autumn (September) and in late spring (May—June).

The trend in mean corpuscular haemoglobin concentration (*MCH*) was reversed in relation to the trend in *RBC* count. The level of haemoglobin concentration had relatively small effect on the *MCH* changes.

g) Leucocytes count

No relationship between leucocytes count and environmental or endogenous factors could be established (Table 1, Fig. 6). The observed values varied in ranges, which had been found by other workers, in small mammals.

3. Discussion

Blood counts in *S. araneus* and *C. glareolus* and related species have been reported previously (Knoll, 1932; Kleinberg, 1952; Koržujev & Koreckaja, 1962) but they were done on only few animals, whose age, sex and conditions of life were not stated.

Previous informations on the effect of changes in environment on blood picture are scarce. Some informations, concerning domesticated animals, cattle (Smith & Kilburne, 1893; Manresa et al. 1934, 1939; acc. to Schalm, 1961), sheep (Bulatova, 1962), guinea pigs (Bender & de Witt acc. to Riddle & Braucher, 1934) suggest that RBC count diminished in winter. Pintor & Grasini (1957) found seasonal changes in the rate of maturation of erythrocytes in rabbits.

Wild animals, living in their natural environment, were studied only exceptionally. Dudziński et al. (1962) studied seasonal changes in blood of the rabbit, *O. cuniculus* and found the decrease in RBC count during the dry season (December—February); the lowest value of haemoglobin concentration coming a little later (January—February). Lucenko (1941 acc. to Gorodeckij, 1962) examined blood picture in reindeer. The minimum in RBC count was noticed in July; it was increased till October and remained on the high level till March, when spring-summer decline begun. This general trend was later confirmed by Gorodeckij (1962).

Of special interest for the problem discussed here are publications by Dawson (1956), Newson (1963) and Sealander (1962). The research of Newson, being conducted on *C. glareolus* in the field conditions, is especially valuable. She has found the highest values for haemoglobin concentration in winter, the lowest — in summer and autumn. Haematocrit values, like the weight of spleen, reached the highest level during summer and early autumn. Newson (1963) excluded breeding, age, weather, ectoparasites, as chief factors affecting the examined indices. Sealander (1962) has studied haemoglobin concentration, haematocrit values and erythrocyte diameters in three species of *Peromyscus*. The study has been concerned with caged animals, kept in natural weather conditions, but maintained on an *ad libitum* laboratory diet. Some measurements have been made also on free-living animals. Sealander observed the highest levels of haemoglobin concentration and haematocrit values, simultaneously, in winter. Fairly good correlation between these indices and microhabitat temperature has been established.

In present research the changes in blood picture of wild animals have been studied throughout the whole year cycle. These changes are due to

status of an individual animal. The status may be influenced by many variables, such as sex, age, diet, climatic factors and others.

It seems that circadian behaviour and connected with it changes in blood during a day cycle (Halberg, 1960; Manresa et al., 1934; 1939; acc. to Schalm, 1961) may be disregarded. All the animals examined were caught at the same time of a day, and the differences caused by small deviations in the time of catching may be supposed to be unimportant.

No significant effect of sex on haematological indices has been observed, confirming thus Wintrobe's (1952) opinion. Kalabukhov's (1933) observations on sexual dimorphism in blood picture in *Citellus pygmaeus* Pallas, 1778 seem to be founded on too small number of examined specimens and neglecting the eventual influences of seasonal and dietary factors.

There was no dependence of blood picture on the age of examined animals. It is well known that in adult animals the age has no effect on haemoglobin concentration or RBC count (Drobkin & Fitz-Hugh, 1934; Enzman, 1934; Dudziński et al., 1962; Kalabukhov & Rodinov, 1934). Juveniles among examined here animals were not numerous, the indices found for them followed general pattern of the population mean.

It is still one point to be elucidated. In very excitable animals the circulation, and consequently RBC count and haemoglobin concentration, can be strongly influenced even by trifling occurrences (Hansen, 1950; Schalm, 1961). The stress and excitement caused in animals by handling them during collecting of blood could play some role in this respect. The procedure of handling animals was, at least to some degree, standardized, so it may be supposed that the effects of this were in all animals similar. It is not possible at present to exclude that excitability of animals is changing throughout the year. It is not, however, possible also to measure a degree of distortion, due to this factor. It should be not, it seems anyhow, overestimated, and may be ignored.

The number of pregnant females, which have been examined, was small. No decrease in Hb and RBC values were noted in these animals, when compared with non-pregnant females caught at the same time.

It appears thus, that observed fluctuations in blood values depend not on age, sex, breeding, circadian behaviour or distortion due to nervous stress. These fluctuations seem to be correlated, on the other hand, with environmental seasonal changes directly, or indirectly through humoral factors.

The dependence of erythropoiesis on the dietary factors has been well established.

The role of protein seems to be especially great (Bethard, 1958; Hahn & Whipple, 1939; Miller et al., 1947). Many vitamins e.g. riboflavin, pyridoxin, niacin, folic acid, thiamin, cobalamin (see Schalm, 1961) influenced this process. On the other hand, some constituents of diet may have detrimental influence on the erythropoiesis. The well known example of the last case is bracken fern poisoning in cattle (Sippel, 1952).

The interrelationship between mechanism of erythropoiesis and humoral factors is more complicated. It is known, nevertheless, that after removal of thymus RBC is increased, without any change in Hb concentration. Similar effect was observed after splenectomy (Riddle & Braucher, 1934). Thyroid, pituitary gland and adrenal glands deficiency affects also haemopoiesis (e.g. Dawson, 1956; Schalm, 1961).

The evaluation of dietary factor is difficult. The availability of the food during the year cycle in forests of southern Poland has been examined by Grodziński (1961), Górecki & Gębczyńska (1962) (see Fig. 7). This picture for the Białowieża National Park (North-eastern part of the country) should be modified, which is illustrated by Borowski & Dehnel's (1952) data on number of insects there.

Some informations on seasonal changes of diet of common shrew, living in Białowieża forest, have been reported by Kisielewska (1963). Her researches, based on helminthological findings, are, however, restricted to some snails and arthropoda diet components. They point to variability of shrew's food during the year cycle, but give no informations on the actual composition of the food in particular seasons.

In this connection the study of intestinal microflora may be used as an additional source of information.

The striking seasonal quantitative and qualitative changes of microflora of the intestine in *S. araneus* and *C. glareolus* have been described elsewhere (Kunicki-Goldfinger & Kunicka-Goldfinger, 1962).

Comparison of the data collected in Fig. 7 seems to suggest that the observed changes in haematological values, especially of haemoglobin concentration, are correlated with changes of diet. It may be presumed, that the change in haemoglobin concentration is the first to appear when diet is changed.

The influence of endocrine glands should not be underestimated (see Fig. 7, for changes in thymus, thyroid and testes — Bazan, 1952; Dzierżykraj-Rogalska, 1952; Wolska, 1952), although the interrelationships between these and dietary factors is undoubtedly highly complicated.

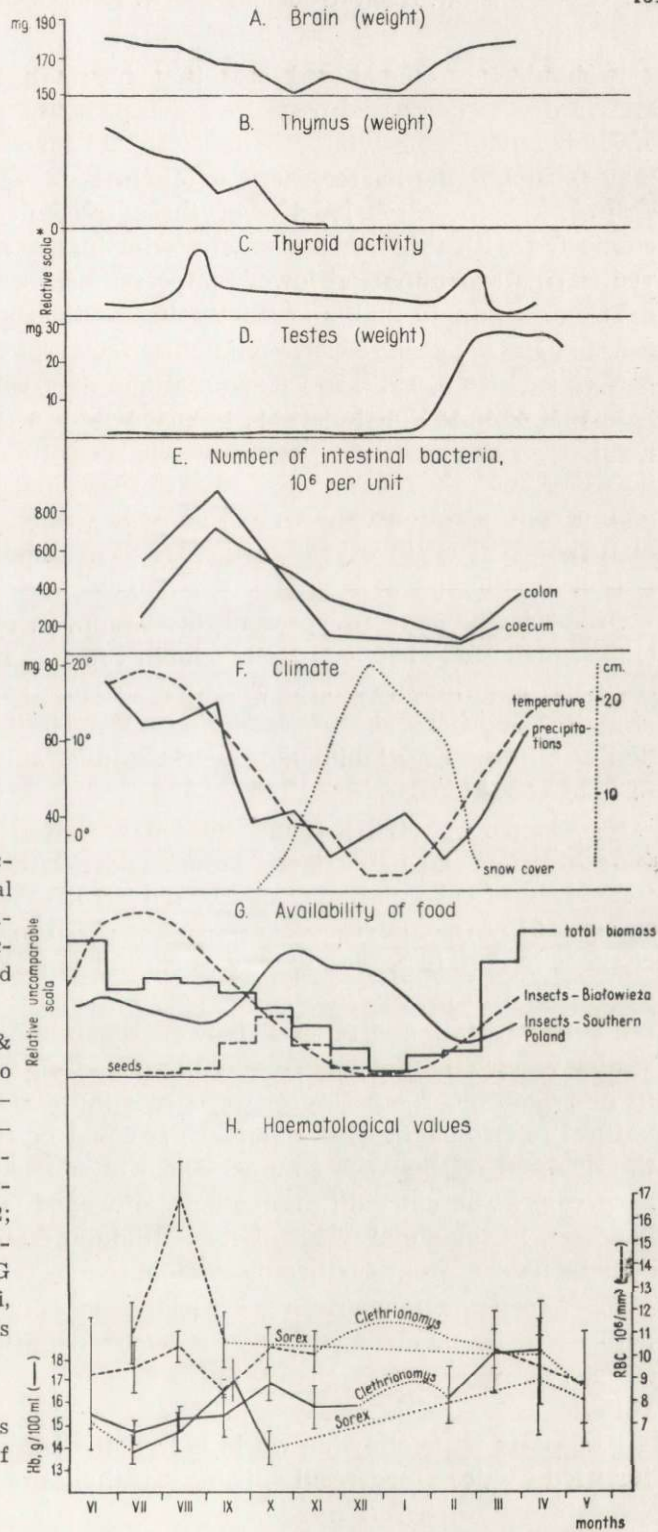


Fig. 7. Comparison of fluctuations of haematological values and seasonal variations of other morphological indices in *S. araneus* and *C. glareolus*.

A — acc. to Bielak & Pucek, 1960; B — acc. to Bazan, 1952; C and D — acc. to Wolska, 1952; — E — acc. to Kunicki-Goldfinger & Kunicka - Goldfinger, 1962; F — acc. to Kowalska-Dyrcz, 1962, modified; G — acc. to Grodziński, 1961, modified; H — this report.

*) Activity of thyroid, as evaluated on the basis of histological data.

It should be remembered also that in small mammals the relative anoxia due to decreased partial oxygen pressure, especially in subnivean environment during winter period (Coulianos & Johnels, 1962), may influence the haemopoiesis through the haemopoietin production in kidney. The increased level of erythrocyte count during winter may be connected with this mechanism and with higher metabolism rate, observed in small mammals at lower temperatures.

The exceedingly high and fluctuating erythrocyte counts in *S. araneus* can be caused by similar environmental factors, by very high metabolism rate (Pearson, 1947) in this animal and its great activity.

In this connection, the study, reported by Newson (1962), should be mentioned. She excluded age, breeding, weather and ectoparasites, as possible factors affecting the pattern of seasonal fluctuations of haemoglobin concentration values in *C. glareolus*. She suggested, that the rate of turnover of erythrocytes is increased in summer, but no satisfactory reason for this has been offered.

Of special interest for present discussion, is, reported by Newson (1962), difference between haemoglobin concentration values in free living voles and the caged ones. The animals were kept in cages in the same climatic conditions as the free-living ones. The haemoglobin concentration was in the caged animals, nevertheless, invariably distinctly lower than in field-conditions.

Sealander (1962) found good correlation between haemoglobin concentration and haematocrit values and mean maximum microhabitat temperature. On this basis, Sealander has suggested, that seasonal changes in haemoglobin concentration of small mammals reflect seasonal changes in metabolism rate. It is worth yet to be noticed, that Sealander found in caged animals distinctly higher mean values of haemoglobin concentration and much narrower separation between summer and winter values, than among free living. According to him, seasonal changes in microhabitat temperature were more pronounced in cages, than in natural environment of the animals. It could be then expected, that also the differences between summer and winter haemoglobin values should be greater. The chief difference between caged and free living animals has been in their diet. This factor, although mentioned, is dismissed by Sealander from further discussion.

The effect of temperature, especially in connection with metabolic activity of small mammals, is not to be neglected. Metabolic activity affects, however, rather the erythrocyte count, than haemoglobin concentration.

It appears that following working hypothesis may be, at least partially, justified by described results, being at the same time not inconsistent

with data of Newson (1962) and Sealander (1962): in wild animals, living in their natural environment, the haemoglobin concentration mirrors the changes of diet; the erythrocyte count is, on the other hand, influenced to a great extent by the rate of metabolism, the activity of the animal and the partial oxygen pressure in its environment.

Fluctuations in mean corpuscular haemoglobin concentration are as marked as in erythrocytes count. The population heterogeneity in the respect of this value is caused by great differences in *MCH* values between late summer minimum and late spring and early autumn maxima. It is evident that measurements of the haematological indices, without proper consideration for seasonal changes, may give misleading or false results.

III. SUMMARY

Haemoglobin concentration (*Hb*), erythrocyte count (*RBC*), leucocyte count (*WCB*) and mean corpuscular haemoglobin (*MCH*) have been measured in *S. araneus* and *C. glareolus* during the year cycle.

In *S. araneus* at the beginning of the spring *RBC* and *Hb* were low, increasing until September, when they reached the highest values. During autumn *RBC* level was low. The data for winter period were too scarce.

In *C. glareolus* there were two peaks in haemoglobin concentration (at spring and early autumn). The lowest values of *Hb* were found during late summer and in winter.

RBC reached highest level at late summer and late autumn. The high level of *RBC* was prolonged through the winter till early spring; minimal values were observed, correspondingly, at early autumn and very late spring.

No correlation was found between haematological values and age or sex of the animals.

The dietary factors and endocrine glands activity were suggested as chief causes of changes in haemoglobin concentration. The metabolic activity and the partial oxygen pressure in the environment of the animals seem to be responsible for changes in the erythrocyte count. The exceedingly high and strongly fluctuating red blood cells count in *S. araneus* may be connected with these factors.

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STRESZCZENIE

Przebadano stężenie hemoglobiny, ilość czerwonych ciałek krwi, średnią zawartość hemoglobiny w krwince i ilość leukocytów u 51 osobników *Clethrionomys glareolus* (Schreber, 1780) i 27 osobników *Sorex araneus* Linnaeus, 1758, złowionych w Białowieskim Parku Narodowym w ciągu całego cyklu rocznego.

U *S. araneus* ilość erytrocytów (RBC) i zawartość hemoglobiny (Hb) były niskie w początkach wiosny, podnosząc się stale aż do września, gdy osiągnęły wartości maksymalne. Ilość krwinek czerwonych w jesieni ponownie spadła. Dane z okresu zimy były zbyt skąpe, by można było statystycznie ocenić istotność wahań (Tabela 1, ryc. 1, 2, 3, 5 i 6).

U *C. glareolus* stwierdzono dwa maksima stężenia hemoglobiny (na wiosnę i wczesną jesienią). Najniższe zawartości Hb znaleziono późnym latem i zimą. Ilość erytrocytów osiągnęła maksima późnym latem i późną jesienią. Wysoki poziom erytrocytów przeciągnął się przez zimę, aż do wczesnej wiosny. Minima obserwowano odpowiednio, wczesną jesienią i późną wiosną (Tabela 1, ryc. 1, 2, 3, 4 i 6).

Nie wykryto zależności między wskaźnikami hematologicznymi a wiekiem lub płcią zwierząt.

Jako główne przyczyny zmian stężenia hemoglobiny, proponuje się uznać czynniki żywieniowe i aktywność gruczołów dokrewnych. Aktywność metaboliczna i cząsteczkowe ciśnienie tlenu w środowisku są, jak się wydaje, odpowiedzialne za zmiany ilości erytrocytów. Wyjątkowo duże i silnie zmieniające się ilości erytrocytów u *S. araneus* mogą być kształtowane przez te czynniki (ryc. 7).