

**Seasonal variations
in hematological values and heart weight in two small mammals,
a mouse: *Apodemus sylvaticus*, and a vole: *Pitymys duodecimcostatus***

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Hematological values and relative heart weight were analyzed in two rodents: the mouse, *Apodemus sylvaticus* (Linnaeus, 1758), and the vole, *Pitymys duodecimcostatus* (de Selys-Longchamps, 1839). The body weight of the two species was similar, 18.60 ± 0.61 g for the mouse, versus 20.14 ± 0.42 g for the vole. The specimens were captured in the same geographic area but in different ecological niches, since the vole is a burrowing animal, and the mouse is not. Hematological variations in both species were not related to age, sex, or gestation. *P. duodecimcostatus* had higher RBC count and Hb and Hc concentrations and lower MCV and MCH values than *A. sylvaticus*. However, MCHC values were almost the same in both species. The seasonal hematological variations observed in *P. duodecimcostatus* are like those found in most small mammals, while the hematological values found in *A. sylvaticus* barely changed throughout the year. The relative heart weight increased significantly in the two species during winter.

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Introduction

The metabolic needs of animal tissues are met by hemoglobin based oxygen transport. For this reason the hematological parameters closely reflect O₂ transport and partly explain organism adaptation for this vital requirement. Although the hemoglobin content of the blood and hematocrit in mammals are relatively constant, the total number of red blood cells and mean cell size varies considerably. An inverse relationship between cell size and number was pointed out by Hawkey (1975). Numerous small red blood cells are often found in those species which inhabit high altitudes or live underground, where the modification is thought to be an adaptation to low oxygen availability by providing increased surface area for gas exchange (Hawkey 1975).

The hematological variations found in different mammalian species have been studied with respect to possible influential factors such as acclimatization to different

temperatures (Sealander 1960, 1962, Lee and Brown 1970), postnatal development (Kostelecka-Myrcha 1966a), altitude (Kalabukhov 1953, Sealander 1964), diet (Kunicki-Goldfinger and Kunicka-Goldfinger 1964), age (Sealander 1964, Wolk 1974, Barnett *et al.* 1979) or stress (Kunicki-Goldfinger and Kunicka-Goldfinger 1964). All these studies refer to the O₂ transportation capacity of the blood as regulated by different external pressures to which the animals are subjected.

The blood parameter variation in relation to seasonal changes has been studied in different species of small mammals such as rats (Sealander 1962, 1964), kangaroo rats (Scelza and Knoll 1982), rabbits (Dudziński *et al.* 1962), shrews (Kunicki and Kunicka 1964, Wolk 1974), and voles (Rewkiewicz-Dziarska 1975). These studies generally found that the hemoglobin and hematocrit values and the number of red blood cells tended to increase in winter and then decrease during summer. This is partly explained by the higher metabolic rates found in these animals in response to their acclimatization to lower winter temperatures.

Another factor contributing to improved circulatory efficiency, and therefore increased tissue oxygenation, is the increase in relative heart weight since this supposes an increased cardiac output per unit of weight. Other factors, such as photoperiod (Puchalski and Heldmaier 1986), acclimatization to cold (Heroux 1961, Malzahn 1974, Sealander 1966), or altitude (Kostelecka-Myrcha 1967) have also been described as influencing relative heart weight.

This study researched seasonal changes in certain physiological parameters related to the transport and delivery of oxygen to the tissues of two small mammals, *Apodemus sylvaticus* (Linnaeus, 1758), a mouse that lives above-ground, and *Pitymys duodecimcostatus* (de Sélys-Longchamps, 1839), a burrow-dwelling vole which lives underground.

Materials and methods

A total of 95 *Apodemus sylvaticus* and 90 *Pitymys duodecimcostatus* were caught in live traps near Alcalá de Henares (Madrid, Spain) between September, 1984 and September, 1985. The animals were anesthetized by ether inhalation and blood was obtained by heart puncture with sterile heparin-rinsed syringes. Red blood cells (RBC) were counted in a Thoma chamber and the hemoglobin concentration (Hb) was determined with the cyanomethemoglobin technique. The hematocrit (Hc) was determined by blood centrifugation in microhematocrit capillaries at 11,000 rpm for 10 minutes. Mean corpuscular volume (MCV) was calculated from RBC count and Hc; mean corpuscular hemoglobin (MCH) was obtained from the RBC count and Hb concentration, and mean corpuscular hemoglobin concentration (MCHC) was calculated with the Hc and Hb concentrations. Once the animal was dead, the heart was extracted, cleaned of adherences and blood, and weighed on a Mettler scale to ± 0.1 mg. The values are expressed as relative weight in mg of organ weight per gram of body weight (mg/g). The age of each animal was estimated by weighing the crystalline lens of the eye fixed in 40% formol for 15 days and then drying it at 70°C. Condylobasal length (CBL) was measured to within ± 0.1 mm.

Data are expressed as means \pm SE and statistical significance was tested by analysis of variance (ANOVA), or by correlation coefficients (*r*), where appropriate. The significance level was set at 0.05%

unless otherwise stated. The calculations were made with a BMDP programme on an "Eclipse" MV4000 computer (Data General).

Results

The hematological values of both rodent species are shown in Table 1. The total results obtained indicate that hematological values for RBC, Hb and Hc in *P. duodecimcostatus* were higher than in *A. sylvaticus*, while the MCV and MCH values were higher in the mouse than in the vole. The MCHC values were similar in both species.

Table 1. Hematological values (mean \pm SE) of *Apodemus sylvaticus*, *Pitymys duodecimcostatus* and statistical significance ANOVA (ns = not significant).

| Variable | Sign. | Males | Females | Pregnant females | Total |
|----------------------------|-------|------------------|------------------|------------------|------------------|
| <i>A. sylvaticus</i> | | | | | |
| RBC ($10^6/\text{mm}^3$) | ns | 9.52 \pm 0.21 | 9.55 \pm 0.21 | 8.96 \pm 0.30 | 9.46 \pm 0.14 |
| Hb (g/dl) | ns | 14.68 \pm 0.25 | 14.96 \pm 0.36 | 13.88 \pm 0.36 | 14.59 \pm 0.18 |
| Hc (%) | ns | 47.19 \pm 0.71 | 47.09 \pm 1.18 | 43.38 \pm 1.33 | 46.59 \pm 0.57 |
| MCV (μ^3) | ns | 48.91 \pm 0.64 | 49.23 \pm 1.14 | 48.75 \pm 1.74 | 48.96 \pm 0.56 |
| MCH (pg) | ns | 15.32 \pm 0.24 | 15.39 \pm 0.39 | 15.56 \pm 0.49 | 15.39 \pm 0.19 |
| MCHC (g%) | ns | 31.34 \pm 0.39 | 32.40 \pm 0.47 | 32.53 \pm 0.59 | 31.88 \pm 0.26 |
| n | | 52 | 25 | 18 | 95 |
| <i>P. duodecimcostatus</i> | | | | | |
| RBC ($10^6/\text{mm}^3$) | ns | 12.13 \pm 0.24 | 11.40 \pm 0.34 | 11.21 \pm 0.49 | 11.72 \pm 0.18 |
| Hb (g/dl) | ns | 15.41 \pm 0.33 | 14.84 \pm 0.33 | 14.88 \pm 0.51 | 15.08 \pm 0.23 |
| Hc (%) | ns | 48.84 \pm 0.73 | 47.22 \pm 1.08 | 46.18 \pm 1.28 | 47.86 \pm 0.56 |
| MCV (μ^3) | ns | 42.14 \pm 0.72 | 42.70 \pm 1.08 | 42.62 \pm 1.25 | 42.41 \pm 0.53 |
| MCH (pg) | ns | 13.29 \pm 0.28 | 13.20 \pm 0.30 | 13.72 \pm 0.57 | 13.82 \pm 0.20 |
| MCHC (g%) | ns | 31.51 \pm 0.46 | 30.80 \pm 0.87 | 32.21 \pm 0.80 | 31.48 \pm 0.37 |
| n | | 46 | 25 | 19 | 90 |

Effects of sex and gestation

No significant differences were observed in the variables analyzed in either sex of these species, although pregnant *A. sylvaticus* did show some decrease in RBC and the Hc and Hb concentrations.

Effect of age

Table 2 shows the correlation coefficient (r) matrix for the different variables in relation to age: condylobasal length (CBL), crystalline lens weight, animal weight and the hematological parameters for each species. Correlations between the hematological variables and the age-related variables were not significant.

Table 2. Correlation coefficient (*r*) matrix of hematological parameters and variables in relation to age (body weight, CBL, cristalline weight) for *Apodemus sylvaticus* and *Pitymys duodecimcostatus* (**p*<0.05, ****p*<0.001).

| Variable | <i>Apodemus sylvaticus</i> | | | <i>Pitymys duodecimcostatus</i> | | |
|---------------|----------------------------|---------|-------------------|---------------------------------|-------|-------------------|
| | Body weight | CBL | Cristaline weight | Body weight | CBL | Cristaline weight |
| Body weight | 1.00 | | | 1.00 | | |
| CBL | 0.94*** | 1.00 | | 0.19 | 1.00 | |
| Cristaline wt | 0.91*** | 0.97*** | 1.00 | 0.60* | 0.03 | 1.00 |
| RBC | -0.21 | -0.19 | -0.22 | 0.22 | -0.08 | 0.22 |
| Hb | -0.13 | -0.06 | -0.14 | 0.30 | -0.06 | 0.18 |
| Hc | -0.19 | -0.04 | -0.21 | 0.21 | -0.08 | 0.12 |

Table 3. Seasonal changes of hematological values and relative heart weight (mean ± SE) of *Apodemus sylvaticus* and *Pitymys duodecimcostatus*. Statistical significance ANOVA (ns = not significant, **p*<0.05, ***p*<0.01, ****p*<0.001).

| Variable | Sign. | Autumn | Winter | Spring | Summer |
|---|-------|--------------|--------------|--------------|--------------|
| <i>A. sylvaticus</i> | | | | | |
| RBC (10 ⁶ /mm ³) | ns | 9.86 ± 0.23 | 9.52 ± 0.31 | 9.39 ± 0.26 | 9.21 ± 0.32 |
| Hb (g/dl) | * | 14.90 ± 0.23 | 14.61 ± 0.37 | 15.03 ± 0.27 | 13.74 ± 0.48 |
| Hc (%) | ** | 49.47 ± 0.54 | 46.13 ± 0.79 | 46.85 ± 0.94 | 43.13 ± 1.03 |
| MCV (μ ³) | ns | 49.83 ± 0.96 | 48.51 ± 1.18 | 50.23 ± 1.16 | 46.95 ± 1.03 |
| MCH (pg) | ns | 15.07 ± 0.34 | 15.25 ± 0.36 | 15.98 ± 0.48 | 14.97 ± 0.34 |
| MCHC (g%) | ns | 30.75 ± 0.37 | 32.24 ± 0.82 | 32.07 ± 0.44 | 32.30 ± 0.52 |
| Relative heart weight (mg/g) | * | 7.92 ± 0.34 | 8.30 ± 0.25 | 7.52 ± 0.29 | 7.36 ± 0.09 |
| n | | 22 | 22 | 28 | 23 |
| <i>P. duodecimcostatus</i> | | | | | |
| RBC (10 ⁶ /mm ³) | ns | 10.85 ± 0.49 | 13.09 ± 0.45 | 11.66 ± 0.26 | 10.93 ± 0.27 |
| Hb (g/dl) | *** | 14.52 ± 0.50 | 15.83 ± 0.29 | 15.93 ± 0.35 | 13.25 ± 0.44 |
| Hc (%) | *** | 46.62 ± 1.52 | 51.81 ± 0.84 | 47.61 ± 0.87 | 43.94 ± 0.85 |
| MCV (μ ³) | ns | 45.07 ± 1.59 | 43.69 ± 1.07 | 41.85 ± 0.87 | 40.72 ± 0.99 |
| MCH (pg) | ns | 13.27 ± 0.30 | 13.54 ± 0.31 | 13.96 ± 0.36 | 12.23 ± 0.47 |
| MCHC (g%) | ** | 31.26 ± 0.51 | 30.07 ± 0.77 | 33.15 ± 0.51 | 30.30 ± 0.73 |
| Relative heart weight (mg/g) | *** | 6.78 ± 0.14 | 7.96 ± 0.25 | 7.20 ± 0.29 | 7.53 ± 0.09 |
| n | | 12 | 23 | 35 | 10 |

Seasonal variations

The seasonal variations in hematological values were studied by two way ANOVA and they were significantly affected by sex or gestation. Table 3 shows both the seasonal hematological values and relative heart weights for *A. sylvaticus* and *P. duodecimcostatus* during the year, as well as their statistical significance. Although RBC were not significant in *A. sylvaticus*, the highest value was found in autumn and the lowest in summer. RBC varied significantly in *P. duodecimcostatus* (*p*<0.05). The summer values were highest and differed significantly from those of the other three seasons.

Seasonal differences in Hb concentration were significant in both *A. sylvaticus* ($p < 0.05$) and *P. duodecimcostatus* ($p < 0.001$). The autumn, winter and spring values for the mouse were similar while those for summer were comparatively lower. This difference between summer and the other three seasons was statistically significant. The Hb values in the vole were high in winter and spring, and lower in summer. The seasonal differences between winter-spring and summer-autumn were significant.

The seasonal differences in hematocrit (Hc) values were significant in both species. The highest Hc value in *A. sylvaticus* was found in autumn after which Hc decreased during winter and spring to its lower value in summer. A significant difference was found between autumn and the other seasons. In *P. duodecimcostatus* the highest value was found in winter and the lowest in summer. Differences between winter and the other seasons were significant.

No significant seasonal differences in MCV values were found in either species. The highest values for this index in *A. sylvaticus* were found in autumn and spring. The values in *P. duodecimcostatus* decreased progressively from autumn through winter and spring to summer.

In *A. sylvaticus* MCH did not vary significantly, although there was a slight increase from autumn to spring which levelled off during the summer. In *P. duodecimcostatus* MCH variations were significant ($p < 0.01$). The value was similar in autumn, winter and spring but lower in summer. The significant differences was between summer, and the other seasons.

A. sylvaticus MCHC values did not vary significantly over the seasons. However, the differences in *P. duodecimcostatus* were significant ($p < 0.001$) with a high MCHC in spring. The difference between spring and the other seasons was significant.

In both *A. sylvaticus* and *P. duodecimcostatus* the seasonal variation in relative heart weight was significant ($p < 0.05$ and $p < 0.001$, respectively), being higher in winter than in summer.

The differences between the winter and spring, and the spring and summer seasons in *A. sylvaticus* were significant. For the *P. duodecimcostatus* the significant difference was between winter and the other three seasons.

Discussion

The quantity of blood hemoglobin is affected by the characteristic energy requirements and metabolic rate of each species. The relations between Hb concentration and body size (Kunicki-Goldfinger and Kunicka-Goldfinger 1964, Sealander 1964, Lee and Brown 1970, Wołk 1974), and Hb and altitude (Kalabuchov 1953, Sealander 1964), is inverse, not allometric. These variables cannot be taken into account in our study, since specimens had similar geographic origin and weights.

The polycythemia presented by *P. duodecimcostatus*, in comparison to *A. sylvaticus*, has low MCV values and can be explained as a mechanism to allow the increase of the total gas exchange. This has been described by different authors (Kostelec-

ka-Myrcha 1966b, Withers 1975, Ar *et al.* 1977, Boggs *et al.* 1984) in various burrowing animals.

Hematologic studies carried out by Palacios (1976) confirm that the number of erythrocytes and Hb concentration are higher in small members of the *Microtinae* family than in the *Murinae* family. According to Palacios, this may be caused by the higher metabolic rate in the *Microtinae*. The MCHC values for both species were similar to those cited by Hawkey (1975) for other mammals.

The effect of sex and gestation on hematological parameters

The lack of sexual dimorphism in the hematological variables of both *P. duodecimcostatus* and *A. sylvaticus* is also true of other small mammals (Sealander 1960, 1962, 1964, Kunicki-Goldfinger and Kunicka-Goldfinger 1964, Kostelecka-Myrcha 1967, Wołk 1974, Scelza and Knoll 1982). During pregnancy, *A. sylvaticus* has lower Hb, Hc and RBC values, while only RBC and Hc decrease in nonpregnant females in *P. duodecimcostatus*. Similar data have been found by Sealander (1964), and Kostelecka-Myrcha (1967) in other small mammals.

Effect of age on hematological parameters

The absence of significant variation with age in the hematological values found in *A. sylvaticus* and *P. duodecimcostatus* agrees with other authors (Kunicki-Goldfinger and Kunicka-Goldfinger 1964, Sealander 1964, Kostelecka-Myrcha 1967, Palacios 1976). Kostelecka-Myrcha (1966a, 1967) observed that the hematological values in *Pitymys subterraneus* and *Clethrionomys glareolus* stabilized one month after birth.

Seasonal variations in hematological parameters and relative heart weight

The seasonal variations in hematological values found in *P. duodecimcostatus* and *A. sylvaticus* follow different patterns, although the lower Hb concentration, Hc, MCV, and MCH values all found during summer.

The seasonal characteristics found in other small mammals for these variables consist of increased Hb and Hc concentrations and RBC values with low MCV values in winter which then reverse during summer. Many authors have related this seasonal variation to environmental acclimatization (Newson 1962, Newson and Chitty 1962, Sealander 1962, 1964, Kostelecka-Myrcha 1967, Lee and Brown 1970, Wołk 1974, Rewkiewicz-Dziarska 1975, Palacios 1976). They suggest that winter cold produces a period of intense thermogenesis that leads to increased respiratory function and, among other adaptations, supposes an increase in Hb concentration and the number of RBC. As a result, capacity for oxygen transport would increase and the total available area for gas exchange would be enlarged.

This scheme for the seasonal variation to hematological parameters is followed by *P. duodecimcostatus* in part with high RBC, Hb and Hc values in the winter-spring period that decrease during summer and autumn. Lee and Brown (1970), in their studies on burrowing rodents, observed that the general lack of available oxygen in the

burrows could worsen during winter and so contribute to the increase in the Hb concentration.

P. duodecimcostatus also showed a significant seasonal variation in MCHC. This hematologic index reflects erythropoiesis and may be affected by diet, particularly Fe deficiencies (Kunicki-Goldfinger and Kunicka-Goldfinger 1964, Bernard and Levy 1983) or by lack of oxygen. In our case, we can only mention these findings since the causes for this variation are not under study here. *A. sylvaticus* did not show the same hematological variation pattern mentioned above, and with the exception of higher autumn Hc, the other hematological variables oscillated only slightly through the different seasons of the year. This lack of seasonal variation is not surprising since there are reports of other rodents that do not follow the winter-summer hematic cycle. Kalabukhov (1953) found a greater Hb concentration in winter than in summer in *A. flavicollis*. Sealander (1966) found no hematological changes in the different seasons in *C. rutilus*. He explained this as being a result of the uniform habitat temperature. Recently, Puchalski and Heldmaier (1986) studied seasonal hematological variations in hamster. They created two experimental groups, one exposed to seasonal and temperature variations and the other had thermoneutral conditions. The differences in Hb concentration between the two groups were not significant, although RBC did varied, suggesting that these parameters are fundamentally influenced by the photoperiod.

In addition to the mechanisms that have been mentioned, there are others that increase blood capacity for oxygen transportation, such as increased respiratory rate or cardiac output. Kostelecka-Myrcha (1967) found greater relative heart weights in those *C. glareolus* that lived at high altitudes, and interpreted this as an adaptation mechanism to increase the oxygen demands at low O₂ pressure since no differences were observed in the hematological values.

Sealander (1966), in *C. rutilus*, and Heroux (1961), and Puchalski and Heldmaier (1986), in hamster, noted an increase in relative heart weight during the cold months, which might be indicative of a seasonal change. The physiological impact of an increase in heart weight is obvious, since improved circulatory efficiency is obtained by an increase in cardiac output per unit of body weight. In our study, we have found seasonal variation in the relative heart weight in the two species, *A. sylvaticus* and *P. duodecimcostatus*, since the greatest relative heart weight was found during winter, and, therefore, could contribute as another factor of seasonal acclimatization.

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