

HEMATOLOGICAL VALUES FOR FREE-RANGING YELLOW-BELLIED MARMOTS

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(Received 18 March 1982)

Abstract—1. Hemoglobin, packed cell volume, erythrocytes, leucocytes, MCV, MCH and MCHC were determined for a population of *Marmota flaviventris* over a period of seven years.

2. There was no significant difference in hematology among years, between sexes, or between seasons for adults and yearlings.

3. Early season juveniles had significantly lower PCV, Hb and erythrocyte counts than did late season juveniles. There were no significant differences in hematological values among adults, yearlings and late season juveniles.

4. Juveniles had significantly lower leucocyte counts than adults and yearlings.

5. PCV of marmots responds to acclimatization.

6. Hematological values of sciurids are adaptive to environmental factors such as hypoxia of burrows and high altitude, temperature and metabolic rate.

7. PCV of yellow-bellied marmots evidences an adaptive response to high altitude when compared to the closely-related woodchuck, *M. monax*.

INTRODUCTION

Although hematological values are known for many wild mammals, few values are based on sampling a large population. Furthermore, only a few studies report variation in hematological values related to age, sex, season or year (e.g. Sealander, 1962, 1964, 1966). Most population studies are of ungulates (e.g. Barrett & Chalmers, 1977; Nieminen, 1980; and references cited therein). Population studies are necessary to determine the amount of natural variation and the adaptive significance of hematological values.

Hematological characters have long been considered to be adaptive to hypoxia, especially that of high altitude. But the extent to which they respond to, or are correlated with, high altitude hypoxia is unclear. For example, the mountain form of the wood mouse (*Apodemus sylvaticus*) had higher hemoglobin and erythrocyte content than either of two plains subspecies (Kalabuchov, 1937), but there was no correlation between hematocrit and altitude of origin for several Chilean rodents (Morrison *et al.*, 1963).

The yellow-bellied marmot (*Marmota flaviventris*) is an especially interesting mammal because it encounters hypoxia both as a burrower and as a resident of high altitudes. Previous studies of this species used only a few animals (Bullard *et al.*, 1966; Winders *et al.*, 1974). This paper reports hematological values collected over several years for several hundred free-ranging marmots.

MATERIALS AND METHODS

The population biology and social behavior of yellow-bellied marmots have been studied annually since 1962 in the East River Valley near the Rocky Mountain Biological Laboratory, Colorado, at an elevation of 2900 m. Marmots emerge from hibernation in early May and immerse in early September. Mating occurs shortly after emergence

and young appear above ground in early July. Each year each marmot in eight study populations was live-trapped and marked for identification. The study areas and marking technique were described in detail elsewhere (Armitage, 1962, 1974). Animals were classified as juveniles (in their first summer), yearlings (in the second summer and one year old) and adults (two years old or older).

When an animal was trapped, a blood sample was collected from a vein running along the inner surface of the thigh. Blood was collected into a vacutainer with potassium oxalate as an anti-coagulant. Because of some lysing of red blood cells, blood also was collected directly into heparinized microhematocrit tubes. Multiple samples were collected from those individuals repeatedly trapped during the active season. Because values from samples collected two weeks or less apart did not differ, the values were combined to produce a single average value. Otherwise, the blood values were treated as separate samples. Only for hematocrit (PCV) were values frequently obtained from the same individual.

Blood samples were returned to the laboratory where the microhematocrit tubes were placed on an International microhematocrit centrifuge and spun for 5 min. Packed cell volume was read on an Adams microhematocrit reader. Hemoglobin was determined colorimetrically by the cyanmethemoglobin method and a Spectronic 20 colorimeter set at a wavelength of 540 nm. Erythrocytes and leucocytes were counted on an A0 Spencer bright line hemocytometer. Blood for counting was diluted in Aloe Visi-Tech red cell or white cell pipettes. Samples in which lysing of cells was evident were not used for cell counts. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to standard procedures (Linman, 1975:86). Most of the blood samples were collected from 1966 to 1972. Additional samples were taken from animals captured in Colorado in 1975 and maintained for a year in the laboratory at Lawrence, Kansas at an elevation of 300 m. A few samples were obtained from marmots live-trapped in the Columbia River Basin of Washington in 1977 and maintained at Lawrence for a year.

The hematological values were organized according to sex, year of sample, and season of the year (early:through

June; late: July to hibernation) and were tested for differences in sex-year-season by a three-level nested ANOVA with unequal sample sizes (Sokal & Rohlf, 1969:281). June was chosen as early season to determine if possible nutritional effects might influence hematological values (Nieminen, 1980). Juveniles were divided into two age groups: early, first four weeks post-weaning; late, second four weeks post-weaning. Differences among age groups were tested by a single classification ANOVA with unequal sample size (Sokal & Rohlf, 1969:208).

RESULTS

For adult marmots, there were no significant differences in the mean values of PCV among the seven years, between sexes, or between seasons. Therefore, all PCV values were lumped. The grand mean was 50.82 (Table 1). For yearlings, there were no significant effects of years, season, or sex; the mean PCV was 50.99. For juveniles, there were no effects of year or sex, but early juveniles had a significantly lower ($P < 0.001$) PCV than late juveniles (Table 1). There was a significant difference among age groups ($F_{3,578} = 89.7, P < 0.001$). Early young had a PCV significantly lower than that of the other groups; there was no significant difference among adults, yearlings and late young. There appeared to be a rapid increase in PCV values in the juvenile population between the fourth and fifth weeks post-weaning (Fig. 1). Repeated samples throughout the summer from the same individuals showed the same pattern of an increase in PCV in one week. As is evident from the range of PCV values (Fig. 1), a few individuals increased this PCV earlier and a few maintained low values throughout the summer.

PCV of Colorado adult marmots maintained in the laboratory for a year was 46.5 (± 4.99 SD). This value was significantly lower than that of 50.82 of the free-ranging adults ($F_{1,214} = 14.4, P < 0.001$). The mean of five samples obtained from Washington marmots maintained in the laboratory for a year was 47.2. This value did not differ significantly from the mean of the Colorado marmots maintained in the laboratory ($F_{1,18} = 0.02, P > 0.75$).

Mean concentrations of Hb ranged from 10.42 in early young to 13.12 in adults (Table 1). There was no significant effect of season on the Hb concentrations of adults and yearlings, but the mean Hb of early

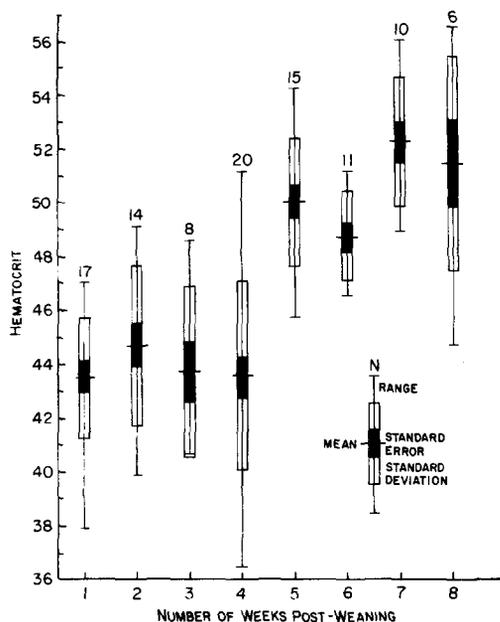


Fig. 1. Hematocrit (PCV) of 35 juvenile yellow-bellied marmots for the first eight weeks post-weaning in 1967. *N* refers to the number of samples.

young was significantly lower than that of all other groups ($F_{6,148} = 4.11, P < 0.001$). There were no other significant differences in mean Hb concentration.

Mean erythrocyte concentration ranged from $4855 \times 10^3/\text{mm}^3$ in early young to $6227 \times 10^3/\text{mm}^3$ in late young. The mean erythrocyte concentration of early juveniles was significantly lower than that of the other groups ($F_{6,175} = 6.35, P < 0.001$). There were no other significant differences. Both Hb and erythrocyte concentrations were lower than values reported elsewhere (Bullard *et al.*, 1966; Winders *et al.*, 1974). Because of the larger sample size, the low values reported in this paper may represent a better estimate of the population mean. However, mean PCV reported in this study is higher than that reported elsewhere, which suggests that there may have been some systematic error in our determination of Hb and erythrocyte values. Such an error should not affect the

Table 1. Mean hematological values for yellow-bellied marmots

Age groups	PCV (%)	Hb (g/100 ml)	Erythrocytes ($10^3/\text{mm}^3$)	MCV (μ^3)	MCH ($\mu\mu\text{g}$)	MCHC (%)	Leucocytes (No./ mm^3)
Adults	50.82(201) ± 4.17	13.12(58) ± 2.03	6160(69) ± 1198	73.26(27) ± 11.4	23.71(43) ± 4.24	26.40(14) ± 4.58	9406(122) ± 4499
Yearlings	50.99(150) ± 3.81	12.57(34) ± 1.60	5858(40) ± 1253	76.84(26) ± 8.40	23.10(19) ± 3.61	25.61(15) ± 2.36	9649(76) ± 4924
Juveniles					24.11(28) ± 5.68		7644(107) ± 4306
Early	44.92(128) ± 3.10	10.42(46) ± 1.81	4855(40) ± 992	80.48(34) ± 8.42		24.32(44) ± 4.09	
Late	50.64(103) ± 2.56	12.90(22) ± 2.69	6227(38) ± 1087	74.18(23) ± 6.95		28.99(9) ± 3.71	

Standard deviation is given below the mean. Sample size (*N*) is given in parentheses following the mean. Values for early and late juveniles are combined when their independent values did not differ significantly

sex, age and yearly comparisons reported in this paper, but use of these data for comparisons with other studies should be made with caution.

Mean corpuscular volume (MCV) differed significantly among the four age groups ($F_{3,106} = 4.41$, $P = 0.01$). The value of 80.48 for early juveniles was significantly greater than the values of the other groups (Table 1). Mean corpuscular hemoglobin (MCH) did not differ significantly among the age groups ($F_{3,86} = 0.24$, $P > 0.75$). Values ranged from 23.12 in yearlings to 24.11 in juveniles (Table 1). Mean corpuscular hemoglobin concentration (MCHC) differed among the four age groups ($F_{3,78} = 4.01$, $P = 0.025$). The value of MCHC for early young (Table 1) was significantly lower than the values of the other groups.

There was no effect of sex or season on leucocyte concentrations, but there was a significant effect among the age groups ($F_{2,293} = 5.77$, $P < 0.005$). Mean leucocyte concentration was lower in juveniles than in adults and yearlings (Table 1). The lower values of juvenile leucocytes and the high values of yearling leucocytes suggested that the difference might be attributed to differential survival. However, there was no significant difference in the mean leucocyte count between young who were recaptured as yearlings and those who were not ($F_{5,68} = 0.87$, $P > 0.5$).

DISCUSSION

Hematological values of many small mammals vary seasonally (Sealander, 1962, 1964, 1966). The lack of seasonal differences in hematological values of marmots is not surprising considering their short active season. Values probably change during hibernation, as they do in the Columbian ground squirrel, *S. columbianus* (Nansel & Knoche, 1972), but no values were obtained for torpid marmots.

The difference in PCV, Hb, erythrocytes, MCV and MCHC between early and late juveniles probably represents a developmental response rather than a seasonal response. Developmental changes are evident in rats (Wells & Webster, 1979). Juvenile marmots are 3–4 weeks old when they emerge from their natal burrow. Therefore, the following question is raised: why do juvenile marmots not develop adult hematological characteristics earlier? They are exposed to low ambient oxygen levels both from high altitude and burrow-dwelling. The answer probably is that the hematological values increase as a result of increased activity which occurs when juveniles are weaned and begin foraging. Above ground activity is minor in the first week, but gradually increases so that after a few weeks the juveniles are active for longer periods during the day than adults. Several authors related high PCV or Hb to activity (Morrison *et al.*, 1963; Lee & Brown, 1970). Therefore, it seems reasonable that activity stimulated the development of adult-level hematological values in late juveniles.

Another question is to what degree do marmot hematological values represent an adaptation to high altitude? One approach is to compare values for marmots with those of other sea-level and high altitude marmots. Bullard *et al.* (1966) considered marmot values similar to those expected for dwellers at sea-level and that hematological values of marmots did

not evidence high altitude adaptation. However, many of the hematological values of marmots are higher than standard values for man, for the opossum (Lewis, 1975), for the snowshoe hare (Dieterich & Feist, 1980), for the bat *Pteropus giganteus* (Lewis, 1977), but are similar to or lower than values for other bats (Caire *et al.*, 1981), and for pronghorn antelope and ungulates in general (Barrett & Chalmers, 1977). Comparisons of such widely different species is not valid as many factors, such as activity, temperature, season and body size affect hematological values (Sealander, 1966; Lee & Brown, 1970; Barrett & Chalmers, 1977; Nieminen, 1980; Scelza & Knoll, 1982). Therefore, only closely-related species should be compared. Thus, hematological values of yellow-bellied marmots were compared with other semi-fossorial sciurids.

The only parameter available for the 11 species for which data were found was PCV (hematocrit). Because PCV is related to body size of small mammals (Sealander, 1965), PCV was plotted against the log of body weight (Fig. 2). There was no significant correlation between these two variables (Olmstead & Tukey's corner test, Sokal & Rohlf, 1969:538). The lack of correlation occurred because this hematological parameter apparently is highly adaptive. The eye-fitted curve was drawn to connect those species who are low altitude dwellers of the temperate zone. The value for *E. minimus*, the smallest species represented, also falls along the line. The three species below the line live in hot environments. In addition, the metabolic rate of *S. tereticaudus* falls below the regression line describing the relationship between body size and metabolism of eight species of spermophiles (Hudson & Deavers, 1973). All the other species, except *C. ludovicianus*, live at moderate to high altitude in relatively cool environments. The metabolic rate of *S. lateralis* falls above the M:W regression line of spermophiles (Hudson & Deavers, 1973). Thus, both environmental temperature and metabolic rate are implicated as factors affecting hematological values, as postulated for other mammals (Sealander, 1962, 1964; Lee & Brown, 1970).

The high PCV of the prairie dog, *C. ludovicianus*, cannot be explained as an adaptation to temperature, altitude or metabolism. The prairie dog lives at low altitudes characterized by hot summers. Thus, its PCV would be expected to fall at or below the line of a PCV:W relationship. This apparent anomaly probably can be explained as an adaptation to the hypoxia of a deep burrow. Oxygen levels in burrows of *S. tridecemlineatus* had a minimal concentration of 13.7 vol % (82.2 mm Hg) with a maximum burrow depth of 1.87 m (Studier & Procter, 1971). The deeper burrows of *C. ludovicianus* may be 5 m deep with tunnels up to 30 m long (Sheets *et al.*, 1971). The burrow plus its many side tunnels and nest chambers constitutes a respiratory dead-space of great magnitude (Vogel *et al.*, 1973). This burrow system probably is the deepest of North American sciurids and during periods when wind-induced ventilation cannot occur; e.g. during snow cover, hypoxic conditions could become severe. The prairie dog has one of the lowest critical PO_2 values coupled with a hemoglobin whose affinity for O_2 is one of the highest reported for a mammal (Hall, 1966). The erythrocyte counts of

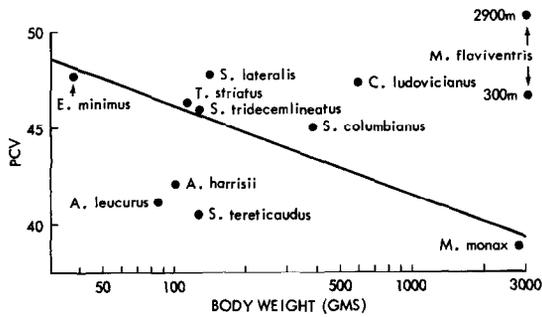


Fig. 2. The relationship between PCV and body weight for 11 species of semi-fossorial sciurids. Body weights are the mean minimum for each species. The diagonal line is eye-fitted to describe a direct relationship between PCV and body size for the three low elevation species, *T. striatus*, *S. tridecemlineatus* and *M. monax*. PCV were obtained as follows: *S. lateralis* (Bullard *et al.*, 1966), *S. columbianus* (Nansel & Knoche, 1972), *Ammospermophilus leucurus* (Lee & Brown, 1970), *Tamias striatus* and *E. minimus* (Sealander, 1964), *M. flaviventris* (this study), *S. tridecemlineatus*, *S. tereticaudus*, *A. harrisi*, *C. ludovicianus*, *M. monax* (Hall, 1965).

prairie dogs are among the highest reported for sciurids (Hall, 1965; Bullard *et al.*, 1966) but lower than that of the fossorial mole rat, *Spalax ehrenbergi* (Ar *et al.*, 1977). Prairie dogs are remarkably insensitive to CO₂ (Withers, 1978). All of these physiological characters are adaptive to hypoxia.

Interestingly, yellow-bellied marmots acclimatized to low elevations have PCV values lower than those of prairie dogs (Fig. 2; Winders *et al.*, 1974). The MCV of yellow-bellied marmots is twice that of the mole rat (Ar *et al.*, 1977). Yellow-bellied marmots have relatively shallow (0.4–0.6 m deep) and short (<5 m) burrow systems (Svendsen, 1976). Thus, no unusual adaptations to burrow hypoxia would be expected.

The higher PCV of the high altitude *M. flaviventris* than that of the low altitude *M. monax* suggests an adaptation to high altitude hypoxia. This difference persists when *M. flaviventris* is acclimatized to low altitudes (Fig. 2; Winders *et al.*, 1974). Because the difference persists in sea-level yellow-bellied marmots, at least part of the high altitude adaptation is genetic. To this genetic base is added the acclimatization response which further increases PCV (Fig. 2) and other hematological values (Winders *et al.*, 1974).

Studies of other rodents demonstrate acclimatization of hematology in response to the simulation of high altitude (Kalabuchov, 1937; Wells & Webster, 1979). However, not all rodents respond to high altitude stress (Kalabuchov, 1937) and rodents taken from high altitudes to sea level may not show reverse acclimatization (Kalabuchov, 1937; Morrison *et al.*, 1963). The lack of acclimatization infers a genetically controlled hematology. The lack of a hematological response to changes in altitude cannot be interpreted as a lack of high altitude adaptation by a species for there are many components of the gaseous exchange and transport systems that can be modified and biochemical adaptations may be the most important (Lenfant, 1973). An animal's hematology is influenced by its body size (probably a function of its metabolic rate), environmental temperatures and environmental

hypoxia. In yellow-bellied marmots, hematological values are influenced by high altitude hypoxia.

Acknowledgements—This research was supported by Grants GB-1980, GB-6123, GB-8526, GB-32494 and DEB78-07327 from the National Science Foundation. My deepest appreciation to David Doll, Jerry F. Downhower, Robert Fleet, Delbert L. Kilgore Jr., Gerald E. Svendsen and Gary Worthen for assistance in trapping animals and collecting and processing blood samples, to D. L. Kilgore Jr. for his helpful suggestions, and to Sharon Hagen for preparing Fig. 2.

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