Transactions of the Illinois State Academy of Science
(1990), Volume 83, 3 and 4, pp. 197-205

Hematological Adaptation to Hypoxia in *Peromyscus* and *Microtus* at High and Low Altitude

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ABSTRACT

1. Values for hematocrit, hemoglobin, RBC numbers and diameter, MCH, MCV, and MCHC were obtained from native populations of *Microtus*, a semi-fossorial rodent, and *Peromyscus*, a non-fossorial rodent, at high and low altitude. Our objectives were to assess hematological adaptation to hypoxia, and to determine whether semi-fossoriality resulted in preadaptation to high altitude hypoxia.

2. There were no differences in the hematological parameters between *Microtus* species at low altitude.

3. Within each genus, high-altitude species had significantly greater hematocrits than their low-altitude congeners.

4. Comparison of the hemoglobin levels of high- and low-altitude animals showed significant differences within *Peromyscus*, but no significant differences within *Microtus*.

5. Erythrocyte numbers did not differ significantly within *Microtus*, regardless of altitude. *Peromyscus*, however, had significantly more red blood cells at 2900 m than at low altitude.

6. MCV increased with altitude within *Microtus* but not within *Peromyscus*. Mean values for MCHC were lower among high-altitude species.

7. These *Microtus* and *Peromyscus* respond differently to altitude; however, differences in these seven hematological parameters provided no

evidence for preadaptation to altitude hypoxia. Rather, these differences probably reflect alternative ways (increased hematopoiesis in *Peromyscus* versus altered red cell volume in *Microtus*) of solving the same problem of low environmental PO_2. This does not, however, preclude preadaption to hypoxia in some other blood parameter(s).

INTRODUCTION

Mammals may experience hypoxia on a day-to-day basis, either at high altitude or within a burrow. Adaptation to low ambient PO_2 may be reflected in the cellular components of the blood. Some mammals alter red blood cell numbers and volume in response to hypoxia (Armitage, 1983; Penney and Thomas, 1975; Hock, 1970; Bullard et al., 1966; Hurtado et al., 1945; Kalabuchov, 1937; Hurtado, 1932), but all species do not respond hematologically (Kalabuchov, 1937; Hall et al., 1936). Bullard (1972) postulated that burrowers may possess adaptive characteristics of the blood that enable them to immigrate into the hypoxic environment of high altitude. We wished to obtain hematological data on fossorial and non-fossorial genera whose ranges encompassed both high and low altitude environments.

The two genera of cricetid rodents used in this study, *Peromyscus* (deer mouse) and *Microtus* (vole) are found over a wide range of altitudes, from sea-level to 4350 m (Snyder, 1985; Hall, 1981). *Microtus* are semi-fossorial (Blair, 1968) whereas *Peromyscus* are chiefly surface-dwellers and non-fossorial (Lackey et al., 1985). Populations of *M. ochrogaster* and *M. pennsylvanicus* inhabit grasslands of the Midwest at elevations below 200 m, and occur sympatrically with *P. leucopus* (M'Closkey and Fieldwick, 1975; Dice, 1922). Populations of *P.maniculatus* and *M. montanus* occur at elevations above 2000 m in the western United States (Stinson, 1978; Armstrong, 1977).

The purpose of this study was to obtain values for hematocrit, hemoglobin, RBC numbers and diameter, mean cell hemoglobin, mean cell volume, and mean cell hemoglobin concentration from native populations of *Microtus* and *Peromyscus* at high and low altitude in order to: (1) assess hematological adaptation to high altitude hypoxia; and (2) test the hypothesis that semi-fossoriality results in preadaptation to high altitude hypoxia.

This study examined some phenotypic expressions of adaptation to hypoxia. If adaptations have occurred as we hypothesized, low-altitude *Microtus* should show blood characteristics that suggest greater adaptation to hypoxia than lowland *Peromyscus*. High-altitude *Peromyscus* should show such adaptations compared to lowland *Peromyscus*, but hematological values of high-altitude and lowland *Microtus* should be similar.

MATERIALS AND METHODS

Hematological values were obtained from native populations of *P. leucopus*, *M. pennsylvanicus*, and *M. ochrogaster* at an altitude of 182 m in Peoria County, Illinois, and of *P. maniculatus* and *M. montanus* at an elevation of 2900 m near the Rocky Mountain Biological Laboratory in Gunnison County,

Colorado. Animals were livetrapped from April to May 1988 in Illinois, and from July to August 1988 in Colorado. Sherman traps were baited with peanut butter-oat mixture, set at dusk, and examined at dawn the following day.

Captured animals were weighed, sexed, and marked with waterproof ink on the venter to prevent duplicate sampling in the event of recapture. Nineteen individuals of each species were sampled. Using microhematocrit capillary tubes, blood samples were taken in the field from the suborbital sinus of animals weighing 18 g or more. One hundred and fifty to 225 µl were collected per animal (two to three capillary tubes).

Blood samples were chilled, and analyzed in the laboratory within 4 hours of collection. Duplicate determinations were performed whenever possible. For the hematocrit (Hct), microcapillary tubes were spun for five minutes at 6500 rpm in a Clay-Adams microhematocrit centrifuge and measured immediately. Hemoglobin (Hb) was determined by the cyanmethemoglobin method on a Welch ChemAnal spectrophotometer set at 540 nm. Erythrocytes (RBC) were counted manually on a hemacytometer with Neubauer ruling. Premeasured diluents for cell counts or hemoglobin determinations were provided in precalibrated Unopette® diluting chambers. Erythrocyte diameter was measured on Wright's-stained dry blood smears using a Zeiss eyepiece micrometer. The diameters of fifty cells were averaged (MCD). Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated following Wintrobe (1932).

Data were analyzed by one-way ANOVA and Student's t-test, with a posteriori comparison of the means by Tukey's HSD. A two-way ANOVA was performed for altitude X genus. Minitab software was used for the analysis of variance and Student's t-test.

RESULTS

In general, hematological values for the high-altitude animals showed slightly less variability than low-altitude animals (Table 1). Among the five species studied, values for *M. pennsylvanicus* showed the greatest variability, especially in hemoglobin and consequently, the MCH calculated from it.

There were significant differences among all species in hematocrit (One-way ANOVA: $F_{4,94} = 40.09$, $P < 0.01$). Within each genus, high-altitude species had significantly greater hematocrits than their low-altitude relatives (Tukey's HSD: $q_r = 14.21, 16.63$; $q_{0.01}(5,90) = 5.66$) (Table 2). There was no difference in hematocrit between *Microtus* spp. at low altitude. Hematocrit values for *Peromyscus* were higher than *Microtus* at both altitudes, but significant differences between the genera were found only at low altitude (Tukey's HSD: $q_r = 6.26$, $q_{0.01}(5,90) = 5.66$; $q_r = 5.05$, $q_{0.01}(5,90) = 4.70$).

Analysis of hemoglobin levels by one-way ANOVA showed significant differences among all five species ($F_{4,94} = 3.89$, $P < 0.01$). Comparison of the hemoglobin levels of high- and low-altitude animals using Tukey's HSD revealed significant differences within *Peromyscus* ($q_r = 2.79$, $q_{0.05}(5,90) = 2.50$), but no significant differences within *Microtus*.

Erythrocyte numbers were significantly different among all five species (One-way ANOVA: $F_{4,94} = 4.14$, $P < 0.01$). Erythrocyte numbers did not differ significantly within *Microtus*, regardless of altitude. *Peromyscus*, however, had significantly more red blood cells at 2900 m than at low altitude (Tukey's HSD: $q_r = 2.52$, $q_{0.01}(5,90) = 1.09$). There were significant differences ($q_r = 1.73$, $q_{0.01}(5,90) = 1.09$) between *Peromyscus* and *Microtus* at high altitude, but not at low altitude. A two-way ANOVA revealed a significant interaction between genus and altitude ($F_{1,75} = 6.08, 5.42$; $P < 0.05$).

There were significant differences among all five species for MCV (One-way ANOVA: $F_{4,94} = 7.64$, $P < 0.01$). Comparison of the means by Tukey's HSD indicated significant differences within *Microtus* between highland *M. montanus* and its lowland congeners ($q_r = 10.25, 8.73$; $q_{0.01}(5,90) = 7.19$); there was no difference within *Microtus* at low altitude. Significant differences between genera were found between *M. ochrogaster* and *P. leucopus* ($q_r = 6.93$, $q_{0.05}(5,90) = 5.96$). For MCV there was a significant interaction between genus and altitude (Two-way ANOVA: $F_{1,75} = 16.44, 10.48$; $P < 0.01$).

There was no significant differences for MCH values among the five species.

Mean values for MCHC were lower among high-altitude species and resulted in significant differences among the five species (one-way ANOVA: $F_{4,94} = 9.90$, $P < 0.01$). There were significant differences between *M. montanus* and *M. ochrogaster* (Tukey's HSD: $q_r = 6.21$, $q_{0.01}(5,90) = 5.33$) and between *M. montanus* and *M. pennsylvanicus* ($q_r = 7.85$, $q_{0.01}(5,90) = 5.33$). Significant differences were found between genera at low altitude: *P. leucopus* and *M. ochrogaster* ($q_r = 4.52$, $q_{0.05}(5.90) = 4.42$) and *P. leucopus* and *M. pennsylvanicus* ($q_r = 6.16$, $q_{0.01}(5,90) = 5.33$). A significant interaction for MCHC was found (Two-way ANOVA: $F_{1,75} = 7.06, 12.26$; $P < 0.01$).

Student's t-tests were performed on MCD values; ANOVAs were not performed due to unequal sample sizes. MCD differed significantly within *Peromyscus* ($t_{1,23} = 2.81$, $P < 0.005$) and between genera at high altitude ($t_{1,36} = 2.43$, $P < 0.01$). There were no other significant differences between or within genera for mean erythrocyte diameter. MCD in *P. maniculatus* and *M. montanus* was lower in this study than in Sealander's (1964). His small sample sizes may account in part for the discrepancy.

Although MCD can give a general idea of red cell size in relation to surface area and cell volume, it is not considered an accurate index by Wintrobe (1932). An increase of 1.0 μ in diameter in a red cell represents an increase of 44% in cell volume (Haden, 1923). Furthermore, cell thickness varies more than does cell diameter (Wintrobe, 1932). Nonetheless, in this study there does seem to be a positive association between MCV and MCD when comparing genera at low altitude. At high altitude, however, this association disappears.

DISCUSSION

The results of this study demonstrated that two related rodent genera, native to high and low altitude, differ hematologically. The question was how hematological differences at different altitudes represent adaptations to hypoxia, and whether semi-fossoriality resulted in preadaptation to the hypoxia of high altitude.

Hematological values for hematocrit and hemoglobin for *M. pennsylvanicus* and *P. leucopus* were similar to those reported in the literature (Dunaway and Lewis, 1965; Sealander, 1964). In this study, the hematocrit for *P. maniculatus* was significantly greater ($t_{1,33} = 4.99$, $P < 0.01$) than that found by Sealander (1964). Hemoglobin and hematocrit for *M. montanus* (Hb = 17.2 g%, Hct = 55.6%) were higher than those found by Sealander (Hb = 14.4 g%, Hct = 46.7%; 1964) although statistical significance could not be tested with his data.

A common and generally expected response of mammals to hypoxia is an increased production of hemoglobin and RBCs. This study confirmed other reports of the increased erythropoietic activity in highland *P. maniculatus*, resulting in higher hematocrit, hemoglobin, and RBC number (Snyder, 1982; Snyder et al., 1982; Hock, 1964). However, polycythemia is not always found among highland rodents (Morrison et al., 1963 a,b; Kalabuchov, 1937) and, indeed, although possessing a higher hematocrit, *M. montanus* did not have a higher number of RBCs than its low-altitude congeners (Table 1).

The RBCs in *P. maniculatus* at high altitude were smaller, but "thicker" than those of low-altitude *leucopus*, since the cells contain the same volume within a smaller diameter (Table 1). MCH and MCHC in *Peromyscus* at high altitude were maintained, therefore, at the same level as at low altitude through an increased production of hemoglobin and RBCs (Table 1). RBCs of *M. montanus* were "thicker" than those of *Microtus* at low altitude; the larger cell volume resulted in a higher hematocrit for *montanus*. The increase in RBC volume, however, was not matched by a commensurate increase in hemoglobin production; mean cell hemoglobin concentration, therefore, was less in high-altitude voles.

At first glance, this does not appear to be an adaptation to hypoxia. A greater hemoglobin concentrating ability is expected in an animal experiencing hypoxia within a burrow (Quilliam et al., 1971). Hurtado (1932), however, proposed that since hemoglobin is distributed at the inner surface of the cell membrane, the increased surface area of a cell of larger volume actually enhances oxygen loading and unloading between RBC and tissue.

It should be recognized that these rodents may respond to hypoxia using mechanisms other than those investigated here. For example, high hemoglobin-oxygen affinity (low P_{50}) is commonly found among burrowers and rodents native to high altitude, including *Peromyscus* (Snyder, 1985; Snyder, 1982; Snyder et al., 1982; Ar et al., 1977; Lechner, 1976; Baudinette, 1974; Bullard, 1972; Hall, 1966, 1965), in addition to an ability to utilize oxygen at a lower critical environmental oxygen tension (Pc) (Rosenmann and Morrison, 1985; Hall, 1966). There is evidence of hypoxic resistance in *M. pennsylvanicus*,

who experienced a smaller increase in respiratory rate under hypoxia than other lowland rodents, including *Peromyscus* (Rosenmann and Morrison, 1975).

This study demonstrated that these *Microtus* and *Peromyscus* respond differently to altitude; however, differences reflected in these seven hematological parameters provided no evidence for semi-fossorial preadaptation to altitude hypoxia. Rather, these differences probably reflect alternative ways (i.e., increased hematopoiesis in *Peromyscus* versus altered red cell volume in *Microtus*) of solving the same problem of low environmental PO_2. Our data do not however, preclude preadaptation to hypoxia in some other blood parameter(s).

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the AAUW Education Foundation. Equipment, supplies, and facilities were provided by the Biology Department of Bradley University. Facilities in Colorado were provided by the Rocky Mountain Biological Laboratory. This work was conducted by S. Wyckoff in partial fulfillment of the requirements for a Master of Natural Science degree at Bradley University.

Bill Kapitko of Jubilee State Park, and the Colorado Division of Wildlife granted permission to trap on government-owned land. We are also grateful to Mr. and Mrs. G. Kailey, Mr. and Mrs. A. Kleczek, and the Farm Credit Services of North Central Illinois for access to private land. The following persons kindly lent equipment and assistance: Dr. R. L. Willey, Dr. E. Gasdorf, Dr. M. R. Lee, Dr. J. E. Hofmann, R. Smith, H. Gasdorf, B. Stoner, W. Wilkins, R. Bjorklund, D. Wyckoff. Dr. E. Gasdorf and two reviewers made helpful comments on the manuscript. S.M.W. is grateful to S. Kellums and S. Nicklin for child care.

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Table 1. Mean Hematological Values ± S.E.

Species	(%) Hct	(g%) Hb	(10^3/mm^3) RBC	(u^3) MCV	(uug) MCH	(%) MCHC	(u) MCD	
M.o. (L)	41.4	15.3	11.3	37.9	13.7	37.3	6.0	(4)
	±1.25	±0.65	±0.57	±1.65	±0.26	±1.60	±0.00	
M.p. (L)	42.6	16.6	11.2	39.5	15.4	38.9	6.1	(2)
	±1.19	±0.97	±0.61	±1.99	±0.94	±1.60	±0.01	
P.l. (L)	47.6	15.6	10.8	44.9	14.8	32.8	6.3	(6)
	±1.32	±0.51	±0.42	±1.55	±0.76	±0.67	±0.01	
M.m. (H)	55.6	17.2	11.6	48.2	14.9	31.1	6.1	
	±1.18	±0.49	±0.31	±1.09	±0.40	±0.78	±0.05	
P.m. (H)	58.0	18.4	13.3	44.1	14.0	31.7	5.9	
	±0.95	±0.38	±0.45	±1.06	±0.38	±0.36	±0.06	

M.o. = Microtus ochrogaster, M.p. = Microtus pennsylvanicus
P.l. = Peromyscus leucopus, M.m. = Microtus montanus
P.m. = Peromyscus maniculatus
L = Low altitude (182 m), H = High altitude (2900 m)
N = 19 except as indicated in parenthesis for some MCD determinations.

Table 2. Comparisons within and between genera in Illinois (182 m) and Colorado (2900 m). Analysis with Tukey's HSD and t-test (MCD only).

Comparison	Hct	Hb	RBC	MCV	MCD	MCH	MCHC
Within Genus							
M.o. vs. M.p.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M.o. vs. M.m.	**	n.s.	n.s.	**	n.s.	n.s.	**
M.p. vs. M.m.	**	n.s.	n.s.	**	n.s.	n.s.	**
P.m. vs. P.l.	**	**	**	n.s.	***	n.s.	n.s.
Between Genera							
M.o. vs. P.l.	**	n.s.	n.s.	*	n.s.	n.s.	*
M.p. vs. P.l.	**	n.s.	n.s.	n.s.	n.s.	n.s.	**
M.m. vs. P.m.	n.s.	n.s.	**	n.s.	**	n.s.	n.s.

M.o. = Microtus ochrogaster (IL)
M.p. = Microtus pennsylvanicus (IL)
M.m. = Microtus montanus (CO)
P.m. = Peromyscus maniculatus (CO)
P.l. = Peromyscus leucopus (IL)
* $P < 0.05$ ** $P < 0.01$ *** $P < 0.005$