Spring 1983

Differential White Blood Cell Count At High Altitude In Microtus montanus nanus

Timothy Wyse
Carroll College

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DIFFERENTIAL WHITE BLOOD CELL COUNT
AT HIGH ALTITUDE IN
Microtus montanus nanus

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology at Carroll College, Helena, Montana

Timothy William Wyse
March 22, 1983
This thesis for honors recognition has been approved for the Department of Biology.

Dr. John Christenson, Director

Dr. Jean Smith, Reader

Mr. Harold Smith, Reader

April 7, 1983
ACKNOWLEDGEMENTS

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My greatest gratitude goes to the Budd Hereford Ranch and especially the Espenchiel family, who not only employed me during this study, but provided for me two summers of extreme character-building. Thanks to Gary, Nancy, Brian, Chad, Jim, Randy, Shane, and Dan for all the help.

I'd also like to thank my roommates, Esquire Jim Staples and Esquire Todd McGovern, who have made my senior year at the Townhouse very special. I'd like to thank my grandmother, my parents, and my brothers and sister for their love and support during these four trying years at Carroll College. I'd like to thank the institute of Carroll College, who has cultured and matured my ideals and thus helped me to achieve my goals. Finally, I'd like to thank Cheryl McNurlin, who's not only been an exquisite typist all these years at Carroll, but also a great friend.
ABSTRACT

Differential white blood cell counts were done on high altitude *Microtus montanus nanus*. The counts were statistically compared to the differential white blood cell counts of three other samples of *Microtus*, one of similar altitude and two of lower altitude. Statistically significant differences existed between the samples. Discussion of altitudinal, hormonal, parasitic, and environmental influences on hematological parameters is included.
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INTRODUCTION

There are many interesting physiological differences that occur within a species in relation to the altitudes they inhabit. Characteristics of adaptation to high altitudes (>1800m, >6000 ft), are greater lung volumes, higher hematocrits, larger capillaries of the lungs, significantly larger volumes of blood in the pulmonary system of the body's total volume, and an unusually large heart which beats slower than average. In addition to circulatory differences, differences also exist in metabolic rate, endocrine function, especially adrenocorticoid functions, the thresholds of the nervous system, and immunological parameters (Hock, 1970). My interest in high altitudes was stimulated by having worked in high altitudes, by noting differences in diets of high altitude residents, and by having suffered from acclimatization to high altitudes. My investigation of a physiological aspect of high altitudes was with white blood cells in montana vole, Microtus montanus nanus (Hall and Kelson, 1959).
LITERATURE REVIEW

Human Studies

In 1968, the Summer Olympic Games were held in Mexico City at an elevation of 2240m (7350 ft). As the athletes prepared for their events, much investigation was done into acclimatization to this high altitude. Hypoxia was found to be the major cause of reduced performance in most athletes and acclimatization was by adjustment to the low oxygen atmospheric pressure (PO$_2$). Physiological adjustments included increased pulmonary ventilation, increased hemoglobin in the blood, increased diffusing capacity of the lungs, increased vascularity of the tissues, and increased ability of the cells to utilize oxygen despite the low PO$_2$. The latter is accomplished by raising the amount of mitochondria and cellular enzymes found within the cells (Guyton, 1976).

Many natives in the Andes and in the Himalayas live at altitudes above 3964m (13,000 ft). One group in the Peruvian Andes actually lives at an altitude of 5338m (17,500 ft) and works a mine during the day at an altitude of 5795m (19,000 ft). Most of these individuals are born at these altitudes and live there
all of their lives. These natives are superior in functioning at these altitudes even when compared to the best acclimatized (for 10+ yr) lowlanders (Guyton, 1976).

These Andeans have descended from generations of inhabitants living continuously at high altitude. They have adjusted completely to an alveolar PO₂ as low as 50mmHg (0.07 atm). Process of acclimatization of the Andean native is believed to begin in infancy. The products of this acclimatization as seen in the adult are that the chest size is especially increased whereas the body size is somewhat decreased, yielding a decreased weight relative to stature. This ratio allows for increased ventilatory capabilities by increasing vital capacity and pulmonary ventilation relative to body mass. Circulatory consequences include increased hematocrits and myoglobin elevated pulmonary arterial pressure to pump blood through a greater expanded capillary system, hypertrophy of the right ventricle of the heart and of the muscular coat of the smaller pulmonary vessels, and sustained hypoxic arteriolar vasoconstriction. The end results of these physiological effects can be demonstrated in the performance of the hard labor required to cultivate the slopes of the Andes, the Andean native's high degree of efficiency well exceeds that of the sea-level worker. His pulmonary ventilation is greater, oxygen consumption is less for a given
workload, pulse rate and blood pressure are lower, and he has a smaller rise in lactic and pyruvic acid levels which compensate for a decreased buffer base (Guyton, 1976; Wintrobe et al., 1976).

The Sherpa of the Himalayas demonstrate man's best survival at high altitudes. These skilled mountain climbers have been observed on expeditions of climbing Mt. Everest, to be able to survive for several hours without oxygen supplement at altitudes as high as 8845m (29,000 ft). It is postulated that perhaps a biochemical anomaly regulates the affinity of hemoglobin for oxygen, a development which has occurred in the llama, yak, and other mountain animals. Others believe the ability of the Sherpa is probably related to better enzyme function, different enzyme ratios, and higher amounts of particular enzymes (Barrie, 1977).

The Peruvian Indians and Sherpas seem to be at an extreme of man's capabilities at high altitude. The majority of people that live at high altitudes, approximately six million, live at an average altitude of 1283m (6500 ft). Residents at these altitudes normally have a higher erythrocyte count than those at sea level. The higher the altitude, the less will be the oxygen tension and therefore the resultant hypoxia will stimulate erythropoiesis. If a resident at sea level quickly ascends to a high altitude, he may at first suffer from hypoxia and show symptoms of dizziness, weakness, and
shortness of breath. Within a few days reticulocytosis is noted and followed by a gradual increase in the erythrocyte count and hemoglobin concentration. After a few months the count may reach erythrocytosis (polycythemia) (Miale, 1972).

A study was conducted on a group of subjects from sea level who were moved to an altitude between 3965m (13,000 ft) and 5795m (17,000 ft). It was first found that a mean reduction in blood volume of 9% occurred, but later returned to normal or higher than normal values. During a six month time interval, hemoglobin concentration showed a progressive rise to a mean of 49% above that of sea level values. It was found, therefore, that erythrocytosis due to high altitudes is accompanied by an increased total erythrocyte mass, but total blood volume is usually not increased. Blood values of the above individuals returned to normal within a month after returning to sea level (Miale, 1972).

Conclusive demonstration of effects of climate or season on leukocyte count is lacking. In the first few days after arrival at high altitudes, some studies showed that certain stimuli, such as infection, influence the production and liberation of neutrophilic series of cells as well as other white blood cell differentials. Experimentally, it has been shown that leukophoresis with its attendant neutrophil loss, causes a rapid acceleration of marrow release. Under other circumstances,
the production of the other forms of leukocytes may be accelerated or repressed (Wintrobe, 1967).

Hormonal influences on leukocytes have been found. Thryoxine increases produce a relative and absolute increase in lymphocytes and an increase in eosinophils. The pituitary adrenocorticotropic hormone (ACTH) through its effect on adrenal secretion, evokes a leukocytic response in man as well as some species of animals. Single injections of ACTH in mice, rats, and rabbits produced within a few hours an absolute lymphopenia, leukocytosis, accompanied by lymphonemia and eosinopenia, has been observed. Slight lymphocytosis and eosinophilia soon develop, however, physiological variations in leukocytes are explained as manifestations of the stimulation of the adrenal cortex. (Wintrobe, 1967).

Factors regulating the production and release of leukocytes are difficult to solve since each leukocyte may be governed in a different way. There is in bone marrow a storage reservoir of neutrophils and in the blood approximately one-half of these cells are not circulating freely. Loss from the blood is by egress rather than senescence and consequently the concentration of leukocytes in venous blood is an unreliable measure of production. Nevertheless, it is clear from clinical and experimental studies that certain stimuli, such as infections, influence the production and liberation of the neutrophil's series of cells. Experimentally,
it has been shown that leukophoresis with its attendant neutrophil loss causes a rapid acceleration of marrow release. Under other circumstances, the production of the other forms of leukocyte may be accelerated or repressed (Wintrobe, 1967).

The pituitary adrenocorticotropic hormone (ACTH), through its effect on adrenal secretion, evokes a leukocytic response in man as well as some species of animals. Single injections of ACTH in mice, rats, and rabbits produced within a few hours an absolute lymphopenia, a reaction which fails to occur in adrenalectomized animals. Adrenocorticosteroids produced the same response in intacted and in adrenalectomized animals. Similar changes in man have been observed. A single intramuscular dose of 0.25 mg of ACTH to normal humans is followed by an increase of circulatory lymphocytes and eosinophils.

Mouse and Vole Studies

*Microtus montanus*, the montane vole, belongs to the order Rodentia, family Cricetidae, and subfamily Microtini. They are boreal in distribution and have evolved into many subspecies. They characteristically inhabit the second ecological mountain level, but may extend both into the alpine tundra or into the tree covered areas. They are associated with vegetation just below the snow line, that of cushion plants, mosses, and lichens, but may extend into pastural lands of cold
climates and mountain regions, such as that found in the Wyoming range. These animals survive by having thick fur with layers of brown fat beneath the skin. They have short legs, mouths, ears, and tails. They shelter in crevasses and roll up in a ball to sleep. All of these features are adaptations to conserve energy and heat (Heinz and Juneticheck, 1976).

The diet of *M. montanus* consists of seeds, grasses, and leaves, but an occasional insect may be eaten. They consume an amount of food equal to their own body weight every 24 hr during the brief summer months. Inability to accumulate sufficient fat reserves before hibernation begins acts as a population selector (Brown, 1970).

The research with *M. montanus* has been very limited. However, altitude studies on mice have been done. Pearson and Pearson (1976) performed a stereological analysis of the ultrastructure of the lungs of *Phyllotis darwini*, a species of mouse widespread in South America, and which has populations that have evolved at both high and low altitudes. In contrast to larger mammals, Pearson and Pearson found that the hematocrits of these two different altitudinal populations did not differ significantly. They did find, however, that the surface/volume ratio of the erythrocytes in the capillaries of the lung was significantly greater in high altitude mice than in low altitude mice. Also the mean diameter of the red blood cells from the high altitude population
was 5.2% smaller, which created a 26% greater surface/volume ratio in capillary red blood cells. Pearson and Pearson concluded that the larger lung found in high altitude mice not only contained more respiratory units, but that the units were either smaller or were modified in some other manner that increased the ratio between surface area of the alveolar epithelium and volume of the alveolar air space. Theoretically, a decrease in spherical alveoli from 56\mu m at low altitude to 44\mu m at high altitude would achieve the differences in surface/volume ratio within the two different populations (Pearson and Pearson, 1976). This study is significant in that it suggests such a reduction in volume is a feature of adaptation and not acclimatization to high altitudes.

Another study by Ahl (1968) examined the North American deer mouse, *Peromyscus maniculatus nebrascensis*. Populations sampled at 3000 ft, 5000 ft, 7000 ft, 9000 ft, and 11,000 ft, showed no significant hematocrit differences. Ahl stated that basically the hematocrit and the amount of hemoglobin in small wild rodents do not increase in response to altitude probably because the hematocrits are so high, even at sea level, that they are close to the theoretical maximum hematocrit for blood to flow as a liquid (60%). Ahl did find, however, that a distinct type of hemoglobin, type S, existed in the mice trapped from higher altitudes which
did not exist in mice of lower altitudes (Ahl, 1968). This information correlates well with that of Pearson and Pearson (1976) and Dieterick and Preston (1977), who found that the erythrocyte is smaller in high altitude mice than that of the house mouse (*Mus musculus*). Perhaps, then, the type S hemoglobin could account for the differences in the size of the erythrocyte of the high altitude *P. maniculatus*.

Dieterick and Preston (1977a, 1977b) published two very similar articles in which three very closely related relatives of *Microtus montanus* were examined for their usefulness as laboratory animals. In the studies, husbandry needs, reproductive behavior and physiologic characteristics were determined for *Microtus pennsylvanicus tananaensis* (the common North American meadow vole), and for *Microtus oeconomus macfarlani* and *Microtus oeconomus operanius* (two subspecies of tundra vole). The two studies showed that the hematological values were similar to those of the house mouse (*Mus musculus*) except for smaller erythrocytes and fewer leukocytes. Organ weights, when compared as a percent of total body parts, differ only slightly from those of *M. musculus*, with these three species all having greater relative amounts of brown fat. Also, in all three species, the muscle glycogen level was similar to that of other northern microtines, but nearly three times greater than that of equatorial rodents. The
main difference found between M. pennsylvanicus and the M. oeconomus ssp. was that M. pennsylvanicus was more hypoxic resistant (Dieterick and Preston, 1977).

In addition to habitat considerations, influences on the hematological system can also come from internal stresses occurring within the animal. One such stress examined by Dobrowlska and Gromadzka (1978), was the influence of the estrous cycle in the common vole, Microtus arvalis. The results suggested that M. arvalis has irregular estrous cycles and that mechanical stimulation can induce ovulation, a characteristic of most small rodents and probably of M. montanus. Even more interestingly, they demonstrated that the hematological values a negative correlation between red blood cell parameters and progesterone concentration existed in the blood of female voles in different estrous cycle periods. When the progesterone levels were high during metaestrous and estrous periods, the erythrocyte numbers and hemoglobin concentration was lower. Yet, on the other hand, when the progesterone levels were high during metaestrous and estrous periods, the total and differential leukocytes were higher. The number of lymphocytes and segmented neutrophils in the blood changed from maximum during estrous to minimum during the proestrous periods. The increase of monocyte number from proestrous to metaestrous was observed, while a decrease was seen during diestrous. Also, a statistically significant
increase of the number of lymphocytes and segmented neutrophils occurred during the estrous period. Therefore, a positive correlation exists between progesterone levels and leukocyte numbers. It is possible, then, that sexual hormones modify the blood parameters. In general, as seen by Lewkiewicz-Dziarska (1975, cited in Dorbowolska and Gromadzka, 1978), a decrease in hemoglobin level and erythrocyte number occurs simultaneously with an increase in leukocyte numbers during the summer breeding seasons (Dorbowolska and Gromadzka, 1978).

A survey of male Microtus montanus trapped at Jackson Hole, Wyoming, only 147km (110 mi), from the capture site of the present study, indicated that 100% of these animals harbored one or more species of endoparasites and/or ectoparasites. Spleen histology and differential white blood cell counts suggested that these animals have persistent parasitic infections, which serve as a stimulus to the immune response (Seed et al., 1976).

Seed et al. (1976) also demonstrated that there was a high percentage of splenomegaly in these Jackson Hole animals. This fact supports the hypothesis that splenomegaly is caused by parasitic infection. Therefore, splenomegaly could possibly be used as a survey marker to determine the extent of parasitism and the condition of white blood cell differentials in field populations. It has also been shown that there was a significant
inverse relationship between spleen size and gonadal weights. It is assumed that smaller gonads denote reduced reproduction potential. It is logical, therefore, that parasitic infection by causing splenomegaly and thus inhibiting reproduction potential may have a great effect on control of population density as well as leukocyte parameters (Seed et al., 1976).
MATERIALS AND METHODS

I studied white blood cell differentials in *Microtus montanus nanus* (Hall and Kelson, 1959), the montane vole, on the Budd Hereford Ranch 8 mi north of Big Piney, Wyoming. The ranch is located at the foothills of the Wyoming Range of the Rocky Mountains, latitude 42° 32.4'N and longitude 110° 06.7'W. The ranch is within the boundaries of Sublette County and has an altitude varying from 2196m (7200 ft) to 2470m (8100 ft). The climate of the area has only 30 to 40 frost-free days a year. The terrain is characterized by gravelly domes intertwined with grassy basins which are fed by creeks and irrigation systems.

I began trapping mice in May. The traps consisted of 13 single aluminum live-traps and three aluminum multi-mouse live-traps. The traps were set in old, abandoned homesteads, animal and machinery sheds, old dumps, near grain bins, and around haystacks. The best baits for the traps consisted of rotting and spilled grains and peanut butter. These provided an aroma that attracted not only mice, but skunks and ground squirrels as well. Oftentimes these latter two animals would push the trap from its original placement.
To capture *M. montanus*, the hand-catch method proved best. In early May, I caught many samples by cleaning up old haystack yards that had hay in them over the winter. By turning over haybales and watching underneath for mice, a quick-handed individual can obtain excellent mouse samples. This method is most easily accomplished if you have an assistant. Likewise, in August, during haying season as the haybaler removes the windrows of hay, mice were exposed and hand-caught in the short, dry meadows. A thick, leather glove provided protection against biting.

Mice obtained by the hand-catch method showed fewer signs of stress than mice caught with other methods. Still, it was necessary to quickly obtain a blood sample. The easiest and best method to obtain an ample representative blood supply off the vole was a razor slash across the neck exposing the carotid. Other more "humane" techniques such as slashing tails or using a needle to draw blood were tried, but found unsuitable for the squirming, short-tailed vole.

A drop of the blood was then placed on a glass slide and a standard blood smear was made. The best smears occurred if the slide was warm. The dried blood smears were stained with Wright's Rapid Stain for 10 sec, and then rinsed with local well water. The slide was dried and labeled. The label noted the date, temperature of the environment, sex, and if female, whether
she was lactating or non-lactating, and approximate age, infant juvenile or adult. The slides were later examined under oil immersion. A differential white blood cell count was made in which monocytes, lymphocytes, neutrophils, eosinophils and basophils were counted until 100 leukocytes were obtained. Three slides upon reasonable examination did not produce 100 leukocytes. The percentage of each type of cell was determined for all individuals in a population of 30. The 30 samples were then combined for the total.
RESULTS

Thirty blood smears of *Microtus montanus nanus* were examined under oil immersion. The 30 samples were composed of 11 adult males, 13 adult females, two of which were lactating, four juveniles and two pups. Twenty-one of the 30 samples were processed before July 1, 1982, and nine were processed after. White blood cell differentials for various aspects of the population are summarized in Table 1.

Table 2 illustrates differential white blood cell counts of the present study and other literature data for *Microtus*.

Table 3 statistically compares the present study samples, which are used as control means, against other literature samples, which are used as experimental means. The means were compared utilizing a two-tailed student-t distribution with a 95% confidence level. The equation used:

\[
t = \frac{\bar{x}_e - \bar{x}_c}{\sqrt{\frac{(N_e-1)s_e^2 + (N_c-1)s_c^2}{N_e + N_c - 2} \left( \frac{1}{N_e} + \frac{1}{N_c} \right)}}
\]

where \(e\) = experimental values and \(c\) = control values.

In order for \(t\) to be *not* statistically significantly
different, \( t \) values must be within \( 1.96 < t < 1.96 \).

Table 4 demonstrates the effect of environmental temperature on the percent of lymphocytes counted within each individual of the population.

A total of 2977 white blood cells was counted. Three were basophils, 54 were eosinophils, 702 were neutrophils, 1990 were lymphocytes, and 228 were monocytes.
Table 1. Differential white blood cell counts\(^1\) from *Microtus montanus nanus* of present study collected at altitudes from 2196m to 2470m

<table>
<thead>
<tr>
<th>Aspect of Population</th>
<th>Character of Sample</th>
<th>No. of Samples</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Pups</td>
<td>2</td>
<td>5.51</td>
<td>67.00</td>
<td>21.50</td>
<td>1.00</td>
<td>--</td>
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<tr>
<td></td>
<td>Juvenile</td>
<td>4</td>
<td>7.52</td>
<td>59.50</td>
<td>22.50</td>
<td>1.00</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>24</td>
<td>8.00</td>
<td>67.00</td>
<td>23.80</td>
<td>0.78</td>
<td>0.08</td>
</tr>
<tr>
<td>Sex</td>
<td>Lactating Female</td>
<td>2</td>
<td>11.50</td>
<td>59.00</td>
<td>28.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Non-lactating Female</td>
<td>11</td>
<td>7.90</td>
<td>68.40</td>
<td>22.70</td>
<td>0.70</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>11</td>
<td>6.45</td>
<td>69.73</td>
<td>23.09</td>
<td>0.73</td>
<td>--</td>
</tr>
<tr>
<td>Time of Capture</td>
<td>May - June</td>
<td>21</td>
<td>5.60</td>
<td>72.20</td>
<td>21.60</td>
<td>0.70</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>July - August</td>
<td>9</td>
<td>9.00</td>
<td>65.00</td>
<td>24.90</td>
<td>.09</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Total Characteristics of the Population  30  7.70  66.80  23.60  1.81  0.10

\(^1\)A total of 2977 white blood cells was counted. Three were basophils, 54 were eosinophils, 702 were neutrophils, 1996 were lymphocytes, and 228 were monocytes.
Table 2. Differential white blood cell counts of *Microtus* comparing data of present study with data from other literature

<table>
<thead>
<tr>
<th>Species</th>
<th>Altitude of Habitat</th>
<th>No. of Specimens</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pennsylvanicus</em></td>
<td>137m</td>
<td>21</td>
<td>5±5</td>
<td>78±14</td>
<td>14±4</td>
<td>3±3</td>
<td>0.2±0.4</td>
</tr>
<tr>
<td><em>M. oeconomus operarius</em></td>
<td>137m</td>
<td>22</td>
<td>1±2</td>
<td>86±10</td>
<td>10.5±9.8</td>
<td>1±1</td>
<td>0.4±1.1</td>
</tr>
<tr>
<td><em>M. montanus</em></td>
<td>1800m</td>
<td>8</td>
<td>5.25±1.22</td>
<td>79.5±2.82</td>
<td>12.25±1.46</td>
<td>3±0.82</td>
<td>--</td>
</tr>
<tr>
<td><em>M. montanus</em></td>
<td>1800m</td>
<td>10</td>
<td>14.84±1.76</td>
<td>45.6±4.36</td>
<td>36.99±3.85</td>
<td>2.50±0.64</td>
<td>--</td>
</tr>
<tr>
<td><em>M. montanus</em></td>
<td>1800m-2135m</td>
<td>23</td>
<td>10.94±1.06</td>
<td>50.79±2.73</td>
<td>34.4±2.36</td>
<td>3.78±0.62</td>
<td>--</td>
</tr>
<tr>
<td><em>M. montanus nanus</em></td>
<td>2196m-2471m</td>
<td>30</td>
<td>7.7±3.9</td>
<td>66.8±9.5</td>
<td>23.6±5.8</td>
<td>1.8±1.3</td>
<td>0.1±0.43</td>
</tr>
</tbody>
</table>

1From Dieterich and Preston (1977a) Fairbanks, Alaska.
3From Seed et al. (1976). Laboratory reared, Jackson, Wyoming.
4From Seed et al. (1976). Laboratory reared and chronically infected (>20.0 days) with *Trypanosoma brucei gambiense*. Jackson, Wyoming.
5From Seed et al. (1976). Wild captured near Jackson Hole, Wyoming.
6From present study (1982). Wild captured near Big Piney, Wyoming.
Table 3. Statistical comparison for small-sample tests comparing statistically significant differences between means of white blood cell differentials of present study\(^1\) with other literature utilizing a 2 tailed student-t test with a 95% confidence level.

SD=statistically significantly different with \(\alpha = .05\).
NDS=not statistically significantly different with \(\alpha = .05\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Altitude</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pennsylvanicus</em> tananae(\textit{sis}^2)</td>
<td>137m</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>NSD</td>
<td>NSD</td>
</tr>
<tr>
<td><em>M. oeconomicus</em> operarius(\textit{arius}^3)</td>
<td>137m</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>NSD</td>
</tr>
<tr>
<td><em>M. montanus</em>(^4)</td>
<td>1800m</td>
<td>NSD</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>---</td>
</tr>
<tr>
<td><em>M. montanus</em>(^5)</td>
<td>1800m</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>NSD</td>
<td>---</td>
</tr>
<tr>
<td><em>M. montanus</em>(^6)</td>
<td>1800m to 2135m</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>---</td>
</tr>
</tbody>
</table>

1 Altitude of population of present study 2196m to 2471m.
5 Seed et al. (1976). Laboratory reared and chronically infected (>20.0 days) with *Trypanosoma brucei gambiense*. Jackson, Wyoming.
Figure 1. Effect of environmental temperatures on the percent of lymphocytes.
DISCUSSIONS AND CONCLUSIONS

The immunological system is a very complex physiological phenomenon. The influences which affect its parameters are vast.

With respect to dietary influences, I suggest that as found with other wild mice, there is a strong selective pressure favoring mice born early in the summer, soon after the females emerge from hibernation. Also, there should be considerable selective pressure against females having a litter near the end of the summer, because both female and offspring would have limited time thereafter to store sufficient energy for the winter. High over-winter survival rates for adults suggest that mice incapable of storing adequate fat reserves are removed from the population (Brown, 1970). The summer of 1982 contained snowy, harsh, wet, cold weather up until late June (National Oceanic and Atmospheric Administration, 1982). Therefore, the mice collected in this study probably were a hardy bunch. Contrarily, hayricks could have acted as a shelter and food supply, upsetting this selective pressure. Regardless, the unusually low population density of the montane vole that existed during this study was probably related to the cold summer
or the cyclic trends of population characteristics of most rodents.

It is very unfortunate that Ahl (1968) named the distinct hemoglobin found in high altitude *P. moniculatus*, type S. Type S is commonly associated with sickle cell anemia. Her work is very significant in that it shows a specific adaptation of the hemoglobin to high altitudes. Such a theory about hemoglobin is also proposed for the Sherpa of the Himalayas. It is important, though, to be cautious when comparing human hematology with mouse hematology. As Ahl found, the hematocrits of wild rodents are at a theoretical maximum whether at sea level or high altitude and, therefore, do not change. This is not the case with humans. Therefore, to draw inferences about blood parameters between the two is erroneous.

Dorbrowolska and Gromadzka (1978) showed a positive correlation between the amounts of progesterone levels and leukocyte numbers. Comparing lactating females and non-lactating females of the present study, notable differences are seen in monocyte, lymphocyte, and neutrophil percents. The progesterone hormonal influence could also be viewed by comparing the male and female white cell differentials as well as the age white cell differentials. Since the study was conducted from May through August, consideration of hormonal influences which occur during the summer breeding seasons, in inter-
pretation of the total results is also essential. Statistical analysis was not performed on these characteristics as the number of samples was not sufficiently large enough to make a statistically significant statement (Table 1).

The study by Seed et al. (1976) showing a 100% parasitic infection in mole *M. montanus* of Jackson Hole, Wyoming could certainly affect the white blood cell differentials of the present study. The Jackson Hole site lies in the Teton Range and has a warmer and moister climate, which would enhance parasitism as compared to the colder, drier climate of the Wyoming range (National Oceanic and Atmospheric Administration, 1982). However, since the two sites lie within a logical radius that would allow parasitic infection to be transmitted, it is possible the population of the present study contained similar parasitic infections. Parasitic infection compounded with the hostile weather conditions of the 1982 summer could have created physiological maximums of the immunological systems in several of the specimens of this study. It could certainly give abnormal differential white blood cell counts and should, therefore, be given consideration when examining the data.

White blood cell differentials of Dieterich and Preston (1977) and Seed et al. (1976), and of the present study are compared (Table 2). The similarities that existed in the study on the three *Microtus* species by
Dieterich and Preston (1977) can probably be safely extended to the subject of this study, *M. montanus*, because of phylogenic relations. Therefore, a comparison of how white blood cell parameters of the six samples in Table 2 differ in response to altitude should be valid.

A consideration of extreme importance that should be given to comparisons of the five samples is the method in which the mice were captured. Dieterich and Preston (1977) and Seed et al. reported that they used live traps. I found that walking an aluminum live trap trap-line twice a day was insufficient. Mice caught during the night or early in the morning suffered from hypothermia and if caught during the day, heat exposure was a factor. To stuff the traps with some type of protection usually only inhibited the function of the trap. To obtain 30 good samples of one species of mouse requires the sacrifice of many mice and wasted trap hours. Therefore, if this study was to be repeated, a self-insulated trap that could maintain physiological equilibrium of its captives for several hours should be used. Even if traps were capable of preserving the physiological equilibrium of their captives, it is highly probable that the captured mouse would still undergo an alarm reaction. Mice obtained by the hand-catch method, as were the majority of mice in this study, showed fewer signs of stress than those caught in traps. The amount of change an alarm reaction from hand catching
causes on the white blood cell parameters is probably minimal compared to that caused by trapping. Consideration of capture technique, and, therefore, the effect of an alarm reaction on white blood cell differentials, should be given when examining the data.

The main concern of this study is with how altitude affects the white blood cell differentials. Demonstration of six samples of Microtus species and lists of their white blood cell differentials is shown (Table 2). A statistical comparison of how the percent of each type of white blood cell of the population of this study compares to those of the other sample is shown (Table 3). It is seen that for the majority of the three high-percentage white blood cell parameters; monocytes, lymphocytes, and neutrophils, all specimens cited differed statistically from my population.

A definite difference occurs between the percentages of white blood cells of the low altitude species, M.p. tananesis and M.o. operarius, and the four high altitude species of M. montanus. Only M. montanus has similar percentages to those of the laboratory reared mice of Seed et al. (1976). This might indicate the role a natural environment has in determining white blood cell differentials. However, it probably can be concluded that high altitude does cause an effect on white blood cell parameters. The effects of high altitude seem to be higher neutrophil percentages, high monocyte per-
centages, and lower lymphocyte percentages as compared to the low altitude samples (Table 2).

The statistically significant differences that occur between the study of Seed et al. (1976) and the present study could be due to altitude. However, it is possible that these differences could be attributed to the parasitic infection in the Seed et al. (1976) samples, thought to be a strain of Babesia, and the different climatic conditions between Jackson Hole, Wyoming and Big Piney, Wyoming, and, perhaps, the 1976 summer and 1982 summer weather. The likeness seen between the monocyte percentages of the present study and that of the laboratory-reared mice of Seed et al. (1976) can probably be accounted for by the relatively large standard deviations (Table 2 and Table 3).

The eosinophil and basophil results are probably statistically questionable due to the low frequency with which these white blood cells occur in the blood. Eosinophils have a dermatropic affinity. Their frequency in the blood of the carotid could therefore be nonrepresentative of their true numbers. To determine if eosinophil numbers really do differ with high altitudes, it would probably be necessary to develop a test for allergenic sensitivity. The major statistical problem with the basophil is also its rarity. Therefore, within and between species, it would seem unlikely that differences in basophil frequency could be determined without
a much larger population study.

The effect of environmental temperature on the percentage of lymphocytes found in each individual of this study is shown (Fig. 1). Lymphocytes were used as an index of the environmental temperature effect since they had the highest percentage of the differential white blood cell count and would therefore have the greatest statistical significance. It seems that there is no determinable correlation between normal environmental temperatures and lymphocyte percentages.

Looking at the immunological system, specifically that of differential white blood cell percentages, one would have to conclude that influences on these indices exist. It would appear that environmental influences (Dieterich and Preston, 1977), hormonal and, therefore, seasonal influences (Dobrowolska and Gromadzka, 1978), and parasitic infections (Seed et al, 1976) all influence the white blood cell differentials in mice and perhaps other mammals. While differential white blood cell counts seem to be partially individual, they could also perhaps be affected by altitude (Table 3). Therefore, I conclude that white blood cell counts differ with altitude. But whether a specific white blood cell percentage differs more significantly than another requires more investigation.

Modern science has made great strides in the understanding of the complex immunological system, yet a
vast amount is still unknown. Studies such as this one may only investigate a minor aspect of the intricate immunological maze, but eventually, with continued research, greater understanding will be gained. Since the immune system defends against disease, an increased understanding of it can only help lead to an increased ability to fight disease and thus result in an increased survival of a species, perhaps that of *Homo sapiens*.
LITERATURE CITED


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