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Hematological variation associated with altitude, season, sexual activity and body in the Uinta ground squirrel

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HEMATOLOGICAL VARIATION ASSOCIATED WITH ALTITUDE, SEASON, SEXUAL ACTIVITY AND BODY WEIGHT IN THE UINTA GROUND SQUIRREL

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INTRODUCTION AND LITERATURE REVIEW

The Relationship of Altitude

Simultaneous with symptoms such as hyperventilation (Sommervell, 1925) and reduced work capacity (Dill, et. al., 1966) an increase in erythrocyte count, hematocrit (Huey and Holmes, 1950) and total blood hemoglobin (Sommervell, 1925; Dill, Terman, and Hall, 1963) amounting to an acute polycythemia was observed in man upon initial exposure to high altitude. Similar increases were obtained in rabbits, sheep (Hall, Dill, and Barron, 1936), rats (Cohen and D'Amour, 1951) and steers (Grover, et. al., 1963) relocated from sea level to altitudes approximating ten to fourteen thousand feet.

The idea that polycythemia is an acclimatization mechanism resulting from brief exposure to the hypoxic stress of high altitude is substantiated by experiments altering the oxygen pressure in simulated altitude chambers. Polycythemia similar to that observed at natural high altitudes was evoked in guinea pigs (Gordon and Kleinberg, 1937; Jensen and Alt, 1945), rabbits (Goodman, 1947; Bancroft, 1949), rats (Altland and Highman, 1951; Fryers, 1952) and mice (Clark and Otis, 1952) by placing the animals in vacuum chambers.

The apparent polycythemic adaptation related to acclimatization to low oxygen pressures does not always appear, however, to be present in permanent residents of high altitude. Sommervell (1925) found that two Tibetan natives had hemoglobin color indices thirty percent less at 16,500 feet than seven Europeans in the process of acclimatization at that altitude. Hall, Dill, and Barron (1936) did not observe a significant difference for erythrocyte count, hematocrit, or total blood hemoglobin when these measurements were performed on Andean mammals native to high altitude and sheep and rabbits at sea level. Likewise, Morrison, Kerst, and Rosenmann (1963) and Morrison _et_. _al_. (1963) failed to find any correlation between altitude and polycythemia when investigating several rodent genera indigenous to various altitudes in the Chilean and Peruvian Andes. Finally, Bullard, Broumand, and Meyer (1966) failed to find higher values for erythrocyte count, hematocrit, total blood hemoglobin, mean erythrocyte volume and mean erythrocyte hemoglobin in woodchucks and ground squirrels at ten thousand feet when these measurements were compared to similar parameters taken on rats at sea level.

On the other hand, Hurtado (1932) not only observed that values for erythrocyte count, hematocrit and mean erythrocyte volume were greater, but that mean erythrocyte hemoglobin was lower among Peruvian natives at 14,800 feet than among individuals at sea level. The lower values in mean erythrocyte hemoglobin were balanced by higher erythrocyte counts; therefore, total blood hemoglobin measurements were similar over the entire altitude range in his study. Kalabuchov (1937) has also demonstrated higher erythrocyte counts for woodmice and other mouse-like rodents living in mountains than for related species inhabiting the plains. Finally, Hock (1964), as cited by Bullard et. al. (1966), has reported higher total blood hemoglobin values for deer mice native to high altitudes when compared to measurements for the same species at lower altitudes.

Genetic variants of hemoglobin protein, separable by electrophoresis, were first discovered in man by Itano and Pauling (1949). Since then, genetic hemoglobin variants for many vertebrate species have been found (Rodnan and Ebaugh, 1957). Hall (1966) has also found that rodents native to high altitude possess hemoglobins with higher affinity for oxygen than similar species resident at lower altitudes. Recently, Dawson (1966) has shown that of the two genetic variants of sheep hemoglobin separable by electrophoresis, type A had a higher oxygen affinity than type B. He also found that those sheep possessing hemoglobin type A had a greater tolerance to hypoxic stress when exposed to a natural altitude of 10,000 feet.

The Relationship of Season and Sexual Activity

Rasmussen (1916) cites the pioneer work of Vierordt (1877), Valentin (1881), Quincke (1882) and Dubois (1893) on the European marmot and his own on the American woodchuck concerning several stages of hibernation and seasonal activity. The Europeans found an initial decrease in erythrocyte number and total blood hemoglobin following arousal from hibernation and a subsequent increase during the summer months until the onset of hibernation. Rasmussen (1916) failed to demonstrate any decrease in erythrocytes following arousal in the woodchuck; indeed, he noticed an eight percent increase above values observed just prior to arousal. He did however, notice a twenty percent decrease in erythrocytes for those animals allowed to eat and drink for a period of time subsequent to arousal which in effect substantiates the observations of the European workers.

In a more recent study, Hock (1952) has observed that relative blood viscosity (which is largely a reflection of the erythrocyte count) was very low in early May and remained low for nearly two weeks. By the first week in June, the relative viscosity was seen to increase steadily until the middle of July when peak values were recorded. Subsequent decreases for viscosity were observed until the onset of hibernation when early May values were again obtained.

Kallen and Wimsatt (1962) working with the little brown bat, not only noticed similar seasonal trends in total erythrocyte volume and hematocrit as described for viscosity by Hock (1952), but also that total erythrocyte volume fell during lactation and rose during post-lactation. An overall hemodilution was observed in women (Caton, et. al., 1951) and rats (Soskind, 1961) during pregnancy and a lactation polycythemia for the rat, cow and burro was reported by Bond (1958).

In a paper electrophoretic study of serum proteins of _Citellus leucurus_ and _C. tereticaudus_, Aurenheimer, Cutter and Atchley (1960) have demonstrated six bands including an albumin, two alpha globulin, two beta globulin and one gamma globulin band. They show different serum electrophoretic patterns when both species are compared, but they do not show intraspecies differences in serum patterns. Blumberg, Allison and Garry (1960) have also examined serum electrophoretic patterns in the Alaskan ground squirrel reporting six bands with no intraspecies differences.

A study concerning the electrophoretic banding pattern of hemoglobin was carried out in the Alaskan ground squirrel (Blumberg, Allison, and Garry, 1960) with a single broad band and no intraspecies differences observed.

The Relationship of Body Weight

Erythrocyte count and total blood hemoglobin increased and mean erythrocyte volume decreased as body weight and age increased in infants (Williamson, 1916), pigs, rabbits (Wintrobe and Schumacker, 1936), cats (Windle, Sweet, and Whitehead, 1940), dogs (Ederstrom and DeBoer, 1946), rats (Donaldson, 1924; Williamson, 1926; Wintrobe and Schumacker, 1936) and guinea pigs (Stewart, Florio, and Mugrage, 1944; Stein and Carrier, 1945). Reticulocytes were reported to have been very numerous in the newborn of cats, pigs, rabbits and rats (Wintrobe and Schumacker, 1936). On the other hand, erythrocyte count decreases with advancing age in calves (Scarborough, 1931; DeLaune, 1939; Reid, Ward, and

Salsbury, 1948) and sheep (Schalm, 1961).

House, Pansky, and Jacobs (1961) have reported alterations in the electrophoretic patterns of serum proteins of the golden hamster in relation to aging. The beta globulin band was observed to diminish steadily throughout the first year. $Alpha_2$ globulin increased after the eighth week until a peak was reached at six months. Animals one year old showed a decreased $alpha_2$ globulin fraction.

Objectives of the Study

Since very few studies have been reported in the literature comparing hematological values in a single species over a wide range of altitude, an objective of this study was to compare measurements of erythrocyte count, hematocrit, total blood hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin and erythropoetic activity (reticulocytes in peripheral blood) for C. armatus trapped at seven stations between 5700 and 10,500 feet. Variation related to seasonal progression, sex, sexual activity and body weight was also analyzed for the hematological measurements mentioned above.

Since genetic variants in hemoglobin exist for many other species, another objective was a comparison of hemoglobin electrophoretic patterns for ground squirrels trapped at various altitudes. Comparative serum electrophoresis was also performed on ground squirrels representative of various altitudes, seasonal activity, sexual activity and intervals of body weight.

MATERIALS AND METHODS

Description of the Study Area

A total of seven stations were designated in the Mt. Timpanogos area near Provo, Utah County, Utah ranging from 5700 to 10,500 feet in altitude. The station at 5700 feet was located west of Midway, Utah on the farm of Mr. Reed Kohler. The 6300 foot station was the sheep meadow of Mr. John C. Stewart above Timp Haven. Stations at 7000, 7600 and 8000 feet were roadside meadows on the Alpine Loop road of Mt. Timpanogos. Horse Flat, approximately one mile southwest from the summit of the Alpine Loop, was selected as the 8500 foot station. The 10,500 foot station was located in the Emerald Lake area at the bottom of the Timpanogos "glacier."

Trapping and Care of the Animals

Ground squirrels were caught by placing wire cage traps beside randomly selected active holes. Trapping was performed at one given altitude on the average of once every seven days. Traps supplemented with oatmeal and peanut butter for bait, were set at six o'clock in the morning before the squirrels commenced their daily activity. After an interval of thirty to sixty minutes, squirrels were collected and transported to the laboratory at the Brigham Young University for examination. The animals were not fed or watered during the time interval of four to five hours between trapping and examination.

Obtaining Blood Samples

Prior to bleeding, ground squirrels were anesthetized with a one percent Nembutal solution (1 ml./100 g. body weight). The abdominal and thoracic cavities were cut open and a sample of blood was taken directly from the heart. One milliliter of blood was immediately placed into a test tube without anticoagulant, and put into a refrigerator for serum isolation. The remainder of the sample (2-4 ml.) was mixed with one milligram of potassium oxalate to prevent coagulation. This sample of oxalated blood was the source for other experiments.

Measurement of Cellular Parameters

Erythrocytes were enumerated by the conventional method following dilution of oxalated blood with Dacie's fluid (Frankel, Reitman, and Sonnenwirth, 1963). Hematocrit percentage was determined by the microhematocrit method. Total blood hemoglobin, expressed in grams per 100 ml. of blood, was determined by the cyanmethemoglobin method of Crosby, Munn and Furth (1954). Reticulocytes were preferentially stained by the new methylene blue technique of Brecher (1949) and expressed as a number per one thousand erythrocytes. Mean erythrocyte volume was determined by the following equation:

$$\text{MEV } (\mu^3) = \frac{\text{Hematocrit Percentage X 10}}{\text{Erythrocyte Count } (10^6/mm^3)}$$

Mean erythrocyte hemoglobin was calculated using the following equation:

$$\text{MEH } (\mu\mu g) = \frac{\text{Hemoglobin (grams/100 ml.) X 10}}{\text{Erythrocyte Count } (10^6/mm^3)}$$

Hemoglobin Electrophoresis

Hemoglobin was isolated by the method of Drabkin (1946) and frozen. Electrophoresis of thawed samples was carried out on six inch Sepraphore III strips (Gelman Instrument Co.) placed in Tris(hydroxymethyl)aminomethane, Ethylenediaminetetraacetic acid, Boric acid buffer of pH 8.6 and 0.1 molar ionic strength. The electrophoretic separation was performed at five hundred volts for seventy-five minutes.

The strips were air dried overnight and then stained with Ponceau R dye for five minutes. Excess stain was immediately removed by three washes with five percent acetic acid, and strips then were allowed to air dry another twelve to twenty-four hours. Next the strips were cleared with colorless immersion oil, placed between two glass slides and scanned in a Gelman-EEL scanner using a green filter. Optical density was plotted on graph paper by hand and a smooth curve was drawn through the points.

Serum Electrophoresis

Serum was poured from coagulated blood twenty-four to forty-eight hours after collection of the original sample. When red cells were present, the serum samples were centrifuged, poured off and frozen for storage until they could be analyzed.

Electrophoresis of the thawed samples was carried out on six inch Sepraphore III strips in Barbital buffer of pH 8.6 and 0.052 molar ionic strength at 190 volts for ninety minutes. The strips were then allowed to air dry and were evaluated in the same manner as hemoglobin electropherograms.

Selection of Animals

Due to the effects of season and body weight on erythrocyte count, volume and hemoglobin, animals trapped early in the season and squirrels less than 250 grams body weight were omitted from the analysis for the altitude study. Ground squirrels possessing a body weight less than 250 grams were omitted from the analysis for the seasonal study.

Statistical Analysis

Differences between comparative mean values were analyzed statistically using the student t-test. Probabilities equal to or less than the 0.05 confidence level were declared significant.

RESULTS

Cellular Parameters

RELATIONSHIP OF ALTITUDE

In the 1966 collection, values for erythrocyte count at different altitudes ranged from 6.83 million cells/mm^3 at 5700 feet to 7.81 million cells/mm^3 at 10,500 feet (see Table 1, page 12). However, variation at each altitude made this difference statistically insignificant.

At the same time values for mean erythrocyte volume at different altitudes ranged from 55.7 μ^3 at 6300 feet to 50.9 μ^3 at 10,500 feet. Again however, due to the extent of variation at altitudes between 5700 and 8500 feet and the small sample size (two animals) taken from 10,500 feet, the correlation between mean erythrocyte volume and altitude was not statistically significant.

Animals resident at altitudes lower than 8500 feet during 1966, possessed approximately half the number of reticulocytes (0.5 to 1.0% of all erythrocytes) of animals secured from 8500 feet. This higher value appeared to be significant ($P=0.05$) when compared to mean measurements from animals resident at all lower altitudes except 6300 feet where large variation made even a twofold difference lack statistical significance. Values for reticulocytes of

Table 1. Comparative mean values for the blood of C. armatus at different altitudes during 1966.

Parameter	Altitude[1]						
	5700	6300	7000	7600	8000	8500	10,500
Erythrocytes ($10^6/mm^3$)	6.83 (0.46)	7.51 (0.41)	7.27 (0.23)	7.41 (0.39)	7.52 (0.57)	7.60 (0.76)	7.81 (0.57)
Hematocrit (%)	38.8 (1.76)	41.2 (1.92)	40.6 (0.17)	41.9 (3.97)	40.1 (3.73)	40.2 (5.25)	39.8 (3.17)
Hemoglobin (g%)	13.3 (1.04)	14.3 (1.00)	13.3 (1.37)	14.7 (1.31)	14.7 (2.09)	14.3 (1.80)	----
M.E.V. (μ^3)	54.1 (2.61)	55.7 (2.39)	54.3 (1.60)	55.1 (6.90)	54.3 (3.87)	54.5 (4.47)	50.9 (0.35)
M.E.H. ($\mu\mu g$)	18.3 (1.17)	19.2 (1.17)	18.4 (1.37)	20.2 (1.27)	19.5 (2.17)	19.8 (1.66)	----
Reticulocytes ($/10^3$ RBC)	----	5.5 (6.36)	5.0 (0.00)	5.0 (4.36)	----	10.0 (5.36)	4.5 (0.71)

[1]Values in parentheses are standard deviations.

ground squirrels native to 10,500 feet were similar to measurements recorded for animals trapped at altitudes lower than 8500 feet.

Variations for hematocrit, total blood hemoglobin and mean erythrocyte hemoglobin did not appear to be related to altitude in the 1966 study.

During 1965, ground squirrels were trapped at altitudes ranging from 5700 to 8500 feet. Reticulocytes were found to increase from 0.5% for squirrels at altitudes below 7600 feet to 1.3% in animals native to 7600 and 8000 feet (see Table 2, page 14). This increase was found to be significant (P=0.05). A mean value nearly twofold higher (0.95%) than measurements taken from squirrels resident at altitudes lower than 7600 feet was also observed in animals native to 8500 feet, but a large amount of variation caused this elevated value to lack statistical significance.

Variations in hematocrit, total blood hemoglobin, erythrocyte count, mean erythrocyte volume and mean erythrocyte hemoglobin measured in 1965 did not appear to be related to altitude.

RELATIONSHIP OF SEASON AND SEXUAL ACTIVITY

In the 1965 study (see Table 3, page 15) erythrocyte count, hematocrit and mean erythrocyte volume were observed to be low at all altitudes in late April, seven to fourteen days after emergence from hibernation. The hematocrit was observed to be elevated 30% (29.8 to 42.1%) and mean erythrocyte volume 25% (41.8 to 52.0 μ^3) by the second week in

Table 2. Comparative mean values for the blood of *C. armatus* at different altitudes during 1965.

Parameter	Altitude[1]					
	5700	6300	7000	7600	8000	8500
Erythrocytes ($10^6/mm^3$)	7.84 (0.96)	7.72 (0.65)	7.75 (0.38)	7.86 (0.39)	7.80 (0.34)	7.50 (0.84)
Hematocrit (%)	40.6 (3.10)	40.9 (2.74)	42.3 (3.02)	42.0 (2.89)	42.2 (2.24)	40.8 (2.87)
Hemoglobin (g%)	14.4 (1.04)	14.9 (1.79)	15.2 (2.98)	14.5 (0.92)	15.4 (2.36)	14.3 (1.50)
M.E.V. (μ^3)	53.1 (4.77)	53.8 (1.87)	53.6 (0.73)	54.6 (3.02)	55.2 (0.30)	54.5 (1.31)
M.E.H. ($\mu\mu g$)	18.2 (1.30)	19.2 (0.42)	20.1 (2.90)	18.4 (1.29)	19.9 (1.02)	18.9 (2.07)
Reticulocytes ($/10^3$ RBC)	5.3 (3.50)	8.0 (2.58)	6.5 (3.32)	11.0 (6.12)	13.0 (4.58)	9.5 (5.50)

[1]Values in parentheses are standard deviations.

Table 3. Seasonal variation of certain blood measurements for C. armatus during 1965.

Parameter	Time of Year (Weeks)[1]												
	April 4	May 1	May 2	May 3	May 4	June 1	June 2	June 3	July 1	July 3	July 4	Aug 1	Aug 2
Erythrocytes ($10^6/mm^3$)	7.00 (0.71)	6.90	---	7.36	7.43 (0.53)	7.75 (0.56)	8.15 (0.34)	7.75 (0.53)	7.40 (1.31)	7.33 (0.79)	7.35 (0.30)	7.29 (0.35)	7.78 (0.44)
Hematocrit (%)	29.8 (2.80)	36.0 (1.41)	36.0 (1.4)	38.8 (6.40)	40.8 (1.95)	41.5 (3.39)	42.1 (4.55)	41.4 (3.73)	41.9 (4.83)	41.0 (4.30)	42.3 (1.55)	39.8 (2.46)	42.7 (2.51)
M.E.V. (^{3})	41.8 (1.34)	50.6 (----)	---- (---)	46.5 (----)	55.5 (5.52)	52.0 (3.00)	52.0 (2.77)	53.4 (2.72)	53.8 (----)	56.7 (1.87)	53.6 (0.36)	55.3 (1.70)	53.5 (2.54)

[1]Values in parenthesis are standard deviations.

June. The differences between early spring and mid-summer values for both hematocrit and mean erythrocyte volume were significant at the 0.01 confidence level. Erythrocyte count was elevated 1.15 million cells per mm^3 by mid-June. Values for hematocrit, erythrocyte count and mean erythrocyte volume remained at peak levels until hibernation. Reticulocytes were not determined in the 1965 study.

Low April values were again recorded in 1966 for the blood of ground squirrels resident between 7000 and 8000 feet (see Table 4, page 17). Hematocrit was observed to be elevated 17% (36.3 to 42.1%), mean erythrocyte volume 8% (51.7 to 55.6 μ^3), erythrocyte count (0.62 million cells), total blood hemoglobin 20% (13.1 to 15.8 g.%) and mean erythrocyte hemoglobin 10% (18.3 to 20.9 $\mu\mu$g). Reticulocyte count on the other hand was found to be 4.5 times lower (2.3 to 0.5%) by mid-May. Differences in reticulocyte count, hematocrit, total blood hemoglobin and mean erythrocyte hemoglobin measurements between April and May were found to be significant at the 0.01 confidence level. Peak values for all blood measurements (with the exception of reticulocyte count) in 1966 were noted by the second week in May. Therefore, measurements corresponding to peak values seen in 1965 were observed a full month earlier during 1966. An apparent deviation from the above seasonal distribution of minimum and maximum values was observed during 1966 in nine squirrels trapped at 6300 feet approximately forty-eight hours after emergence from hibernation. These animals poss-

Table 4. Seasonal variation of certain blood measurements of *C. armatus* from altitudes between 7000 and 8000 feet during 1966.

Parameter	Time of Year[1]				
	April 4th Wk.	May 1st Wk.	May 2nd Wk.	May 3rd Wk.	May 4th Wk.
Erythrocytes ($10^6/mm^3$)	7.13 (0.47)	7.17 (0.38)	7.75 (0.24)	7.58 (0.44)	7.44 (0.19)
Hematocrit (%)	36.3 (3.55)	38.3 (1.15)	42.1 (3.82)	41.9 (3.96)	40.8 (0.71)
Hemoglobin (g%)	13.1 (1.07)	13.1 (0.93)	15.3 (1.68)	15.8 (0.00)	15.1 (0.50)
M.E.V. (μ^3)	52.8 (2.76)	51.7 (2.97)	54.2 (4.47)	55.6 (8.39)	54.7 (1.20)
M.E.H. ($\mu\mu g$)	18.3 (0.87)	18.6 (2.69)	19.8 (2.38)	20.9 (1.20)	20.2 (0.14)
Reticulocytes ($/10^3$ RBC)	23.0 (5.34)	20.0 (7.04)	17.0 (8.50)	5.0 (4.36)	5.0 (0.00)

[1]Values in parentheses are standard deviations.

essed mid-summer values for the blood measurements investigated.

Values (see Table 5) for hematocrit, erythrocyte count, total blood hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin and reticulocytes appeared to be similar for both sexes. The stage of sexual activity in the female was not reflected by hematological variation.

Table 5. Comparative mean values of certain blood measurements for sexual types and stages of sexual activity in _C. armatus_ during 1965 and 1966.

Parameter	Ground Squirrel Type			
	Males	Inactive Females	Pregnant Females	Lactating Females
Hematocrit (%)	40.0	41.4	39.4	40.3
Hemoglobin (g%)	14.2	14.5	14.3	14.7
Erythrocytes (10^6)	7.66	7.34	7.33	7.56
MEV (μ^3)	54.5	55.5	53.0	54.7
MEH ($\mu\mu g$)	18.8	19.2	19.2	19.2
Reticulocytes (/10^3 RBC)	14.0	13.0	14.0	9.0

RELATIONSHIP OF BODY WEIGHT

Mean values for hematocrit (29.3 and 41.0%), total blood hemoglobin (10.7 and 14.6 g%) and erythrocyte count (4.87 and 7.55 million cells) were observed to be higher as larger ground squirrels were examined until peak values were obtained in 250 gram animals (see Table 6, page 19). All of

Table 6. Relationship of body weight to certain blood measurements for *C. armatus* in 1965 and 1966.

Parameter	Ground Squirrel Size[1]						
	< 100 g.	100 - 150 g	150 - 200 g	200 - 250 g	250 - 300 g	300 - 400 g	> 400 g.
Erythrocytes ($10^6/mm^3$)	4.87 (----)	5.07 (0.16)	6.27 (0.61)	6.85 (0.91)	7.55 (0.62)	7.44 (0.76)	7.50 (0.85)
Hematocrit (%)	29.3 (0.36)	33.0 (3.61)	37.8 (3.14)	39.1 (3.79)	41.0 (3.97)	39.9 (3.35)	39.1 (3.21)
Hemoglobin (g%)	11.0 (----)	10.7 (1.06)	12.8 (0.94)	13.6 (1.72)	14.6 (1.62)	14.5 (1.05)	14.0 (1.40)
M.E.V. (μ^3)	59.5 (----)	65.0 (4.95)	63.4 (5.57)	60.1 (6.17)	53.4 (2.46)	54.4 (3.70)	53.4 (4.50)
M.E.H. ($\mu\mu g$)	21.6 (----)	21.0 (1.00)	20.7 (1.49)	20.2 (1.90)	19.0 (1.25)	19.4 (1.63)	18.8 (2.05)
Reticulocytes (/10^3 RBC)	50.0 (----)	35.5 (0.71)	38.7 (18.4)	29.0 (24.8)	22.1 (9.49)	12.5 (8.37)	12.0 (8.37)

[1]Values in parentheses are standard deviations.

the above differences were significant (P=0.01) and no higher values were recorded for heavier animals. Values were lower for mean erythrocyte volume (65.0 and 53.4 μ^3), mean erythrocyte hemoglobin (21.6 and 18.8 $\mu\mu g$) and reticulocytes (5.0 and 1.2%) were noted as larger ground squirrels were examined until minimal values were obtained in 250 gram animals. The above differences noted for mean erythrocyte volume and reticulocyte count were significant (P=0.01) and no smaller values were found in heavier animals.

Electrophoretic Patterns

HEMOGLOBIN

Among the ground squirrels trapped at altitudes between 7000 and 8500 feet, 63% had a hemoglobin electrophoretic pattern of two bands (see Figure 1, page 21). The first and more slowly moving band migrates at the same speed as bovine hemoglobin A which was used as a control. A second band, moving slightly faster than bovine hemoglobin A, was also demonstrated. The remaining 37% of the squirrels trapped at these intermediate altitudes displayed an electrophoretic pattern of a single band; the migration speed was similar to the slow and fast moving bands with nearly equal frequency (see Table 7, page 21). Hemoglobin isolated from all but five of the squirrels from 5700 and 6300 feet migrated as a single, slow-moving band. The other five squirrels at these lower elevations possessed a single

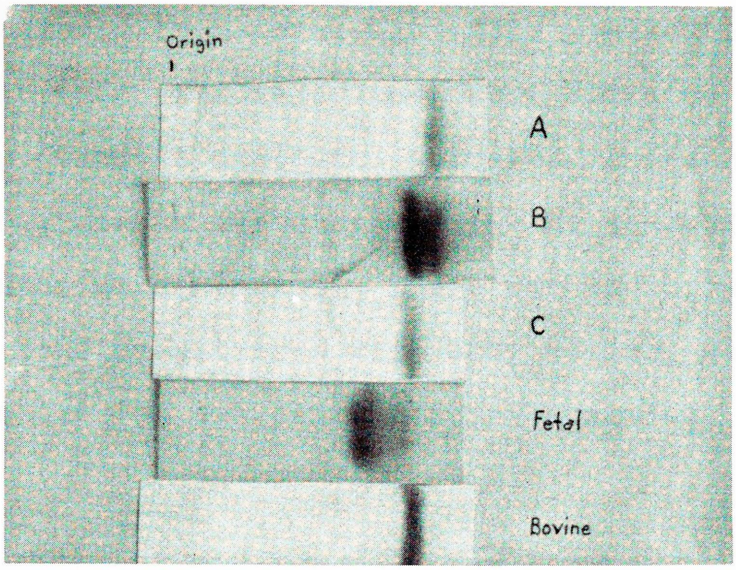

Fig. 1. Comparative hemoglobin electrophoretic banding patterns of C. armatus and Bovine hemoglobin A. A. Ground squirrel number 140 (lactating female from 8000 ft.). B. Ground squirrel number 103 (pregnant female from 7600 ft.). C. Ground squirrel number 137 (yearling female from 5700 ft.).

Table 7. Hemoglobin electrophoretic banding patterns of C. armatus from different altitudes.

Banding Pattern	Altitude				
	5700	6300	7000	7600-8000	8500
Slow Band	5	8	3	2	3
Fast Band	1	4	1	1	4
Both Bands	0	0	7	15	5
Total	6	12	11	18	12

band migrating at a speed similar to the fast moving band.

Hemoglobin from fetal squirrels yielded a pattern of two bands in which the major component migrated slower than either band found in postnatal animals. A second, smaller component migrated a distance similar to that of the slow band in postnatal squirrels. There was no correlation of hemoglobin banding patterns with body weight, sex, sexual activity or time of emergence from hibernation.

SERUM

The sera of all ground squirrels examined in 1965 and 1966 exhibited an albumin, an alpha globulin, two beta globulin and one gamma globulin band. Twenty percent of the males examined and one sexually inactive female had this pattern of only five bands (see Figure 2, page 23). A second band in the alpha globulin range was seen in the remainder of the males and sexually inactive females (see Table 8, page 24). Another band in the beta globulin region was seen in one third of the males and 93% of the sexually active females. Animals smaller than 250 grams body weight also tended to lack the extra beta globulin band. No correlation of serum banding patterns with altitude or time of emergence from hibernation appeared to be present.

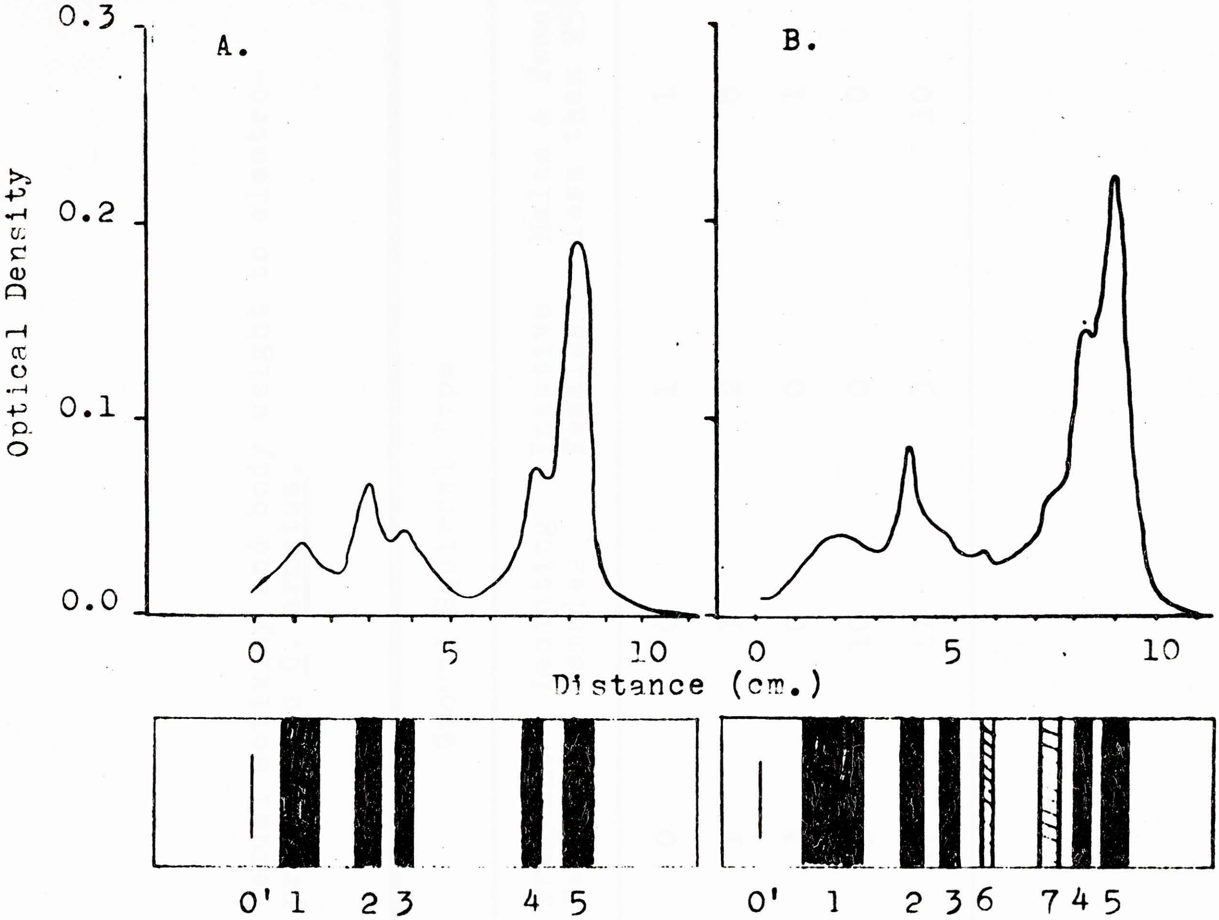

Fig. 2. Scans (upper) and drawings (lower) of electropherograms illustrating two banding patterns for serum proteins in C. armatus. A. Ground squirrel number 36 (male). B. Ground squirrel number 29 (lactating female). O' designates the origin for the scans and electropherograms. Band 1 is the gamma globulin fraction. Bands 2, 3 and 6 are beta globulins. Bands 4 and 7 are alpha globulins. Band 5 is the albumin fraction.

Table 8. The relationship of sex, sexual activity and body weight to electrophoretic banding patterns of serum proteins in *C. armatus*.

Banding Pattern	Ground Squirrel Type				
	Males	Pregnant Females	Lactating Females	Inactive Females	Males & Females less than 250 g
Bands 1-5	5	0	0	1	1
Bands 1-5 plus 7	10	1	1	2	8
Bands 1-5 plus 6	1	3	0	0	1
Bands 1-7	7	13	10	0	0
Total	23	17	11	3	10

DISCUSSION

The Relationship of Altitude

The results obtained in this study did not support the suggestion that a permanent polycythemia is present in C. armatus resident at high altitudes. When mean values were compared for all study areas during one given year certain trends were noted. These included a positive correlation between erythrocyte count and altitude up to 10,500 feet, a negative correlation between mean erythrocyte volume and altitude to 10,500 feet (1966) and a positive correlation between reticulocyte count and altitude up to 8000 feet (1965 and 1966). Values for reticulocytes were significantly higher in animals native to the range of 7600 to 8500 feet than for animals at lower altitudes in both 1965 and 1966, but the lowest count of all was recorded in 1966 for squirrels resident at 10,500 feet. These animals were trapped three weeks later than squirrels at any other altitude. Since reticulocyte count was observed to be minimal by midsummer, season, not altitude was found to have been a primary cause of variation for erythropoetic activity. Due to variations encountered at each study area during a given year and particularly during the two year span of this study, the trends mentioned above did not reach the significant level. Seasonal trends offer evidence that the amount of time that elapsed between emergence from hibernation and capture might have been a primary cause of variations found at all stations. In 1966 ground squirrels were first seen almost three weeks earlier at 5700 and 6300 feet than at 8500 feet. This might have put squirrels from the 8500 foot level behind those animals at lower altitudes in their seasonal activity and development and would have introduced some variation in values recorded for the altitude study. The time of emergence from hibernation also may have varied at a given altitude, providing variation for blood measurements at a single study area.

The Relationship of Season and Sexual Activity

The seasonal trends for hematocrit and mean erythrocyte volume at all altitudes in 1965 and for hematocrit, total blood hemoglobin and mean erythrocyte hemoglobin at altitudes between 7000 and 8000 feet during 1966 roughly paralleled the changes in blood viscosity observed by Hock (1952) on the Alaskan ground squirrel, with the exception that he observed relative viscosity to be minimum just prior to hibernation. Peak seasonal values reported here persisted throughout the summer and were still high when the animals entered hibernation. Early season minimal values for the blood measurements just mentioned and high reticulocyte counts during the same period coupled with peak levels for the above blood measurements and minimal values for reticulocytes at mid-May was evidence that increased erythropoetic activity was directly related to the sustained increases in hematocrit and hemoglobin. Increases in the blood hematocrit have been related to the release of erythrocytes from the spleen and other depots (Mann and Drips, 1917) and to a hemoconcentration due to lowered plasma volume (Svihla, Bowman and Retinour, 1953; Soumalainen and Granstrom, 1955); however, high levels of erythropoetic activity might have been required if the hematocrit increase was to be sustained over a long period of time.

In an early study, Rasmussen (1916) observed that erythrocyte number is high upon emergence for the woodchuck and minimal soon after intake of food and water. Hibernation studies of Svihla et. al., (1953) and of Soumalainen and Granstrom, (1955) pointed to a high erythrocyte count after onset of hibernation and minimum erythrocyte count after arousal in the Alaskan ground squirrel and the golden hamster. Thus, evidence is available that values for erythrocyte count are high upon arousal from hibernation, decrease due to a hemodilution and increase again as the active season progresses. In this study nine animals at 6300 and one at 8500 feet were trapped within approximately forty-eight hours after emergence. These animals were the only ones known to have been trapped within a week after emergence and they all yielded high values for all blood parameters. Reticulocytes, moreover, were at a peak level in these animals indicating that a high level of erythropoetic activity was present upon emergence.

Pregnant and lactating females demonstrated mean values similar to those of males and sexually inactive females for all blood measurements. Therefore, a pregnancy hemodilution and a lactation polycythemia did not appear to be present in *C. armatus*.

The Relationship of Body Weight

The results for erythrocyte number, volume and hemoglobin indicated a shift to more and smaller cells with less hemoglobin in each erythrocyte as ground squirrels increased in size. High values for reticulocytes in small animals (less than 250 grams) indicated high erythropoetic activity in those squirrels undergoing erythrocyte replacement. The patterns of hematocrit, total blood hemoglobin, mean erythrocyte volume, reticulocyte and erythrocyte counts described for *C. armatus* are comparable to those reported for the human infant (Williamson, 1916), rabbits, pigs (Wintrobe and Schumacker, 1936), rats (Donaldson, 1924; Williamson 1926; Wintrobe and Schumacker, 1936; Soskind, 1961) and guinea pigs (Stewart, Florio, and Mugrage, 1944; Stein and Carrier, 1945).

Hemoglobin Electrophoresis

Blumberg, Allison and Garry (1960) have reported a single band with no intraspecies differences in the hemoglobin electrophoretic pattern for the Alaskan ground squirrel. In our study three different hemoglobin bands (one from fetal squirrels and two from postnatal animals) were observed. These bands possibly represented genetic variants for ground squirrel hemoglobin with slightly differing protein primary structure as demonstrated initially by Ingram (1956). Another possibility was alteration of protein subunit association, which might have occurred in dilute solutions (Svedburg and Hedenius, 1934) resulting in double molecules or ruptured chains. The realization of these possibilities would complicate electrophoretic analysis.

Serum Electrophoresis

The serum electrophoretic pattern of six bands for *C. armatus* agrees closely with the work of Aurenheimer, Cutter, and Atchley (1960) on *C. leucurus* and *C. tereticaudus*. Aurenheimer *et. al.* and Blumberg's group, however, demonstrated no intraspecies differences in electrophoretic patterns. In this study, patterns of five to seven bands were observed. In addition to the six bands described by both Aurenheimer's and Blumberg's groups, a light band in the beta globulin region was also demonstrated in this study in sexually active females and in one third of the males. This band was lacking in sexually inactive females and in ground squirrels smaller than 250 grams body weight. No correlation could be found between the presence of the extra beta globulin band and sexual activity in the male since males were very difficult to catch in the spring while they were sexually active.

CONCLUSION

A specific altitude polycythemia did not appear to have been present in _C. armatus_.

Hematocrit, erythrocyte count, mean erythrocyte volume and total blood hemoglobin were low early in the season and high by the second week in May. These peak values persisted for the duration of the summer. Reticulocyte counts remained high until mid-May when values for the other parameters were maximal indicating a high erythropoetic activity in the early spring.

A pregnancy hemodilution and a lactation polycythemia were not observed.

Ground squirrels less than 250 grams body weight underwent gradual replacement of erythrocytes (from larger to smaller) as larger animals were examined. Erythrocyte replacement coincided with higher erythropoetic activity indicated by elevated reticulocyte counts at this time.

Hemoglobin electropherograms of one or two bands were observed in postnatal squirrels. Fetal squirrel hemoglobin had two bands; the major component migrated slower than the slowest postnatal band.

Serum electropherograms varying from five to seven bands were observed for _C. armatus_. A band in the beta globulin range possessed by 92 percent of the sexually active females and one third of the males was also demonstrated. This band was not found to be present in sexually inactive females or ground squirrels smaller than 250 grams body weight.

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HEMATOLOGICAL VARIATION ASSOCIATED WITH ALTITUDE, SEASON, SEXUAL ACTIVITY AND BODY WEIGHT IN THE UINTA GROUND SQUIRREL

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by
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ABSTRACT

Hematological comparisons were made on the Uinta ground squirrel (Citellus armatus) trapped at different altitudes and during various stages of seasonal activity in 1965 and 1966. Hematological variation due to sex, sexual activity and body weight were also analyzed. Ground squirrels were secured from the Mt. Timpanogos area near Provo, Utah County, Utah. Erythrocyte count, hematocrit, total blood hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin and reticulocyte counts were measured using standard procedures; banding patterns of hemoglobin and serum proteins were determined by means of electrophoresis with polyacetate strips.

A significant elevation in erythrocyte count, hematocrit and total blood hemoglobin amounting to a polycythemic condition was not observed in C. armatus native to high altitudes (8500 to 10,500 feet).

Erythrocyte count, hematocrit, total blood hemoglobin, mean erythrocyte volume and mean erythrocyte hemoglobin increased steadily through the month of May until peak values were reached which persisted for the duration of the summer. Reticulocytes in peripheral blood were most numerous shortly after emergence from hibernation and remained so until erythrocyte counts reached a peak indicating accelerated erythropoetic activity during this period.

Values for cellular measurements were similar in both sexes and in all stages of sexual activity in the female. A hemodilution due to pregnancy and a lactation induced polycythemia were not found in this study.

Increases in erythrocyte count, hematocrit and total blood hemoglobin coupled with decreases in mean erythrocyte volume and mean erythrocyte hemoglobin pointed to a gradual replacement of large erythrocytes with smaller ones in yearling ground squirrels of less than 250 grams body weight. Reticulocytes were most numerous in animals less than 250 grams body weight which indicated an accelerated erythropoetic activity in these ground squirrels.

Hemoglobin electrophoretic patterns of one or two bands were found in ground squirrels secured from altitudes between 7000 and 8500 feet. A single band was the only pattern observed in ground squirrels secured from 5700 and 6300 feet. This band was seen to migrate at a speed similar to the slower band just mentioned. Fetal ground squirrels possessed an electrophoretic pattern of two bands; the major component migrated slower than either band present in postnatal squirrels.

Serum electrophoretic patterns varied from five to seven bands. A band in the beta globulin region possessed by 92% of the sexually active females and one third of the males was demonstrated.