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# VARIATION IN HEMOGLOBIN TYPES IN THE DEER MOUSE (*PEROMYSCUS MANICULATUS*) ALONG AN ALTITUDINAL GRADIENT

David Wasserman<sup>1</sup> and Donald J. Nash<sup>1</sup>

ABSTRACT.—Deer mice (*Peromyscus maniculatus*) were captured along an altitudinal gradient that extended from 5,000 feet (1524 m) up to 11,000 feet (3353 m) in central Colorado during August and early September, 1976. Starch gel electrophoresis of deer mouse hemoglobin followed no clear trend that would indicate that slight biochemical differences in the molecule help facilitate adaptation to the decreased  $pO_2$  that exists at that altitude.

Organisms that live at high altitudes must in some way adapt to the hypoxic conditions and meet their oxygen needs. Gluecksohn-Waelsch (1960) suggested that multiple hemoglobins may differ in physical properties and thus facilitate environmental adaptations. In one known mutant human hemoglobin, hemoglobin Rainier, on which a histidine residue replaces a tyrosine residue at one point, oxygen affinity is greatly enhanced (Stamatoyannopoulos et al., 1968). Studies by Ahl (1968) and Sawin (1970) looked at the electrophoretic patterns found in deer mouse hemoglobin along an altitudinal gradient. In both of these studies a predominance of diffuse (double)-banded individuals were found at low altitude. High altitude groups were characterized by a predominance of single-banded individuals. If the hypothesis put forth by Gluecksohn-Waelsch (1960) is correct, then the patterns discovered in the earlier studies should be found along other altitudinal clines if these biochemical differences in the hemoglobin molecule facilitate greater oxygen-binding efficiency.

## METHODS

A total of 67 *Peromyscus maniculatus* were trapped from 27 July through 8 September 1976 in Larimer and Pitkin counties, Colorado, at altitudes of 5,000 feet, 6,500 feet, 8,000 feet, and 11,000 feet. Two populations were sampled at each altitude.

Blood samples were collected from the orbital sinus using heparinized capillary tubes. The blood was centrifuged immediately, and both cells and plasma were frozen with dry ice and later transferred to a freezer and stored at  $-20\text{ C}$ .

Hemoglobin was analyzed by vertical starch gel electrophoresis, using the method of Smith (1968). Following electrophoresis, gels were sliced, fixed, and stained with bromophenol blue.

## RESULTS AND DISCUSSION

A number of studies have dealt with the electrophoretic variants in both the serum and cellular fractions of the blood of the deer mouse. Most studies demonstrated that *Peromyscus maniculatus* has at least two electrophoretically separable hemoglobins (Foreman 1960, Ahl 1968, Sawin 1970). Studies of the genus *Peromyscus* showed that there are variations in the double-banded phenotypes (Rasmussen 1970, Selander et al. 1971). A third hemoglobin variant was reported in one wild mouse captured near Flagstaff, Arizona, in 1967 (McCracken and Foreman 1971). Recent papers have verified the existence of the third variant and three triple-banded phenotypes (Jensen et al. 1976, Maybank and Dawson 1976). It should be noted that the triple-banded individuals were either Foreman's original stock or low altitude populations (600–4700 ft.).

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In previous studies, low altitude deer mice were shown to either have a predominance of individuals exhibiting the diffuse phenotype (type R) or only the diffuse phenotype (Ahl 1968, Sawin 1970). At high altitudes (above 7,000 feet) the single-banded phenotype (type S) was the predominant variety found (Ahl 1968, Sawin 1970). Results of the present investigations do not agree with the previous studies; however, the sampling techniques made it possible to ascertain if the variation found was between altitudes and/or intraaltitudinal in character.

In the populations that were sampled, there was no discernible change in frequencies of the phenotypes over the 6,000 ft. range (Table 1). Population A (5,000 ft. sample) had three individuals that exhibited the single-banded phenotype, but all other individuals in the 5,000 ft. group exhibited the diffuse phenotype. Throughout the remainder of the populations sampled, all individuals exhibited the diffuse phenotype except for two individuals in population I (11,000 feet) that exhibited a triple-banded variety of hemoglobin. It was not possible to ascertain which of the three triple-banded phenotypes as outlined by Jensen et al. (1976) and Maybank and Dawson (1976) was present. It appears to be the first report of the existence of triple-banded hemoglobin phenotypes in high altitude populations.

In light of these findings, the hypothesis put forth by Gleucksohn-Waelsch (1960), which suggests that multiple hemoglobins may differ in physical properties and thus facilitate environmental adaptations, must be questioned. While the two studies noted

above found a high frequency of single-banded individuals at higher altitudes, the present results do not follow a similar pattern.

The genetic polymorphisms found along altitudinal gradients could be a product of genetic drift or varying selective pressures in different environments. The findings suggest that the structural variations found in the beta chain of the hemoglobin molecule in *P. maniculatus* do not infer a greater ability on the part of the organism to exist at high altitudes. At the present time it is not known if these slight structural differences manifest themselves in any manner in the deer mouse. The hypothesis put forth by Gleucksohn-Waelsch (1960) could be tested in an alternate fashion by comparing the O<sub>2</sub>-binding capacities of equal volumes of blood of each phenotype.

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TABLE 1. Hemoglobin variants and frequencies at trapping sites within altitudes.

Altitude in feet	Population	Type S	Type R	Type F	Total
5000	A	3 (.50)	3 (.50)	—	6
	E	—	7 (1.0)	—	7
6500	C	—	5 (1.0)	—	5
	D	—	6 (1.0)	—	6
8000	F	—	6 (1.0)	—	6
	G	—	9 (1.0)	—	9
9500	J	—	8 (1.0)	—	8
	K	—	7 (1.0)	—	7
11000	H	—	6 (1.0)	—	6
	I	—	5 (.70)	2 (.30)	7

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