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Zoology

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A COMPARISON OF VARIOUS BLOOD PROPERTIES OF DIFFERENT ALTITUDINAL AND GEOGRAPHICAL POPULATIONS OF ARIZONA POCKET GOPHERS (THOMOMYS)

by

David Wilson Salter

A Dissertation Submitted to the Faculty of the

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In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by DAVID WILSON SALTER

entitled A COMPARISON OF VARIOUS BLOOD PROPERTIES OF DIFFERENT ALTITUDINAL AND GEOGRAPHICAL POPULATIONS OF ARIZONA POCKET GOPHERS (THOMOMYS)

be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY

Wm. J. McCauley — Dissertation Director — 4/21/70 — Date

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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Finally, I would like to thank my wife, Rondi, for her competent photographic assistance and, most of all, for her compassion during the vicissitudes of the past year.

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ABSTRACT

Blood characters were compared in five different altitudinal and geographical subspecies of Thomomys bottae in Arizona. Included were: erythrocyte number, hematocrit, gram percent hemoglobin, oxygen capacity, oxygen affinity and hemoglobin electrophoretic migration patterns. Blood comparisons were made between two montane populations, and these in turn were compared with data from three populations at lower elevations. In addition, several blood coefficients and the electropherograms of T. bottae were compared in detail with those of Sigmodon hispidus. Where appropriate, data from these species were compared with data for other rodents in the literature.

In general, T. bottae had high oxygen affinities relative to body size. The relatively high affinities can probably be correlated with the fossorial habits and wide altitudinal distribution of this species. The oxygen capacities and hemoglobin binding capacities (ml O_2/gm Hb) of this species were considerably higher than those of Sigmodon hispidus which is often sympatric with T. bottae and of similar size. The differences in the hemoglobin binding capacities between T. bottae and S. hispidus suggests in the latter species the presence of inactive hemoglobins and/or interference mechanisms.

The multiple hemoglobin bands bound in S. hispidus did not confer any special oxygen binding advantages over the single band found in all subspecies of T. bottae. In fact, by comparison, the gopher hemoglobin appeared to be more efficient with regard to oxygen loading at saturation.

Although the correlations between hematocrit, gram percent hemoglobin and oxygen capacity were found to be relatively uniform among the five geographical subspecies of T. bottae, significant variability in all of the individual blood parameters examined was observed within and between populations. Not all of the interpopulation differences could be ascribed to particular environmental conditions without further investigation. However, two high altitude blood adaptations appeared to be operating in T. bottae--either of which appeared to be sufficient at about 2743 meters: (1) consistently high oxygen affinities, and (2) consistently high hemoglobin production--hence, high volume percent O_2 levels.

INTRODUCTION

Knowledge of the characteristics of blood and, more specifically, of hemoglobin in mammals that are continuously or periodically exposed to varying degrees of hypoxia has been experimentally pursued for many years. Most of the attention has been focused on the oxygen capacities and hemoglobin-ligand affinities of bloods operating in conditions of low alveolar oxygen tensions. More recent speculations have been concerned with the possible function of multiple hemoglobins in the hemoglobin-oxygen relationship. Most of these investigations have involved the blood characters of diving, cave dwelling, or high altitude species. However, only scant attention has been given to the blood adaptations of fossorial rodents--especially fossorial rodents that occupy wide altitudinal distributions.

The special microenvironmental conditions associated with the subterannean existence of fossorial mammals have, fairly recently, stimulated investigations into the physical nature of burrow atmospheres. McNab (1966) studied the burrows of five species of fossorial rodents and found that the O_2 concentrations varied from 15 to 20 percent and the CO_2 levels fluctuated from 0.5 to 2.0 percent. He also found that soil porosity and the number of animals per burrow greatly influence gas compositions. Kennerly (1964) found the CO_2 content of Geomys burrows

to be exceedingly high (2.3 percent highest recorded) while O_2 was usually 21 percent but varied to 6 percent. Studier and Baca (1968), studying five species of rodents in simulated burrows, found that the rodents occupying the burrows lowered the partial pressure of oxygen (P_{O_2}) by 10 to 15 mm Hg and increased CO_2 levels by 0.5 to 1.5 volume percent. Hayward (1966) found the typical gas concentrations in sealed European rabbit burrows were 7-8 percent CO_2 and 13-14 percent O_2.

In addition to the unusual atmospheres associated with burrow microenvironments, the lower oxygen tensions associated with increases in altitude tend to create different physiological and anatomical requirements for high altitude populations. Interpolation from Bard's (1961) graph, plotting atmospheric pressure versus altitude, shows that the P_{O_2} drops from 159 mm Hg at sea level to approximately 110 mm Hg at 2743 meters and becomes dangerously low at 6096 meters (P_{O_2} = 75 mm Hg). At reduced atmospheric pressures, alveolar oxygen tensions (which are lower than atmospheric tensions) may not be sufficient for adequate oxygen loading in pulmonary blood unless adaptive mechanisms are operating.

Morphological modifications such as reduced (or absent) eyes, specialized forelimbs and digits, reduced pelvic girdles, etc., in fossorial rodents are well known. However, much less is known with regard to their physiological adaptations. McNab (1966) found that fossorial rodent species (including Geomys) generally have lower

metabolic rates. He suggested a correlation between lower metabolic rates and decreasing the tendencies of anoxia and acidosis in burrow inhabiting rodents. Hall (1965) found higher oxygen affinities in the blood of the most fossorial of seven species of Sciuridae.

Most of the work on adaptation to high altitude has been done on man (Hall 1936 and 1937, Keys, Hall and Barron 1936, Mitchell and Edman 1951, Hock 1970) and large mammals (Hall, Dill and Barron 1936, Bartels et al. 1963). Not all of the findings of these authors necessarily agree but, in summary, several possible anatomical and physiological adaptations to oxygen stress were suggested. Some of the more important adaptations are: (1) larger chests, lung volumes, and hearts, (2) greater dilation of pulmonary capillaries and increased lung blood pressure, (3) increased respiration and cardiac output, (4) increased hemoglobin production and physiochemical changes in hemoglobin allowing for changes in oxygen capacity and oxygen affinity, respectively. Much less data exists for small mammals (i.e., rodents). However, considerable hematological and some anatomical data for high altitude populations of Peromyscus maniculatus are reported by Hock (1962) and Gough and Kilgore (1964). The relevant findings of all these investigations will be discussed later.

To my knowledge, no detailed hematological work has been done on pocket-gophers--either from the standpoint of their fossorial habits or altitudinal distribution. Hence, this research was done using

Thomomys bottae in an effort to: (1) determine the oxygen capacities, affinities and other hematological properties of these pocket gophers in general, (2) compare these data with those of other fossorial and non-fossorial rodents, (3) correlate differences in the various blood parameters of several gopher populations with their altitudinal distributions, and (4) examine the hemoglobin electrophoretic migration patterns of several geographical gopher populations and perhaps correlate any differences found with differing oxygen affinities--also, compare these patterns with those of other rodents, in an effort to elucidate the relationship between different hemoglobins and their oxygen binding efficiences.

Thomomys bottae proved to be an opportune species for this investigation since it is entirely fossorial and occupies a wide altitudinal range in Arizona. There are approximately 41 subspecies (demes) of T. bottae in Arizona according to Cockrum (1960), and they are found at elevations of from 38 meters to over 2750 meters in some of the mountain ranges. The karyotypes of nine of these subspecies have been worked out by Patton and Dingman (1968). They found that the diploid number for all populations studied was uniform (2N = 76), however considerable variation in chromosome complements was found between populations. The absence of acrocentrics in T. b. modicus compared to the 9 pairs of acrocentrics in T. b. alienus represented the extremes. A correlation between the karyotypic variation and morphological variation in this species was suggested by these authors.

The two mountain populations of T. b. grahamensis and T. b. catalinae were selected and compared with their lower elevation counterparts of T. b. alienus and T. b. modicus, respectively. The range boundaries (Figure 1) of T. b. alienus are, to varying extents, coincident with those of the other three subspecies. Thomomys b. albatus is geographically isolated from these previously mentioned subspecies and was selected primarily because it represents the lowest elevation in Arizona (Yuma).

MATERIALS AND METHODS

Collection of Animals and Blood Preparation

The pocket gophers used in this research project were trapped with ten specially designed (Howard 1952) live gopher traps. Fresh gopher mounds were located and their burrow entrances exposed. Various grains (oats, barley, milo, etc.) were used to bait the burrow entrances and traps. The traps were inserted in the burrow entrances and covered with soil. The cotton rats used in this study were trapped with standard Sherman collapsible live traps baited with oatmeal. Trapped animals were kept in captivity in holding cages in the mammalogy animal quarters. Gophers used for altitudinal comparisons were processed within 48 hours after their arrival at the animal quarters. Trapping localities for the five principal populations (subspecies) of Thomomys bottae are presented in Figure 1. Subspecific designations for the populations studied were determined principally on the basis of geographical location after Cockrum (1960). The specific information on trapping localities, number and sex ratio of animals trapped at each locality, is given in Appendix I.

Animals were weighed on an Ohaus Triple-Beam Balance and injected with Nembutal (Sodium Pentobarbital, Abbott Laboratories, North

Figure 1: Trapping localities and ranges for the five principal subspecies of Thomomys bottae. -- Range boundaries were extrapolated from Cockrum (1960), p. 107. Thomomys b. catalinae and T. b. grahamensis are located in the Santa Catalina and Pinaleno Mtns., respectively. Elevations are given in parentheses; circles are trapping localities; heavy lines are the ranges.

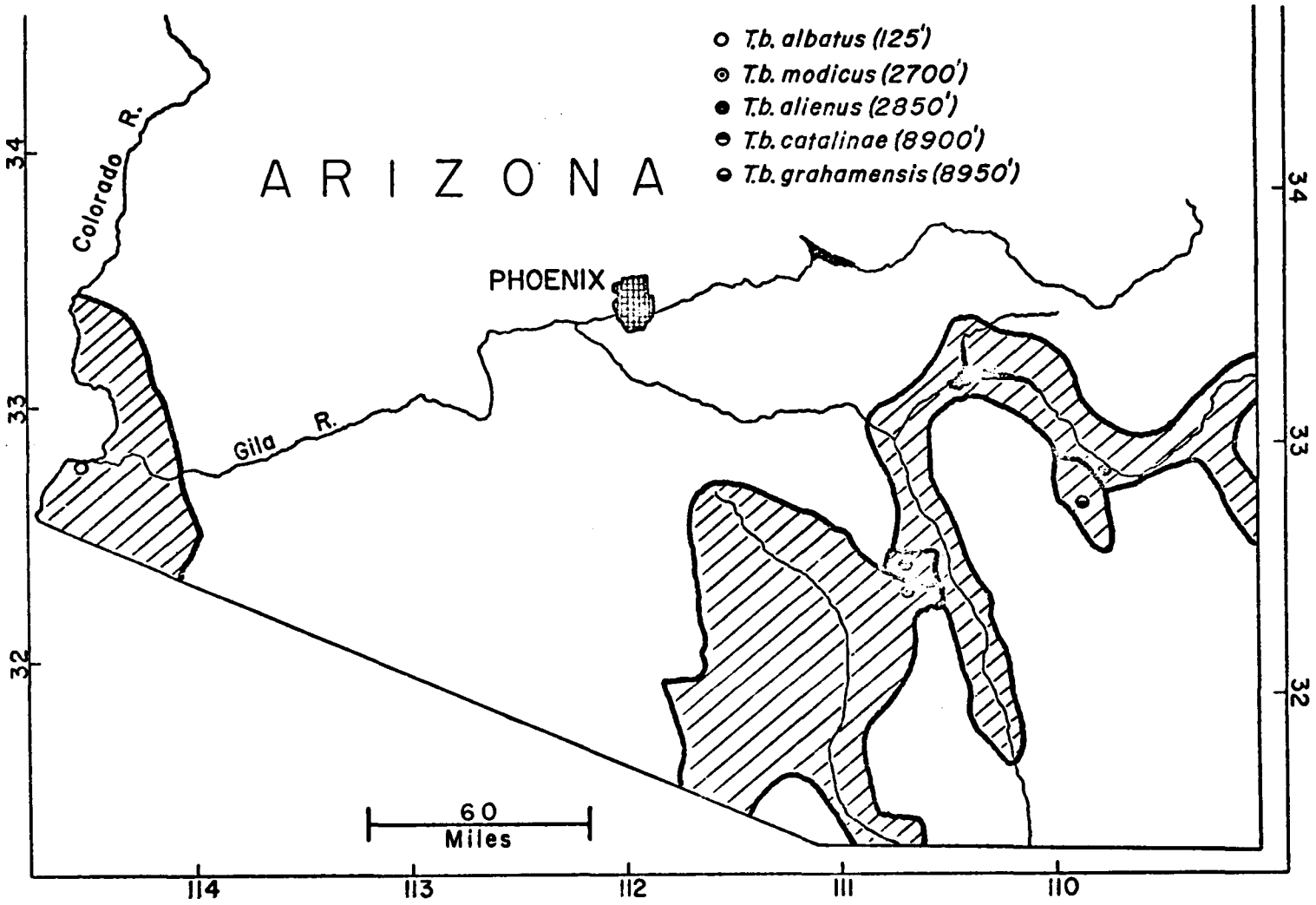

Figure 1: Trapping localities and ranges for the five principal subspecies of Thomomys bottae.

Chicago, Ill.) at a dosage of between 50 and 75 mg/kg. Blood was withdrawn from the anesthetized animals via cardiac puncture using disposable syringes wetted with heparin (NK Heparin, Medical Chem. Corp., Los Angeles, Calif.). Pooling the blood from two or more animals was unnecessary since sufficient blood volumes could be obtained from single individuals for the various analyses.

Erythrocyte Factors and Gram Percent Hemoglobin

Hematocrit Determination

Hematocrit values were determined by using heparinized capillary tubes (Van Waters and Rogers, Inc., Los Angeles, Calif.). Blood samples were centrifuged at 11,000 rpm for 10 minutes in an International Hemacrit Centrifuge (International Equipment Co., Boston, Mass.). Percent erythrocytes was read directly from an Adams Micro-Hematrocrit Reader (Clay-Adams, Inc., New York, N. Y.).

Measuring Erythrocyte Diameters

Dry blood smears on standard microscope slides were emersed in methanol for 30 seconds followed by a 45 minute staining period in a 1:50 dilution of Giemsa's Blood Stain (Matheson Coleman and Bell, Norwood, Ohio). The stained slides were dried and coverslips applied with Permount. Erythrocyte diameters were measured under oil emersion with a Spencer Binocular Microscope fitted with an ocular micrometer.

A minimum of 50 erythrocytes were randomly selected and measured for each animal investigated.

Erythrocyte Count

Erythrocytes were counted by using Unopette (Becton, Dickinson and Co., Rutherford, N. J.) disposable blood diluting pipettes and reservoirs in combination with a hemocytometer (Max Levy Co., Philadelphia, Pa.). Blood samples were diluted 1:200 in isotonic saline (0.85 percent NaCl). The hemocytometer was charged with the diluted blood and the number of erythrocytes in 80 of the smallest grid squares was determined under the microscope. The formula

$$\frac{\#\ \text{RBC's in 80 sq.}}{80} \times \underset{(a)}{200} \times \underset{(b)}{4000} = \#\ \text{RBC's/cu mm blood}$$

was used to compensate for the dilution factor (a) and volume of a single counting square, (b) (volume per square = 1/4000 mm^3).

Gram Percent Hemoglobin Determination

The cyanmethemoglobin method (Crosby, Munn, and Furth 1954) was used to determine gram percent hemoglobin. A 20 lambda sample of blood was mixed with 5 ml of cyanmethemoglobin reagent (1.0 gm $NaHCO_3$, 0.2 gm $K_3Fe(CN)_6$, 0.05 gm KCN to 1 liter with H_2O) which lysed the corpuscles and converted hemoglobin to cyanmethemoglobin. Percent transmittance of this solution was measured on a Bausch and Lomb Spectronic 20 at a wavelength of 540 mu. Percent

transmittance was converted to gram percent hemoglobin by interpolation from a standard curve. The standard curve was established by reading and plotting the percent transmittance of three blood samples with known hemoglobin concentrations ranging from 7.2 to 14.0 gram percent. The hemoglobin concentrations of these samples were verified at a local hospital clinic.

Determination of O_2 Capacity and Affinity

Oxygen Analysis

The Roughton and Scholander (1943) syringe technique for microgasometric estimation of oxygen in the blood was used for the determination of both oxygen capacity and oxygen equilibrium curves. A pipette and analyzing syringe are manufactured (Phipps and Bird, Inc., Richmond, Va.) especially for this technique.

The blood sample to be analyzed was drawn into a 39.3 cu mm pipette (Figure 2 B) and transferred to the analyzing syringe (Figure 2 C) where it was laked in a ferricyanide solution (12.5 gm $K_3Fe(CN)_6$, 3.0 gm $KHCO_3$ and 0.5 gm Saponin powder dissolved in H_2O to 50 ml). An acetate buffer (70 gm $NaC_2H_3O_2.3H_2O$ dissolved in 100 gm H_2O and 15 cc glacial acetic acid) was drawn down on top of the blood and ferricyanide mixture. The reagents and blood were mixed by shaking for 3 minutes. The addition of the acetate buffer generated an excess of CO_2 which liberated oxygen from the hemoglobin. The plunger of the

syringe was free to slide out as gases were generated inside the barrel. A 10 percent NaOH solution was then added which selectively absorbed CO_2, leaving O_2, N_2, CO and trace gases in the barrel. The volume of these remaining gases was measured (V_1) in the capillary portion of the syringe (each division = 0.393 cu mm). A pyrogallol solution (15 gm powdered pyrogallol added to 100 ml 20 percent NaOH) was drawn into the syringe which selectively absorbed O_2, and the remaining gas bubble was measured (V_2). The difference between the two volumes (V_1-V_2) was inserted into the formula

$$(V_1-V_2 - c) \times f = \text{volume percent } O_2$$

which compensated for temperature, aqueous vapor pressure, and barometric pressure (f factor) and the amount of oxygen dissolved in the reagents (c). Values for f were found in the table prepared by Peters and Van Slyke (1932). The amount of dissolved oxygen in the reagents was determined by working the procedure described above without the blood sample. The value of c was found to be between 1.0 and 1.1 volume percent. The oxygen analysis procedure was carried out at least twice for each separate oxygen capacity determination.

Equilibration Tonometer Design

A tonometer (Figure 2 A) was used for both oxygen capacity and oxygen equilibrium curve determinations. The tonometer consisted of a 20 cc glass syringe (without plunger) fitted at the top with a stopcock.

Figure 2: Photograph of the equilibration tonometer (A), 39.3 mm^3 delivery pipette (B), and syringe gas analyzer (C).

Figure 3: Photograph of the vacuum-manometer apparatus. -- This photograph shows the manometer (center) and the equilibration tonometer partially submerged in the water bath (right). Vacuum tubing running from the flask (far left) is connected to a faucet aspirator.

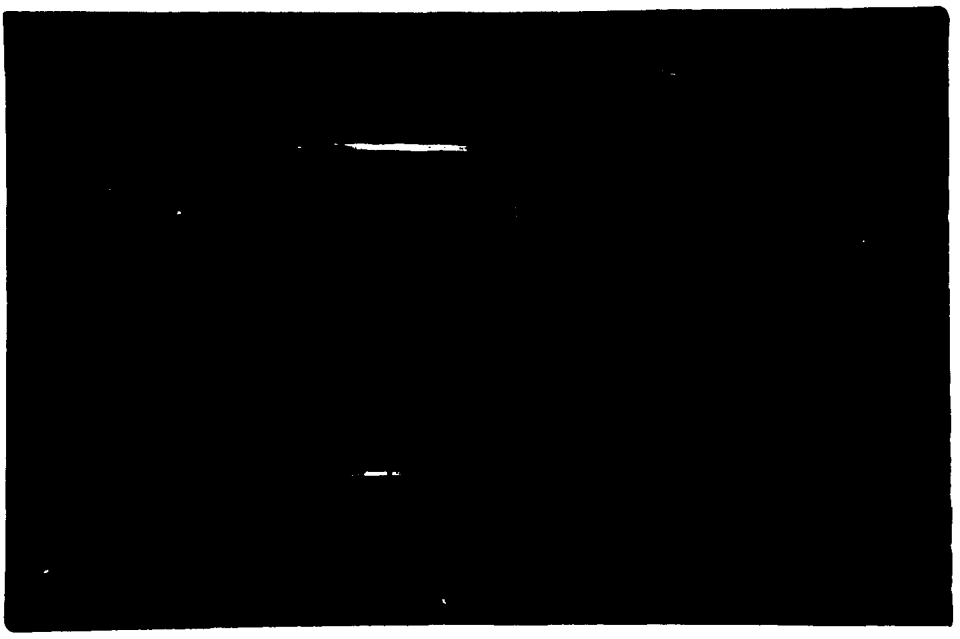

Figure 2. Photograph of the equilibration tonometer (A), 39.3 mm^3 delivery pipette (B), and syringe gas analyzer (C).

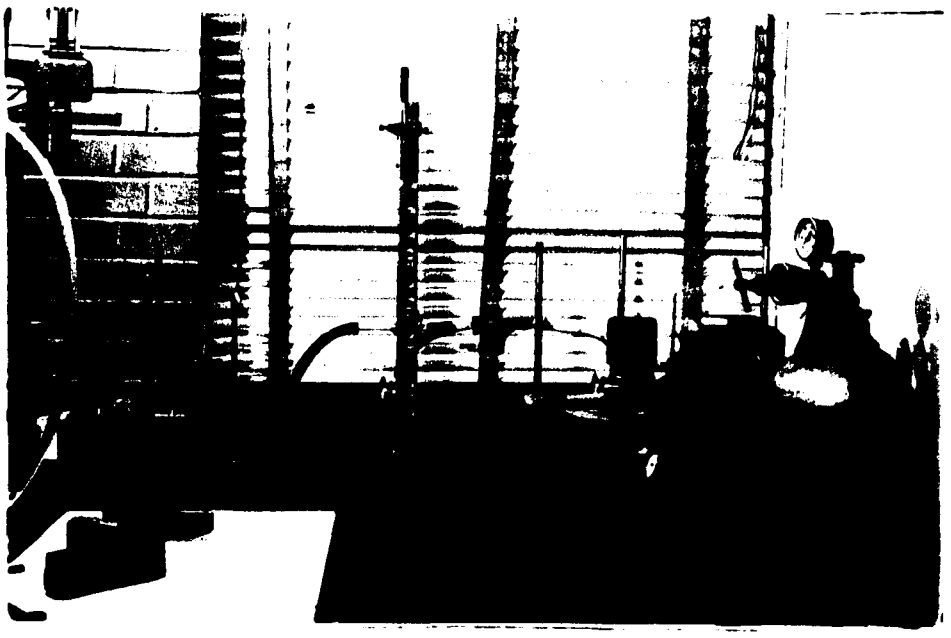

Figure 3: Photograph of the vacuum-manometer apparatus.

The nozzle of the syringe was fitted with a 1 1/2 inch section of Tygon aquarium tubing sealed at the opposite end with a small glass plug. Small samples of blood could be easily drawn into the section of the tubing from the tonometer barrel, and removed for analysis.

Oxygen Capacity Determination

Oxygen saturation of hemoglobin was achieved by rotating 2.5 cc blood samples in the tonometer in a water bath at 37°C for 15 minutes. The stopcock valve was left open during the rotation so that the tonometer chamber was in direct communication with the atmosphere above the water bath. Equilibrating blood in pure O_2 was found to be unnecessary since atmospheric concentrations of O_2 were more than sufficient for completely saturating the hemoglobin. Plasma evaporation was negligible during the rotation period and will be discussed more fully with reference the to equilibrium curve procedures. The pH of the blood at oxygen capacity was determined to the nearest .01 pH unit by using a Beckman (Model G) pH meter.

Determination of Oxygen Affinity

Vacuum-Manometer Apparatus. Construction of this apparatus can been seen in Figure 3. The mercury cistern of the manometer was continuous with a vacuum line which was connected to a faucet aspirator at one end and the tonometer at the other end. With the tonometer connected and all stopcock valves closed (except the tonometer's valve),

the gas pressure in the closed system was read directly from the millimeter scale attached to the mercury column.

Equilibrium Curve Procedure. Four arithmetically spaced oxygen partial pressure values were used to determine the oxygen equilibrium curves. These values were 65, 45, 25, and 5 mm Hg P_{O_2}. Based on the principle that dry air is 20.95 percent O_2 (Krogh 1941), the dry hypobaric pressures used to yield the above values were 309.0, 214.0, 119.0, and 23.8 mm Hg, respectively. These values were then adjusted by compensating for P_{H_2O} at equilibration temperature and correcting for the rising mercury levels in the cistern as the mercury column of the manometer was drawn downward by evacuation.

Gases were slowly withdrawn from the tonometer via the aspirator until the desired pressure was reached. The pressure was held at this level for five minutes to allow for complete pressure equilibration and to check for possible leakage. The stopcock valve on the tonometer was then closed and the tonometer disconnected from the apparatus. The tonometer was rotated in the water bath for 15 minutes at 37°C. At the completion of the equilibration period, a small sample of blood was removed from the tonometer and analyzed for oxygen. Hematocrit readings were taken before and after the complete equilibrium curve determinations. Plasma evaporation during the 1 1/2 to 2 hours required to complete a curve was minimal. The highest increase in blood concentration recorded was only 4.0 percent.

Each 2.5 cc blood sample used for the equilibration curve data was measured for oxygen capacity and equilibrated at all four P_{O_2} tensions beginnig at 65 mm Hg and working down. Percent saturation of hemoglobin at each of the reduced P_{O_2} levels was calculated by dividing the reduced volume percent O_2 values by oxygen capacity. The pH determinations were made for all individual curves to the nearest .01 pH unit. The P_{50} values (P_{O_2} at 1/2 saturation of hemoglobin) for all individual curves were determined by plotting each curve on graph paper and interpolating the P_{O_2} at 1/2 saturation. All of the P_{50} values determined at the differing equilibration pH's were correct to pH 7.40 by using the correction factors of Severinghaus (1965) for human blood. A complete analysis of the equilibrium curve procedure is given in the discussion section of this paper.

Electrophoresis

Preparation of Samples

Fresh, 1.0 cc samples of heparinized blood were mixed with isotonic saline (0.85 percent NaCl) and the erythrocytes were separated from the plasm by centrifuging for 15 minutes at 1550 rpm in a Precision Clinical Centricone (Precision Scientific Co.). This procedure was repeated three times. A volume of barbital buffer, pH 8.60 (10.3 gm/l Na Barbital, 1.4 gm/l barbital in CO_2-free H_2O) equal to that of the

packed, washed erythrocytes was added. The buffered erythrocytes were prepared for storage by bubbling carbon monoxide through the sample for 3 minutes, transferring the sample to a labeled vial, and freezing at -7°C. Freezing lysed the corpuscles and, in combination with the CO treatment, prevented denaturization during the long storage periods.

Electrophoresis Apparatus

Electrophoretic separation of hemoglobins was accomplished by using an EC470 Vertical Gel Electrophoresis Cell (E-C Apparatus Corp., Philadelphia, Pa.). The EC470 cell consists of upper and lower buffer chambers separated from each other but both in contact at opposite ends with a sheet of 7 percent acrylamide gel (migrating medium). Electrodes in the buffer chambers were connected to a constant voltage power supply (E-C power supply EC454). Both chambers were filled with Tris-Na_2EDTA-Borate buffer, pH 8.40.

Electrophoresis Procedure

Detailed electrophoretic procedures and recipes for the gel and various solutions used, are given in the E-C Technical Bulletin 141.

Hemoglobin samples were prepared for analysis by thawing the frozen samples into centrifuge tubes and spinning in an International Clinical Centrifuge (International Equipment Co., Needham Hgts., Mass.) at 7000 rpm for 20 minutes. Fifty lambda samples of the supernatant were removed and diluted in 250 lambda of barbital buffer

(same as described on p. 16). These dilutents were mixed with equal volumes (300 lambda) of 40 percent sucrose solution. Different hemoglobin samples inserted in the same gel were quantitized via the cyanmethemoglobin method (p. 9). Hemoglobin concentrations in all slots were made approximately equal by selecting the appropriate delivery pipette volumes (5-10 lambda).

All samples analyzed were run against a Human A hemoglobin standard (Hyland Laboratories, Los Angeles, Calif.). The cell was connected to the power supply and run at 70 amps for 2 hours. Temperature of the gel was maintained at 4-6^{o}C. At the completion of the run, the gel was removed and placed in a peroxidase stain specific for hemoglobin. The hemoglobin bands were developed in the dark for 20 minutes, removed and photographed. The migration distances were measured in mm and compared with Human A.

RESULTS

General

A total of 94 gophers, 18 cotton rats and 23 white rats were processed during this study. Gopher trapping success was generally found to be highest during the mid to late afternoon trapping sessions. In the five principal populations trapped, females out numbered males. Pelage color ranged from almost white (T. b. albatus) to very dark brown (T. b. catalinae and T. b. grahamensis) as can be seen in Figure 4. Average body weights of the five populations ranged from 101.9 gm (T. b. catalinae) to 123.6 gm (T. b. albatus). All data collected are summarized in Appendices II through V. Data for the separate sexes were not segregated because no consistent differences in their blood characters were observed. The cotton rats were not involved in the altitudinal study and, in most cases, only their major blood coefficients are presented. Discussion of the white rat data is confined to the Analysis of Methods section (pp. 37-40).

Blood Characteristics

Erythrocyte Factors and Gram Percent Hemoglobin

Erythrocyte Count. A gradual increase in the averages of the number of erythrocytes per cu mm of blood was found with increasing

Figure 4: Pelage colors of the five principal subspecies of Thomomys bottae used in this investigation. -- Specimens shown were selected to represent the typical pelage color for each population.

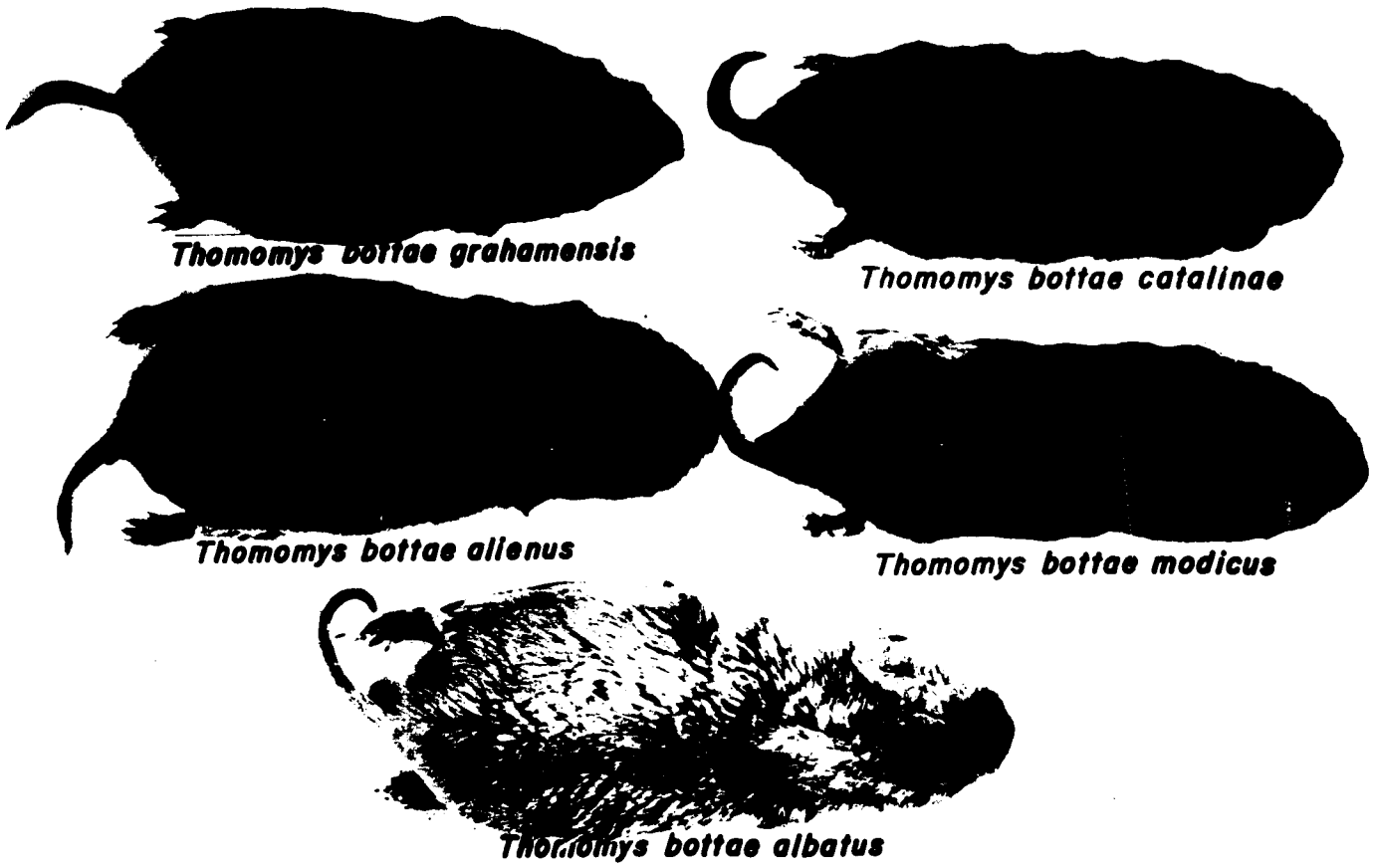
Thomomys bottae grahamensis
Thomomys bottae catalinae
Thomomys bottae alienus
Thomomys bottae modicus
Thomomys bottae albatus

Figure 4

habitat elevations (Figure 5). However, there is considerable overlapping of standard errors and a significant difference exists only between T. b. grahamensis and T. b. albatus. No consistent correlation was found between erythrocyte count and hematocrit (Figure 6).

Erythrocyte Diameters. Erythrocyte diameter measurements were done late in the investigation and time and snow conditions in the mountains did not allow a complete sampling of all populations or sufficient sample sizes for adequate statistical analysis. However, the superficial analysis indicates that T. b. modicus may have significantly smaller average corpuscle diameters than the other subspecies of T. bottae examined (Table 1). Also, these data suggest that the corpuscle size of T. bottae does not differ significantly from that of Sigmodon hispidus.

Hematocrit Values. Hematocrit averages of the two montane populations of T. bottae were higher than those of the lower elevation populations, although only T. b. catalinae was found to have significantly higher values than all other populations studied (Figure 6). The greatest contrast was between T. b. albatus and T. b. catalinae at elevations of 38 meters (125 ft.) and 2713 meters (8900 ft.) respectively. Although not given in the tables, the plotted hematocrits for S. h. eremicus (Figure 11) averaged 42.7 percent.

Gram Percent Hemoglobin Values. The general pattern of plotted gram percent Hb data closely parallels those of hematocrit for the

Figure 5: Erythrocyte count data for five subspecies of Thomomys bottae. -- Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t_{0.95}$ SE. Sample sizes are given above the ranges.

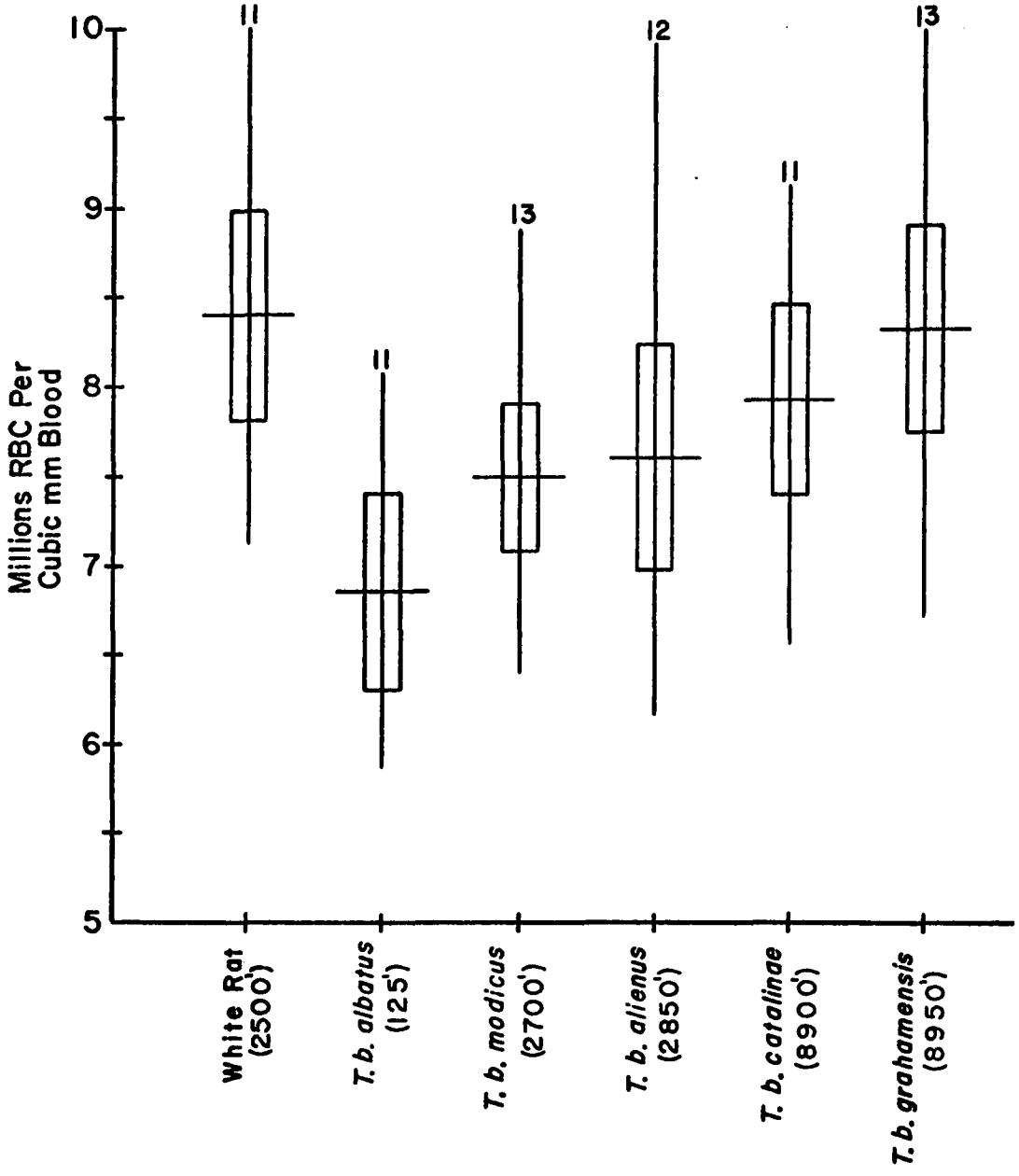

Figure 5: Erythrocyte count data for five subspecies of Thomomys bottae.

Table 1

Erythrocyte Diameters for Thomomys bottae and Sigmodon hispidus

Species	Sample Size[1]	Ave. Diameter (in microns)	Range (in microns)
T. b. albatus	3	6.39	4.48 - 7.84
T. b. modicus	3	5.89	4.48 - 7.28
T. b. alienus	1	6.48	5.60 - 7.84
T. b. catalinae	3	6.47	5.05 - 7.84
S. h. eremicus	4	6.42	4.48 - 8.96
S. h. confinus	3	6.40	5.05 - 7.84

[1]A minimum of 50 erythrocytes were measured for each animal examined.

Figure 6: Hematocrit values for five subspecies of Thomomys bottae. -- Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t0.95$ SE. Sample sizes are given above the ranges.

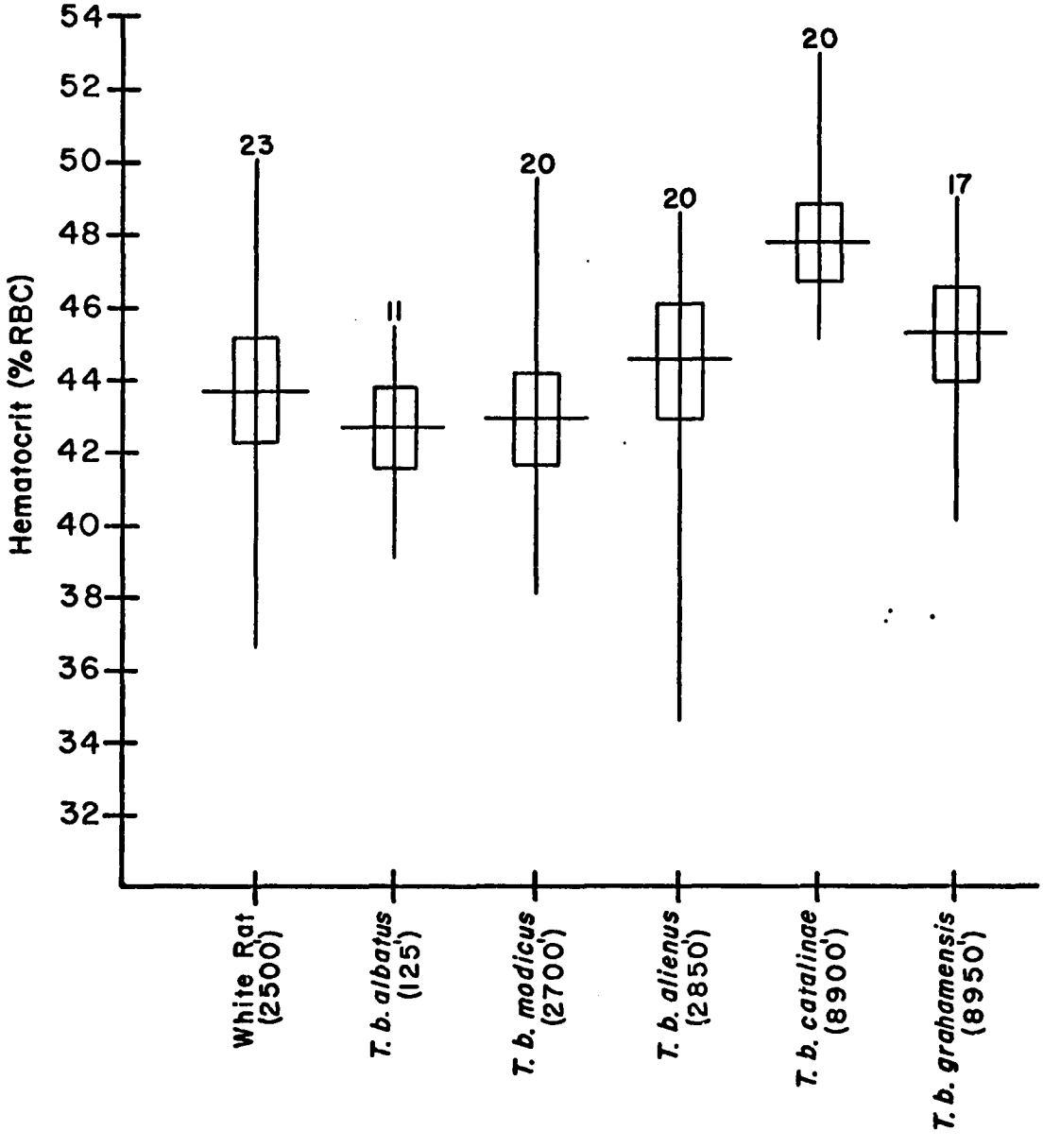

Figure 6: Hematocrit values for five subspecies of Thomomys bottae.

five subspecies of T. bottae (Figure 7). A significant difference was found only between T. b. catalinae and T. b. albatus. Surprisingly, the mean of T. b. grahamensis was slightly lower than that of T. b. alienus. Although not given in the tables, S. h. eremicus and S. h. confinus had means of 12.9 and 13.0 gram percent, respectively.

Gram Percent Hb Per 100 ml RBC Coefficients. This coefficient represents the correlation between gram percent hemoglobin and hematocrit. As in all the blood coefficient data, only the subspecies of T. bottae that differed the most are presented graphically with Sigmodon hispidus (Figure 8). The complete data are represented in Appendix III. Little intraspecific variability was found in T. bottae and S. hispidus with regard to the grams of hemoglobin per unit of hematocrit. However, the latter species was found to have significantly lower values.

Oxygen Capacity and Affinity

Oxygen Capacity Values. Plotted oxygen capacity data followed the same general pattern as the hematocrit and gram percent Hb data for the five subspecies of T. bottae (Figure 9). Thomomys b. modicus exhibited the lowest overall values, contrasting significantly with the high values of the geographically close but montane population of T. b. catalinae. However, the montane population of T. b. grahamensis exhibited only slightly higher capacities than the geographically close but lowland population of T. b. alienus. The plotted

Figure 7: Gram percent hemoglobin data for five subspecies of Thomomys bottae. -- Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t0.95$ SE. Sample sizes are given above the ranges.

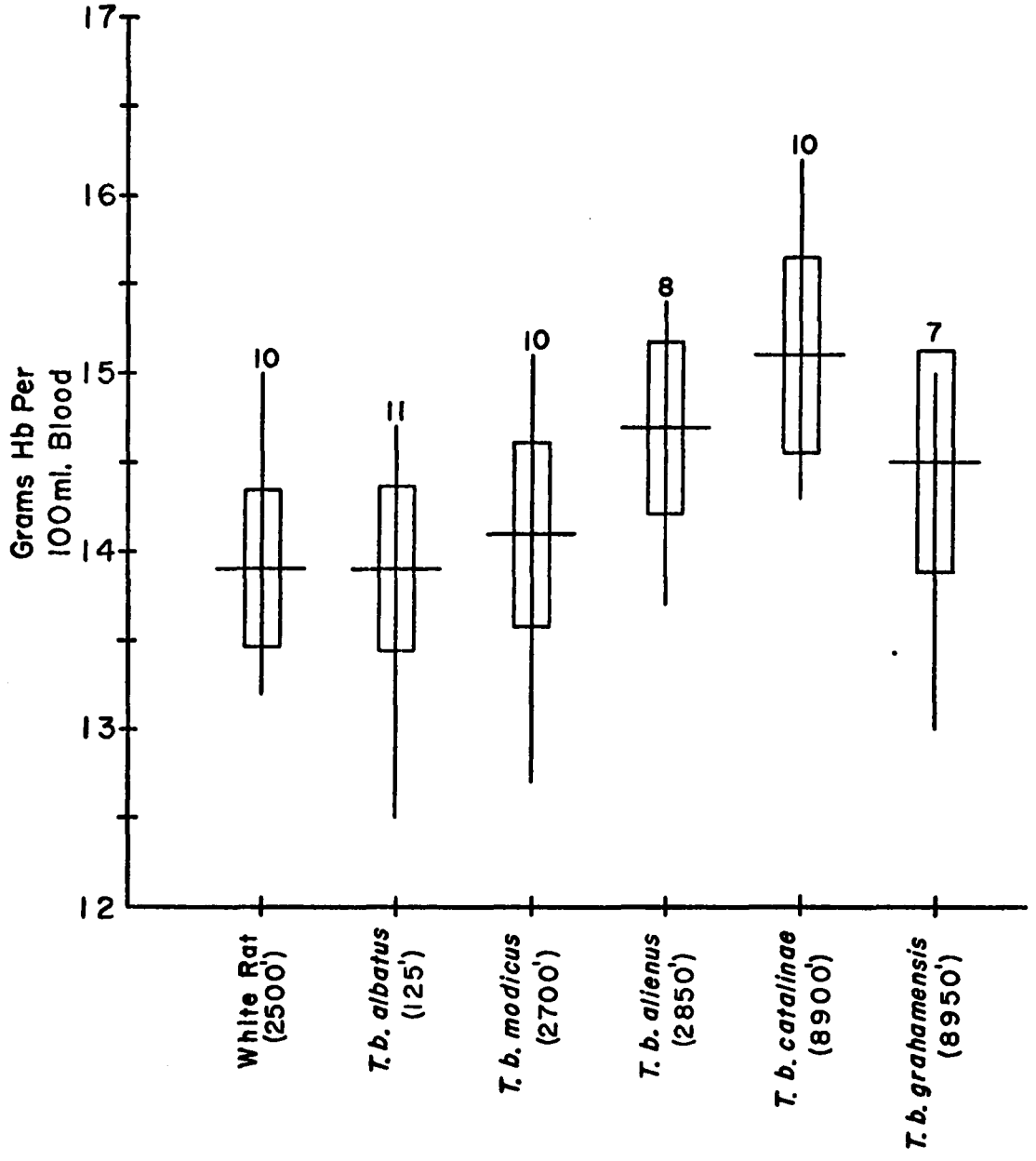

Figure 7: Gram percent hemoglobin data for five subspecies of Thomomys bottae.

Figure 8: A comparison of the gm Hb/100 ml RBC coefficients among the white rat, Thomomys bottae and Sigmodon hispidus. -- Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t0.95$ SE. Only the population of T. bottae that differed the most are plotted. Sample sizes are given above the ranges.

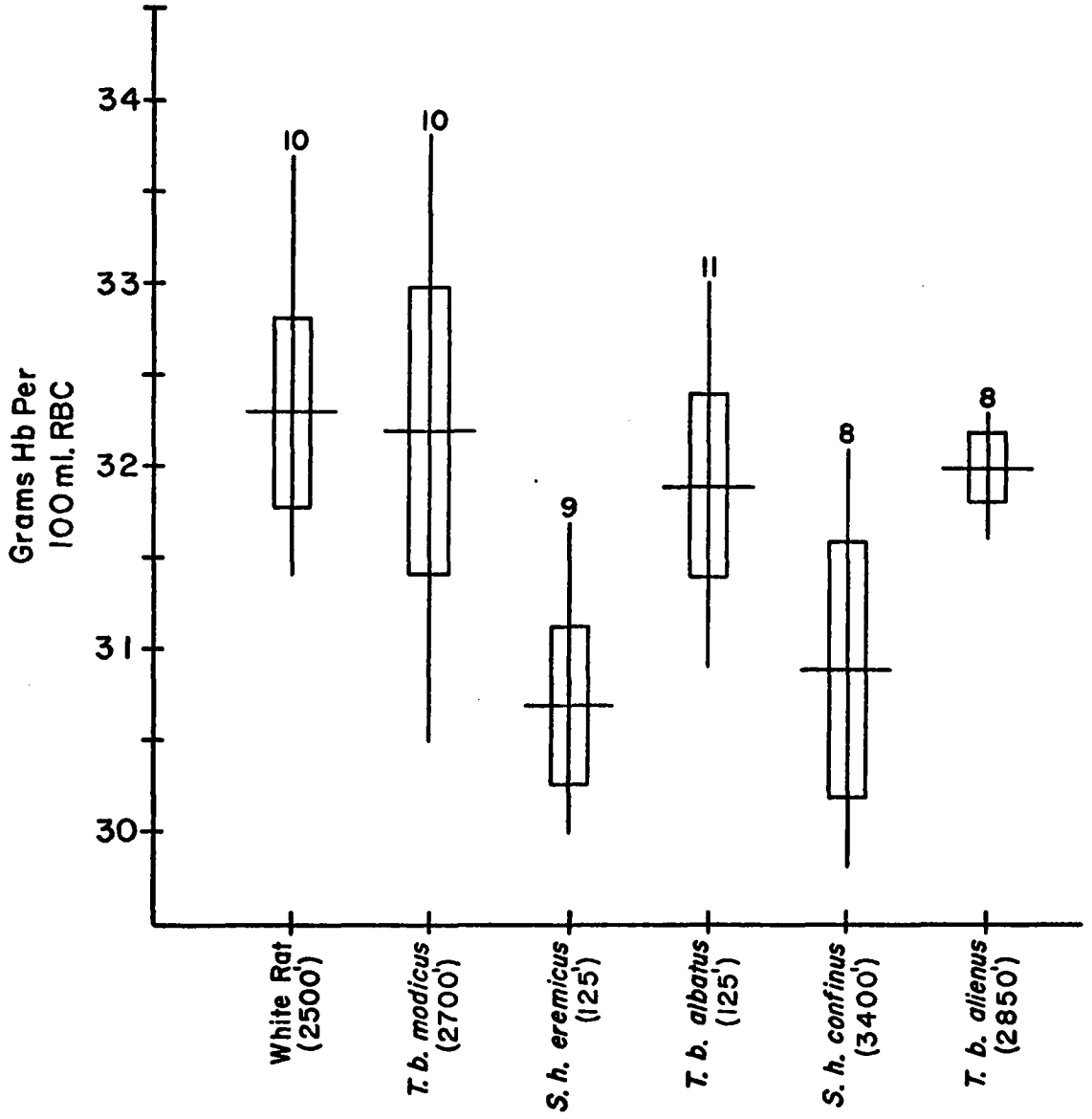

Figure 8: A comparison of the gm Hb/100 ml RBC coefficients among the white rat, Thomomys bottae and Sigmodon hispidus.

Figure 9: Oxygen capacity data for five subspecies of Thomomys bottae. -- Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t0.95$ SE. Sample sizes are given above the ranges.

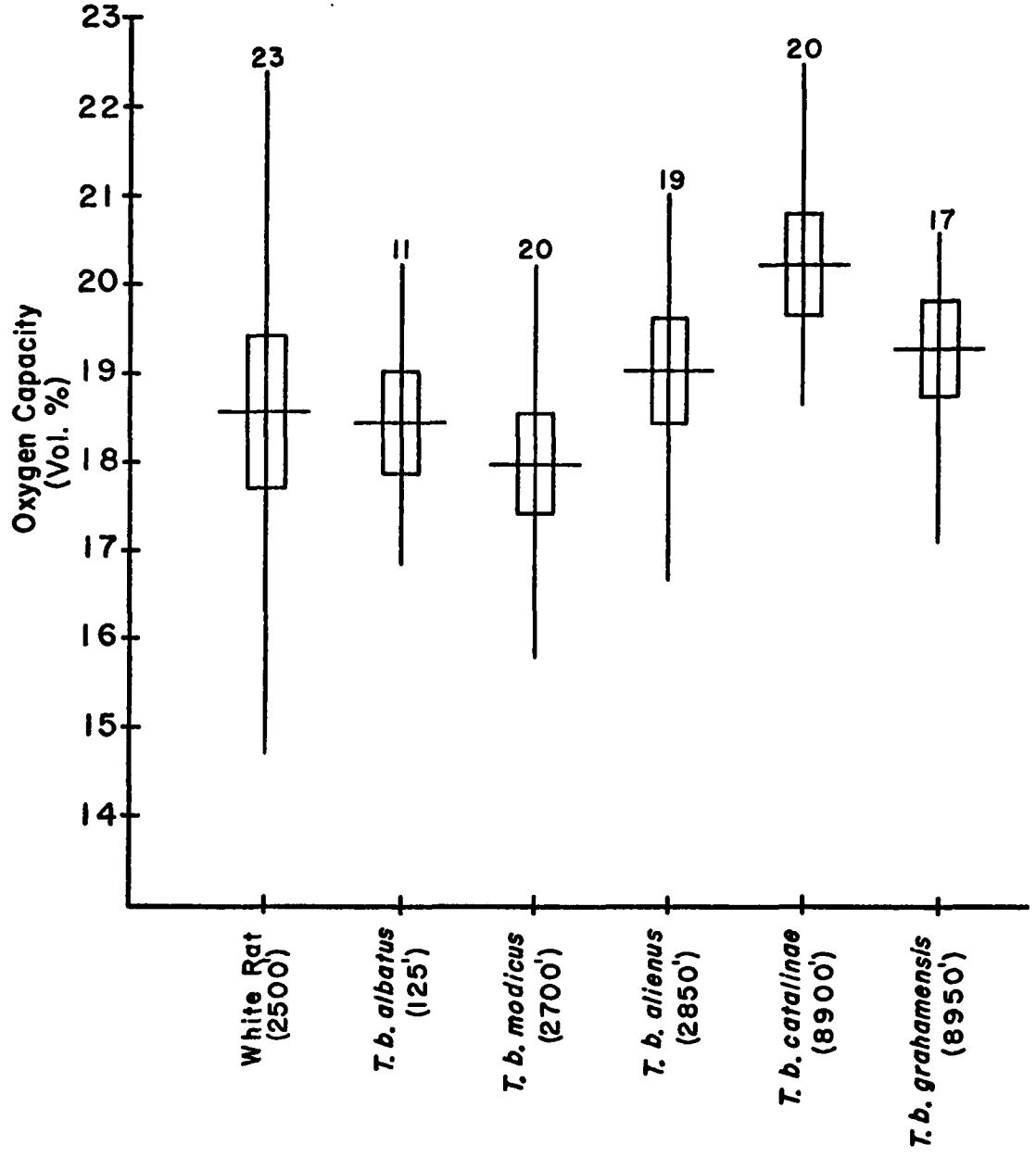

Figure 9: Oxygen capacity data for five subspecies of Thomomys bottae.

oxygen capacity data for S. hispidus eremicus (Figure 11) averaged 15.42 volume percent compared to the average of 18.43 volume percent for the sympatric T. b. albatus. The average oxygen capacity for 8 individuals of S. h. confinus was 15.62 volume percent.

The pH values of whole blood following oxygen capacity determinations fell within 7.75 to 8.20 for all animals investigated.

ml O_2 Per 100 ml RBC Coefficients. These data can also be expressed as volume percent saturation of erythrocytes and represent the correlation between oxygen capacity and hematocrit (Figures 10 and 11). The plotted data in Figure 11 exhibits the relationship between these two parameters and compares in detail the two sympatric populations of T. b. albatus and S. h. eremicus. Although the hematocrits were similar for these two populations, the oxygen capacities differed significantly, giving S. h. eremicus a lower ml O_2/100 ml RBC ratio. Little intra-specific variability was found in T. bottae or S. hispidus although the values of the former were significantly higher than those for the cotton rats.

ml O_2 Per Gram of Hb Coefficients. The correlation between oxygen capacity and gram percent hemoglobin is considered as the hemoglobin binding capacity at 100% oxygen saturation. This coefficient was found to be especially valuable since it indicates the overall efficiency of hemoglobins with regard to their oxygen binding properties. No consistent correlation could be made between altitudinal distribution

Figure 10: A comparison of the ml O_2/100 ml RBC coefficients among the white rat, Thomomys bottae and Sigmodon hispidus. -- Only the populations of T. bottae that differed the most are plotted. Vertical lines represent the ranges; horizontal lines the means; and rectangles the $\bar{x} \pm t0.95$ SE. Sample sizes are given above the ranges.

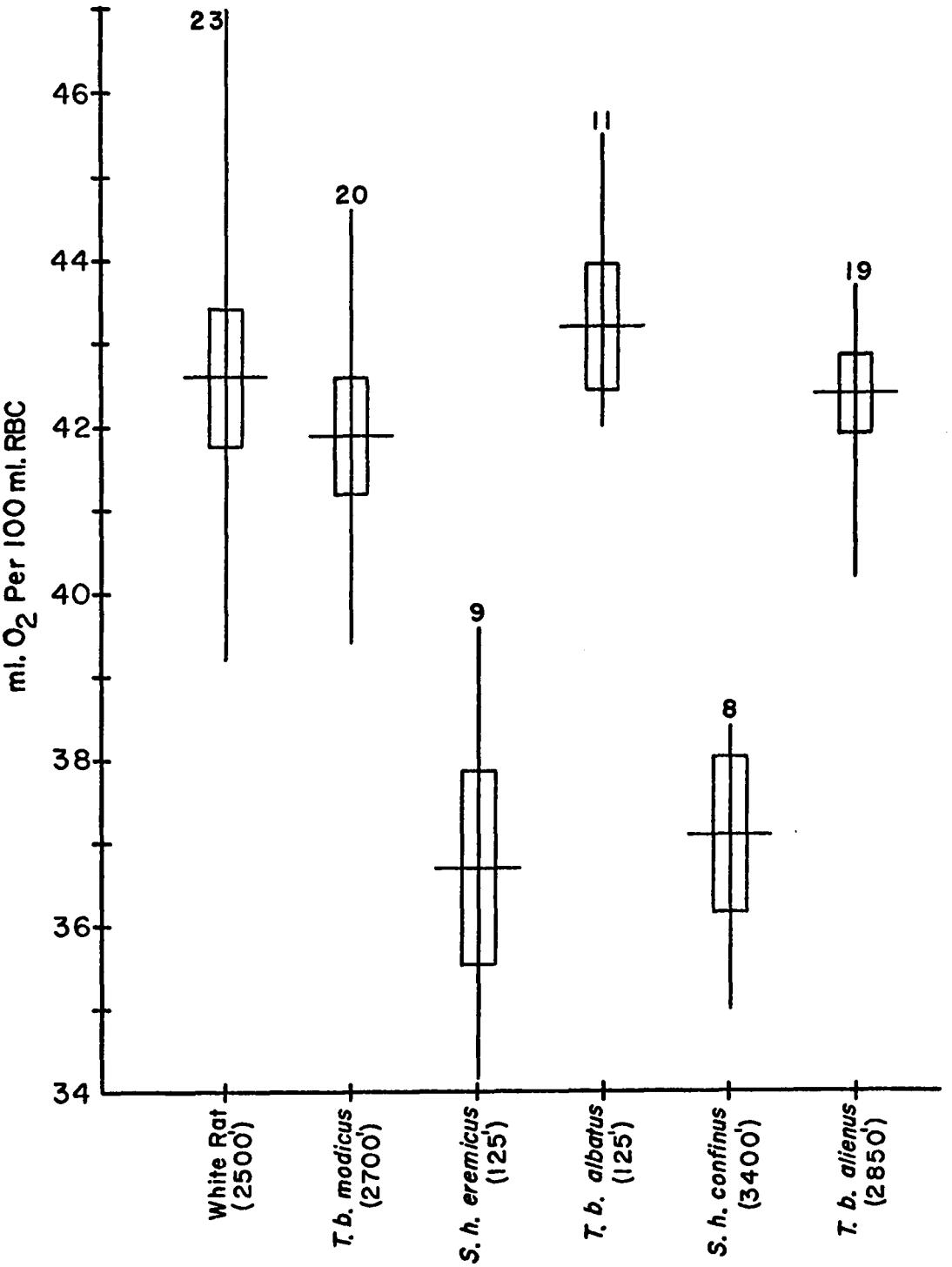

Figure 10: A comparison of the ml O_2/100 ml RBC coefficients among the white rat, Thomomys bottae and Sigmodon hispidus.

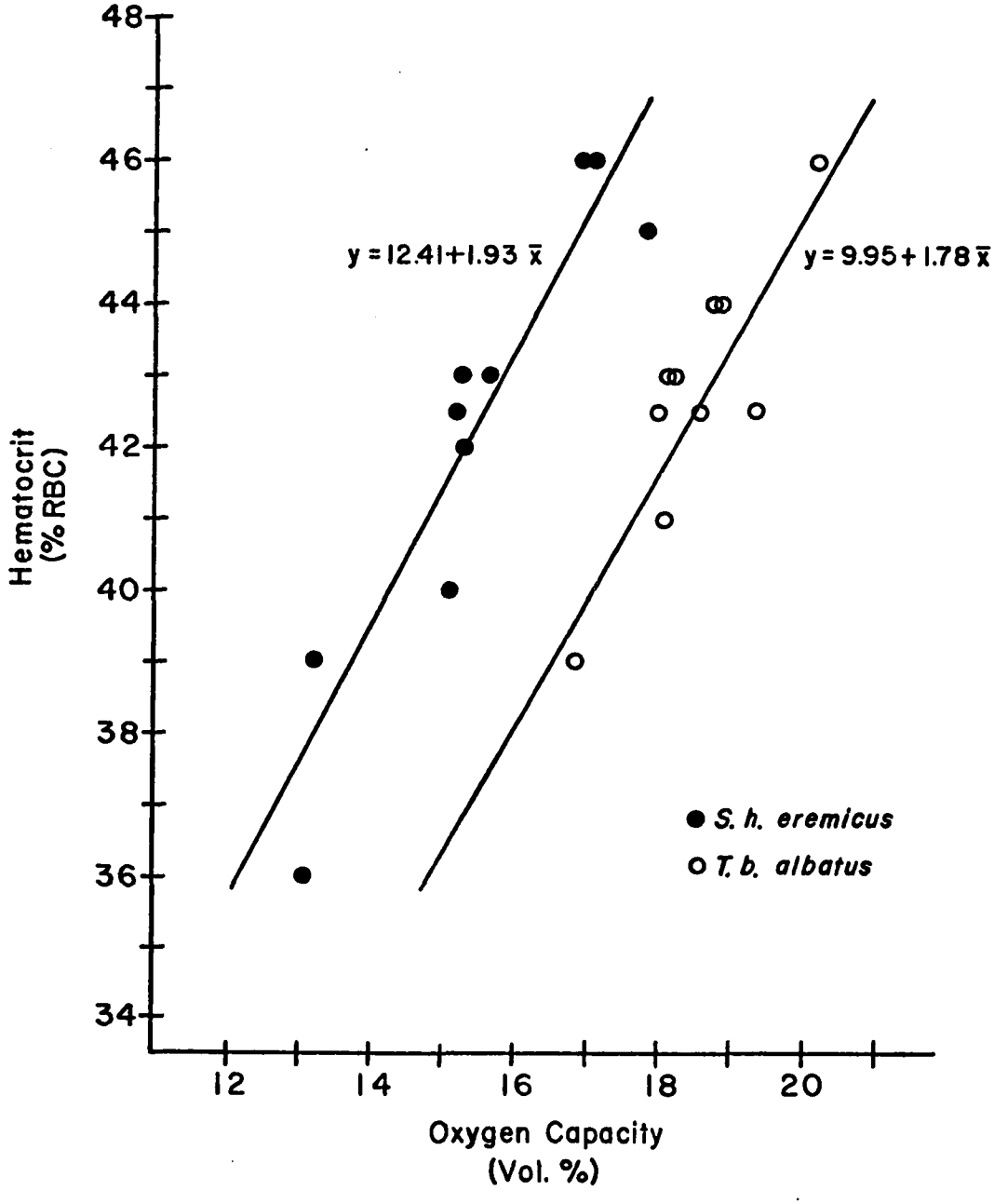

Figure 11: Plotted hematocrit and oxygen capacity data for S. h. eremicus and T. b. albatus--both trapped from the same field near Yuma, Arizona. -- Body weights of the two species were very similar. Regression lines are fitted to the two sets of points and represent the ml O_2/100 ml RBC coefficients for the two species.

and binding capacities in T. bottae (Appendix III). However, binding capacity values for all populations of T. bottae were significantly higher than the two populations of S. hispidus (Figure 12).

Oxygen Affinity as Indicated by P_{50} Values. The P_{50} values (P_{O_2} at 1/2 sat. Hb) of all individual equilibrium curve determinations before and after pH corrections are given in Appendix V. Applying the Severinghaus (1965) correction factors to the initial high pH curves reduced the variability in all populations with the exception of T. b. alienus. Also, shifting the curves to the right at a pH of 7.40 accentuated some of the interpopulation differences that were suspected before pH corrections were made. The validity of using the human blood pH correction factors is discussed in the Analysis of Methods section.

Statistical analyses of the P_{50} values for each of the six populations investigated are given in Appendix IV and graphically illustrated in Figure 13. These data show strong similarities between the white rat, T. b. modicus, and T. b. catalinae--all of which are to the right (= less oxygen affinity) of the three similar curve positions of T. b. albatus, T. b. alienus and T. b. grahamensis. This latter population was found to exhibit considerable consistency with regard to the individual oxygen affinities.

The maximum observed increase in blood concentration during the equilibrium curve procedure was 4.0 percent. Preliminary

Figure 12: A comparison of the ml O_2/gm Hb coefficients (binding capacities) among the white rat, Thomomys bottae and Sigmodon hispidus. -- Only those populations of T. bottae that differed the most are presented here. Vertical lines represent the ranges; horizontal lines the means; and rectangles $\bar{x} \pm$ to0.95 SE. Sample sizes are given above the ranges.

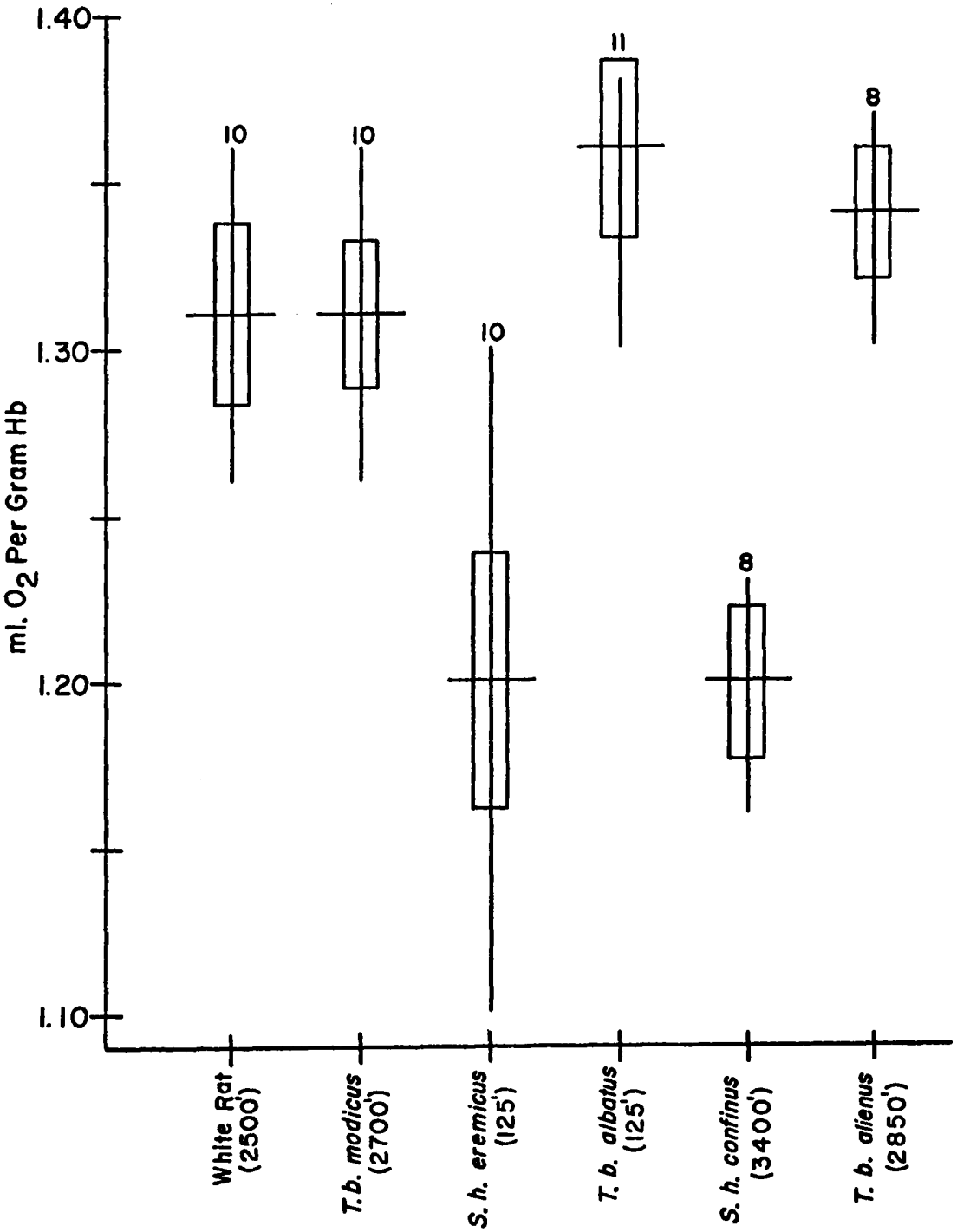

Figure 12: A comparison of the ml O_2/gm Hb coefficients (binding capacities) among the white rat, Thomomys bottae and Sigmodon hispidus.

Figure 13: P_{50} values corrected to pH 7.40 for five subspecies of Thomomys bottae and the white rat. -- Values are based on the partial pressure of oxygen at 1/2 saturation of hemoglobin. Vertical lines represent the ranges; horizontal lines the means; and rectangles $\bar{x} \pm t0.95$ SE. Sample sizes are given above the ranges.

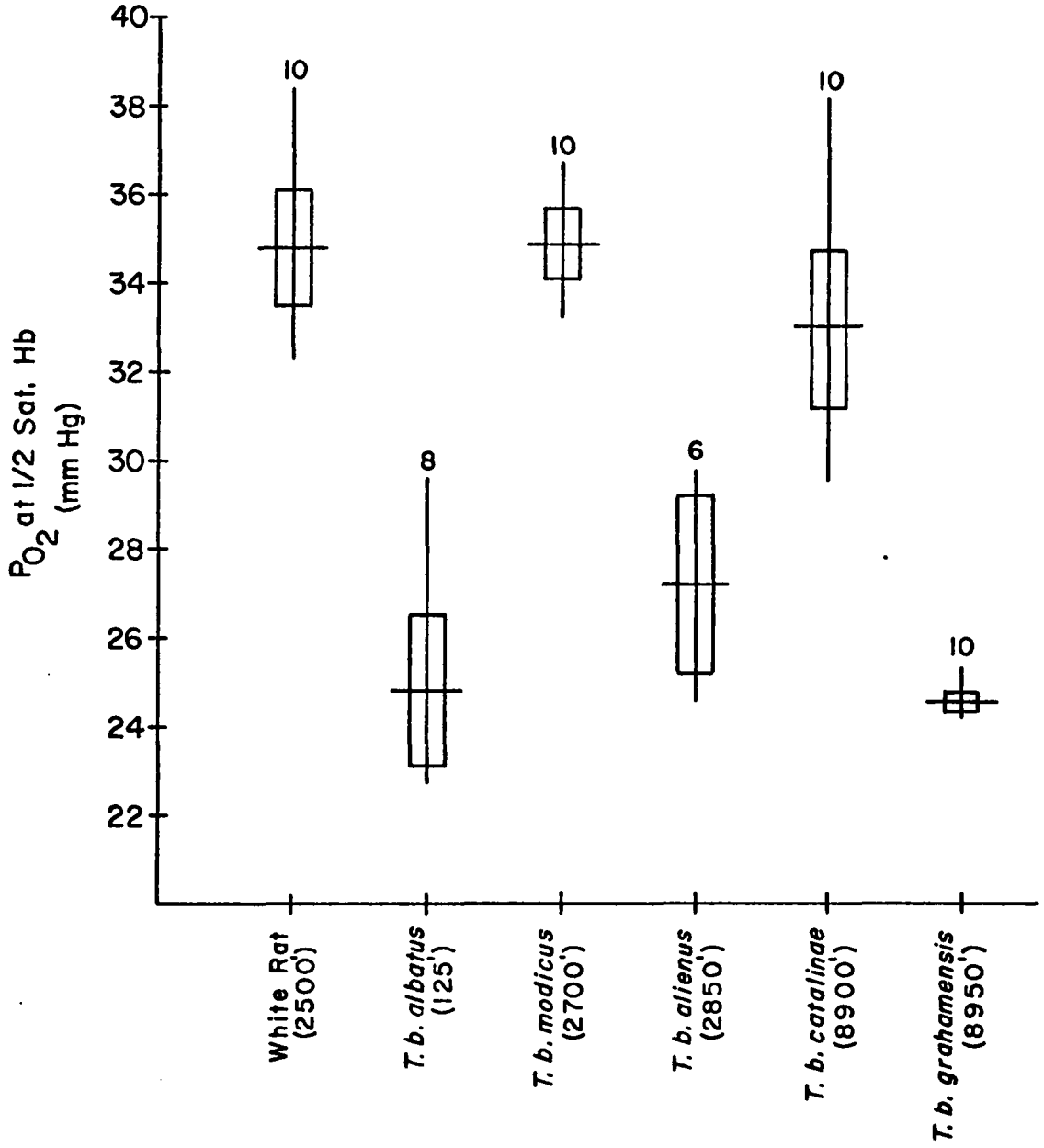

Figure 13: P_{50} values corrected to pH 7.40 for five subspecies of Thomomys bottae and the white rat.

calculations using the white rat data showed this degree of error to be insignificant. For example, correction for a white rat curve that exhibited a 4.0 percent increase in blood concentration changed the P_{50} value from 19.7 to 19.9 mm Hg P_{O_2} at pH 7.92. Since most hematocrit readings taken before and after the equilibrium curve determinations exhibited smaller increases than 4.0 percent, compensations for small evaporative errors were considered unnecessary.

Electropherograms

Electrophoretic separation of the various rodent bloods exhibited single hemoglobin bands with identical mobilities (R_a values of 0.90) for Cratogeomys castanops lacrimalis, the white rat, and the seven subspecies of Thomomys bottae (Figure 14). However, Sigmodon hispidus eremicus and S. h. confinus were found to have multiple hemoglobin bands (Bronander 1970). S. h. eremicus exhibited four bands (2 major and 2 minor) with R_a values of 0.99*, 1.117, 1.38*, and 1.50 (*major bands). Sigmodon h. confinus exhibited three bands (2 major and 1 minor) with R_a values of 0.99*, 1.19*, and 1.36.

Several individuals from each population of S. hispidus and T. bottae (except T. b. extenuatus) were subjected to electrophoretic analysis, and no significant intrapopulation variability was observed.

An interesting difference was observed in the blood samples of S. hispidus and T. bottae during the electrophoresis sample preparation

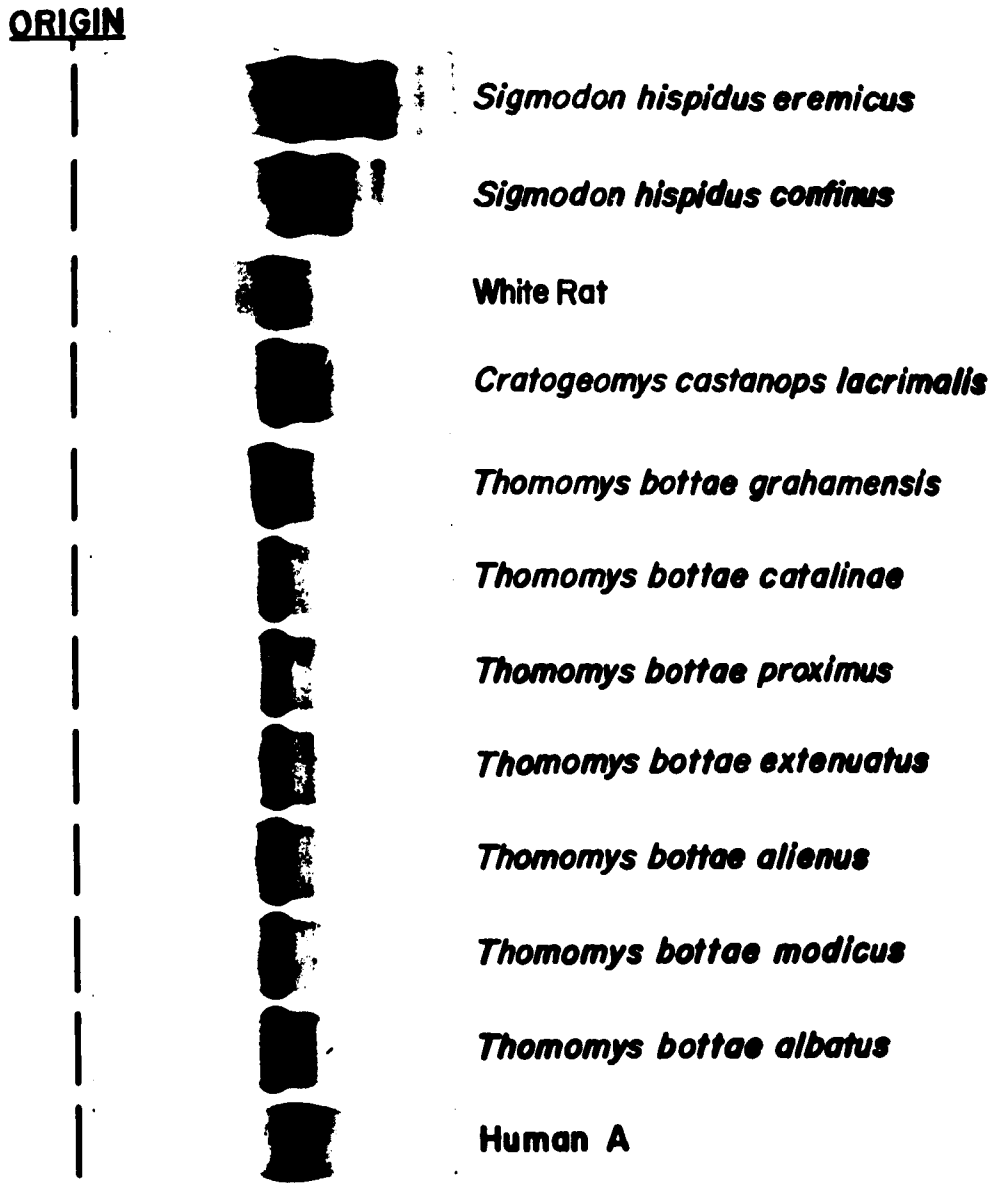

Figure 14: Combined electropherograms of the white rat, 2 subspecies of S. hispidus, 7 subspecies of T. bottae, and Cratogeomys. -- The R_a values for the two subspecies of S. hispidus were 0.99, 1.17, 1.38, 1.50, and 0.99, 1.19, 1.36 for S. h. eremicus and S. h. confinus, respectively. R_a values for the remaining species were approximately 0.90. All samples were run against the human hemoglobin A standard (bottom).

procedures. The thawed blood samples of S. hispidus after 20 minutes of centrifugation at 7000 rpm exhibited large, whitish pellets of corpuscle debris (stroma) below the supernatant. T. bottae samples, however, exhibited a denser supernatant and a complete absence of cell debris.

DISCUSSION

Analysis of Methods

The white rat data in the literature provided a convenient means for checking several of the techniques used in this investigation. Although considerable variability is undoubtedly manifested in the different strains of laboratory rats, the similarities of results of this work with those in the literature will indicate which techniques are comparable.

In this study the average hematocrit value for the white rat of 43.6 percent is somewhat lower than the 46.0 percent reported by Albritton (1952). However, the gm Hb/100 ml RBC coefficient of 32.0 given by the same author compares closely with the average of 32.3 found in this investigation. Gardner (1947) reports for adult white rats a gram percent Hb value of 14.4 versus the 13.9 given in Appendix II, and an average erythrocyte count of 8.5×10^6/cu mm blood versus 8.4×10^6/cu mm obtained in this study. Hematocrit values appear to be variable among the different strains of white rats (Altman and Dittmer 1961), and differences in average hematocrit could be responsible for the discrepancies in gram percent Hb and RBC counts observed.

Burke (1953) and Gjonnes and Schmidt-Nielsen (1952), using the syringe method, found the oxygen capacity of the adult white rat to

average 18.1 and 18.6 volume percent, respectively. These data compare closely with the average of 18.56 volume percent found in this investigation.

Most of the oxygen equilibrium (dissociation) curves in the literature have been determined spectrophotometrically using buffered hemoglobin solutions. Two fairly recent spectrophotometric techniques are described by Gordy and Drabkin (1957) and Haab, Piper and Rahn (1960). There is considerable discussion in the literature with regard to the oxygen affinity differences between hemoglobin solutions and whole blood. Sullivan and Riggs (1967) found that a given species may have a greater oxygen affinity than another using whole blood but a lower oxygen affinity when hemoglobin solutions are compared. Forbes and Roughton (1931) stated that the P_{50} of whole blood is often higher (i.e., curve is to the right) than that of hemoglobin solutions. Paul and Roughton (1951) found that the P_{50} value for whole sheep blood was 7.2 mm Hg at 6°C, whereas a hemoglobin solution of equal concentration and under the same conditions exhibited a P_{50} of 5 mm Hg. Christensen and Dill (1935) found different results with whole blood and hemoglobin solutions in birds. The hemoglobin solutions were found to have much less affinity for oxygen than whole blood at the same pH. These authors state that "from a physiological point of view the nature of the oxygen dissociation curve can be best studied in whole blood."

The gasometric technique used with whole blood in this investigation proved to be more informative than the spectrophotometric techniques since both oxygen capacity and oxygen affinity values were obtained from the same blood samples. Thus, the combined effects of hemoglobin affinity and quantity could be estimated.

The high equilibration pH's observed in this study were probably due to the steep CO_2 concentration gradients between the blood and equilibration atmospheres. Carbon dioxide diffusing from the blood would alter the acid-base equilibrium to a more basic condition. The use of Severinghaus's (1965) human blood pH correcting factors is considered a valid means for reducing the variability in the gopher and white rat P_{50} values caused by the differing equilibration pH's. In standardizing the P_{50} values at pH 7.40, it is fully realized that the resulting curve positions are at best only rough approximations since the magnitude of the Bohr effects of the gophers and white rats were not known. However, the white mouse data of Gray and Steadman (1964) suggest that the human Bohr effect factors may not be greatly in error when applied to the P_{50} values of this particular species. The P_{50} of the mouse at pH 7.20 can be closely approximated by multiplying the P_{O_2} value obtained at pH 7.40 by the human Bohr factor of 1.22 (from Severinghaus 1965).

Since the equilibrium curve techniques used in this study were different from those in the literature, a comparison of the specific P_{50} values obtained with those values presented in the literature was not

attempted. Interpopulation comparisons of Thomomys bottae and the comparison of this species with the white rat, are possible because all individuals were subjected to precisely the same procedures.

Although acrylamide gel electrophoresis yields the greatest resolution compared to the many other electrophoretic procedures (Massa, Tentori and Vivaldi 1967), interpretation of the protein fractions from a molecular standpoint is not possible without further elaborate analysis. Therefore, it can not necessarily be assumed that the multiple bands obtained, using the methods described in this paper, represent complete and different hemoglobins. However, regardless of whatever the detailed molecular composition of the bands may be, the technique is valid and useful for demonstrating evidence of gross hemoglobin differences between populations.

A Comparison of Pocket Gophers with Other Rodents

Hemoglobin Oxygen Affinities

Schmidt-Nielsen and Larimer (1958) report a general correlation between body size and blood oxygen affinities in mammals. The P_{50} values were determined on whole blood and found to decrease with increasing body size. The horse, for example, was found to have a much higher affinity (P_{50} of 25 mm Hg) than the deer mouse (P_{50} of 49 mm Hg). Foreman (1954), using buffered hemoglobin solutions, found little correlation between oxygen affinity and body size in 12 species of

rodents. The discrepancies between these two studies may be partially explained on the basis of the differences found between the affinities of whole and diluted blood. Also, Foreman (1954) was working with a much smaller size range. This same author also found considerable variation in hemoglobin oxygen affinities even among species closely related phylogenetically and having similar habits and habitats.

In this study, the P_{50} values obtained for the white rat and five subspecies of Thomomys bottae indicated that the oxygen affinities of T. b. catalinae are similar to that of the white rat. T. b. albatus, T. b. alienus, and T. b. grahamensis exhibited greater affinities. This takes exception with the general body size-oxygen affinity relationship found by Schmidt-Nielsen and Larimer (1958) since gophers are generally less than one-half the size of adult white rats. In contrast, the cotton rat (Sigmodon), which is of comparable size and often sympatric with gophers, was found to have a lower oxygen affinity than the white rat (Schmidt-Nielsen and Larimer 1958).

Because of the unusual atmospheric conditions that have been found in gopher burrows (see Introduction), it is not surprising to find greater oxygen affinities in this fossorial species. Hall (1965) found in a survey of seven species of squirrels (Sciuridae) that the most fossorial species (Cynonmys ludovicianus and Spermophilus tereticaudus) had the highest oxygen affinities. Bartels, Schmelzle, and Ulrich (1969) also found high oxygen affinities relative to body size in moles, which they

attributed to the possible hypoxic conditions associated with burrowing habits. Gjonnes and Schmidt-Nielsen (1952) found that the oxygen equilibrium curve of the kangaroo rat (Dipodomys spectabilis) did not differ significantly from that of the white rat although the average body weights of the two species differed by over 100 grams. Although kangaroo rats spend much less time in their burrows than gophers, it appears that both species have high oxygen affinities relative to their body sizes.

Less pronounced Bohr effects in blood with high oxygen affinities were found by Foreman (1954). On the basis of this observation, it may be reasonable to suggest that the Bohr effects in gopher blood are less pronounced than those of the white rat. If so, then the corrected P_{50} differences found between these two species would be further accentuated at pH 7.40. Less pronounced Bohr effects in fossorial species would seemingly enhance oxygen loading in the lungs under conditions of high CO_2 concentrations generally found in burrows. However, Hall (1964) observed in the prairie dog (Cynomys ludovicianus) -- one of the more fossorial of the sciurids -- a Bohr effect greater than those of most other mammals. Obviously, much more work needs to be done in this area.

Oxygen Capacity Factors

Although the determination of oxygen capacity without additional blood data is not always meaningful as a means of comparing

hemoglobin efficiencies, this information does represent the sum effect of various blood factors at hemoglobin saturation. Burke (1953) found no taxonomic relationship in the orders and families of those mammals for which oxygen capacity data have been collected. Nor did he find any correlation between oxygen capacity and body size in adult mammals. He did find, however, that the oxygen capacities of very young white rats were significantly lower than those measured for adults. The same situation for gophers was suggested in my study by an oxygen capacity of 11.0 volume percent for a 33.0 gm juvenile compared to an average of 17.98 volume percent for adults in the same population.

The 17.98 to 20.23 volume percent range of averages for the oxygen capacities of the five subspecies of T. bottae studied, indicates that even the low elevation populations have higher oxygen capacities than many other rodents discussed in the literature. Only gasometric determinations are considered. Burke (1952) reports mean oxygen capacities of 15.6 volume percent for Cricetus auratus, 17.5 volume percent for Neotoma floridana, and 11.7 volume percent for Cavia sp. Gjonnes and Schmidt-Nielsen (1952) report a mean oxygen capacity for Dipodomys spectabilis of 17.5 volume percent. Larimer (1959) found values of 15.06 and 14.53 volume percent for two individuals of Sigmodon hispidus. The results of Bronander's work with two subspecies of S. hispidus, exhibited averages of 15.62 and 15.42 volume percent.

The coefficient that represents the relationship between gram percent hemoglobin and oxygen capacity was found to be very useful for measuring the binding capacity efficiencies of hemoglobins. This coefficient represents the observed ml O_2 bound per gram of hemoglobin in whole blood--not the theoretical binding capacity based on the iron-oxygen relationship in the hemoglobin molecule. Bernhart and Steggs (1943), using the gram percent iron content of human blood, calculated that 1.0 gm of hemoglobin will bind with approximately 1.36 ml O_2. This is interpreted to be the theoretical binding capacity of hemoglobin under optimal conditions. If this theoretical maximum binding capacity can be applied to all mammalian hemoglobins, then any value that falls below 1.36 ml O_2/gm Hb in whole blood suggests the presence of inactive hemoglobins and/or interference mechanisms.

Barker (1957), Nahas (1958), and Sealander (1964), used the values of 1.35, 1.34, and 1.36 ml O_2/gm Hb, respectively, to derive oxygen capacity data from gram percent hemoglobin readings. It is my opinion that such calculated capacities must be used with caution because they do not take into account the possibility of inactive hemoglobins and/or interference mechanisms. Most methods for determination of gram percent hemoglobin do not differentiate between the ferric (inactive) and ferrous (active) states of hemoglobin. Hence, there can be very large discrepancies between the calculated and actual oxygen capacities. For example, in the work with S. hispidus

(Bronander 1970), the oxygen capacities determined gasometrically were 12.5 to 13.0 percent lower than the values calculated from the gram percent hemoglobin data. Larimer (1959) found 4.7 and 9.2 percent discrepancies in two individuals of S. hispidus when gasometric and calculated techniques were compared.

The range of population averages for the hemoglobin binding capacities in T. bottae was found to be 1.31 to 1.36 ml O_2/gm Hb. Both subspecies of S. hispidus exhibited means of 1.20 ml O_2/gm Hb. Hence, T. bottae was found to have a more efficient blood from the standpoint of the volume of oxygen carried per unit of hemoglobin at 100 percent saturation. This may suggest a significant percentage of inactive hemoglobin (methemoglobin) in S. hispidus. Methemoglobins have been found to occur commonly in the blood of man, horses, dogs, and snakes (Prado 1946) and many species of lizards (Pough 1969). Prado (1946) found methemoglobins in 82 percent of the dogs examined and they represented from 3.5 to 20.5 percent of the total hemoglobin present.

An observation made during the electrophoresis blood preparation procedures suggests that an interference mechanism may also be operating in the oxygen-hemoglobin relationship of the cotton rats. As previously mentioned (p. 36), the buffered and lysed blood of the cotton rats after centrifugation, exhibited a very conspicuous pellet of stroma below the supernatant. The gopher blood, however, exhibited no stroma debris whatsoever following centrifugation. Since both bloods were

treated precisely the same, it appears that the nature of the erythrocyte membrane may differ between the two species. It may be that the corpuscle membrane of the pocket gopher is thinner and/or more fragile than that of the cotton rat. A more fragile membrane may, upon lysing, disperse into fragments that are too small to be centrifuged out at 7000 rpm. A higher stroma/hemoglobin ratio in the corpuscles of the cotton rats may somehow interfere with the oxygen diffusion and/or hemoglobin binding processes resulting in a reduction in the hemoglobin binding capacity. The supposition of a higher stroma/hemoglobin ratio in the corpuscle of the cotton rat is supported by the lower gm percent Hb/100 ml RBC coefficient found in this species. Thomomys bottae exhibited population averages ranging from 31.6 to 32.3 gm Hb/100 ml RBC compared to the averages of 30.7 and 30.9 found in the two populations of S. hispidus. This discrepancy could not be explained on the basis of corpuscle size difference since both species exhibited very similar averages and ranges with respect to their erythrocyte diameters (Table 1).

Interpretation of the Electropherograms

The uniformity of the hemoglobin electrophoretic migration patterns among the eight populations of Geomyidae--including Cratogeomys and seven subspecies of T. bottae--suggests that these apparently similar hemoglobins have been stable through time and

efficient within the wide range of environmental extremes inhabited by these animals. This uniformity strongly contrasts with the considerable variability found in the hemoglobin electrophoretic patterns in the genus Sigmodon (Bronander 1970). Both subspecies of S. hispidus used for comparison in this study exhibited conspicuous multiple hemoglobin bands.

The physiological significance of multiple hemoglobins has been fertile ground for speculation for many years. Much of the speculation has been with reference to possible oxygen affinity advantages of multiple hemoglobins in situations of oxygen stress (e.g., high altitude). Mitchell (1969) suggested a possible correlation between multiple hemoglobins and greater oxygen affinities in high flying, migratory bats. Out of seven vespertilionid bats found to have multiple hemoglobins, four were migratory and had wide altitudinal ranges. Ahl (1968) found that a greater percentage of a particular hemoglobin in Peromyscus maniculatus was associated with the higher altitude populations. Foreman (1964), however, found no correlation between oxygen affinity of specific hemoglobins and their electrophoretic mobilities in nine species of rodents. Naughton et al. (1963) found two separate hemoglobins in Dorset sheep which had different oxygen affinities.

The single hemoglobin band found in Thomomys bottae appears to represent a more efficient condition, with respect to the hemoglobin oxygen binding properties, than the multiple bands found in Sigmodon

hispidus. This supposition is based on the significantly low hemoglobin binding coefficients for S. hispidus compared to the high coefficient values for T. bottae (p. 45). Therefore, in this particular comparison, multiple hemoglobin bands cannot be correlated with greater efficiency.

It is apparent that much more must be learned about the nature of the individual electrophoretic bands in situations where multiple hemoglobins are postulated. An obvious approach would be to isolate the various bands in sufficient quantities for oxygen capacity and oxygen affinity analysis. It may be that some of these bands represent less efficient hemoglobins (e.g., methemoglobins) or various degradation products of a single hemoglobin at the particular pH used.

Blood Adaptations to Altitude in Pocket Gophers

General Considerations

Since only blood adaptations were considered in this study, it must be kept in mind that many other anatomical and physiological compensatory mechanisms are generally operating to some extent in both native and acclimatized high altitude populations (see Introduction). Therefore, an apparent deficiency in one or more blood characters may be compensated for by other factors.

Although correlations between hematocrit, gram percent hemoglobin and oxygen capacity were found to be relatively uniform among the five geographical subspecies of Thomomys bottae

(Appendix III), significant variability in all of the individual blood characters examined was observed within and between populations. Not all of interpopulation differences can be ascribed to particular environmental conditions without further investigation. However, two adaptive blood mechanisms appear to be operating in the mountain populations: (1) consistently high oxygen affinities and/or (2) consistently high hemoglobin production--hence, high volume percent concentrations of oxygen.

Oxygen Affinities and Capacities

In the montane (2728 M) population of T. b. grahamensis which was not found to have consistently high oxygen capacities, the comparatively high affinity for oxygen compensated for this deficiency by increasing the percentage of hemoglobin saturation in the lungs. Conversely, the consistently high oxygen capacities of the montane (2713 M) T. b. catalinae compensated for the lower oxygen affinity by loading greater volumes of oxygen per unit volume of blood. This latter situation may be of greater advantage since a lower oxygen affinity, concurrent with larger oxygen volumes being carried, releases more oxygen to the tissues at the lower oxygen tensions in the capillaries.

Since, in general, the gophers exhibited high oxygen affinities relative to their body size, it is possible that concurrent higher affinities and capacities are not physiologically mandatory until altitudes well in

excess of 2728 meters are reached. The elevations used in this investigation may fall in a transition zone where either high oxygen capacities or high affinities will suffice.

Hall et al. (1936) found that two mammals native to high altitude (llama and vicuna) exhibited much greater oxygen affinities than their counterparts as sea level. However, the hematocrits and erythrocyte counts were similar between the two altitudinal populations. It is not certain whether the high affinities found in native high altitude mammals represent adaptations to alpine conditions or preadaptations which permitted invasion of higher elevations (Bartels et al. 1963). The phenotypic flexibility of oxygen affinity in mice is indicated by the work of Tribukait and Brummer (1967). They subjected mice to a simulated altitude corresponding to 6000 meters for 10 days, and found that the normal equilibrium curve was shifted 14.2 mm Hg to the left. Return to the original affinity was observed after 4 to 6 weeks in norr al atmospheric conditions. This indicates that sufficient oxygen stress will elicit a shift of the equilibrium curve in the direction of greater affinity. Even though in small mammals high metabolic rates are usually associated with low oxygen affinities (Schmidt-Nielsen and Larimer 1958), it appears that conditions of hypoxia will shift the emphasis of oxygen affinity to loading in the lungs.

Keys et al. (1936) determined the positions of oxygen equilibrium curves for man in both native high altitude residents and

acclimatized individuals from low elevations. They found that the individuals acclimatized for 2 1/2-3 months at 6,000 meters exhibited equilibrium curves that were to the right of their original curves at sea level. The positions of the curves for 11 long-time residents were, in general, within the normal range for man at sea level. These workers suggested that the alteration of the oxygen equilibrium curves in the acclimatized individuals may represent an intermediate stage in final adaptation. Hall (1936) observed no significant change in the hemoglobin affinity for oxygen in nine men with a change in altitude from sea level to 6,000 meters. He concluded that hemoglobin produced in the body during acclimatization to altitude does not differ from that produced at sea level. However, other studies have suggested an increase in fetal hemoglobins with acclimatization to high altitudes (Dill 1938, Huisman 1958). In most cases reported (Altman and Dittmer 1961) acclimatized or native men at high altitude exhibited significant increases in hematocrit.

Other Blood Factors

The montane population of T. b. catalinae exhibited consistently high hematocrits when compared to all other gopher populations studied. This, of course, was manifested in the significantly high oxygen capacities also found in the same population. However, the gradual increase in erythrocyte number with altitude observed in the five

gopher populations was not necessarily accompanied by proportional increases in hematocrit and gram percent hemoglobin. Although the data are incomplete, corpuscle size may be responsible for at least some of the discrepancies. For example, the slightly higher erythrocyte count of T. b. catalinae over that of T. b. modicus was not proportional to the strongly contrasting hematocrits between these two populations. The same discrepancy was observed between T. b. albatus and T. b. modicus--both having similar hematocrits but differing erythrocyte counts. The erythrocyte diameter measurements suggested a smaller average corpuscle size for T. b. modicus which would increase the number of erythrocytes possible per unit of hematocrit. Smaller erythrocytes are suspected in T. b. grahamensis which exhibited the highest erythrocyte counts but significantly lower hematocrits than T. b. catalinae. This would perhaps be advantageous in oxygen loading because of the increased surface/volume ratio in smaller erythrocytes. Gough and Kilgore (1964) found that Peromyscus populations from 2700 meters in Colorado had smaller erythrocytes and higher erythrocyte counts than those from Louisiana.

Acclimatization experiments in the literature help elucidate the genetic nature of the polycythemic response to high altitude. Kalabuchov (1937), working with wood mice (Apodemus) in Russia, transferred montane (1500-1800 M) animals to the plains (300 M) and observed initial decreases in erythrocyte numbers followed by increases to

somewhat above the original levels. However, acclimatizing lowland animals in the mountains resulted in a 109 percent increase in the erythrocyte numbers. Hock (1962) transferred deer mice (Peromyscus) captured at sea level to 3900 meters and found that their acclimatization resulted in hematocrits and erythrocyte counts that were very similar to those of the native mice at the same elevation. These same animals when transferred back to sea level, were found to restore their blood characters to the original state. Translocating native high altitude mice to sea level exhibited no significant decrease in hematocrits after 90 days of acclimatization. This author (Hock 1962) states that although the results of the last experiment may suggest genetic adaptation to high altitude, it may also be that there is an absence of anything in the low altitude condition that would foster the destruction of excess erythrocytes. Because of the lack of any obvious barriers to gene flow between different altitudinal populations of deer mice, Hock (1962) concludes that the response to high altitude is phenotypic (acclimatization) rather than genotypic. A similar situation may be hypothesized for Thomomys bottae in Arizona. Gene flow among the different altitudinal demes appears to have been sufficient to maintain the specific status of these populations and similarities of hemoglobins and basic blood coefficients.

SUMMARY AND CONCLUSIONS

Various blood properties of five different geographical and altitudinal populations of Thomomys bottae were examined. Blood comparisons were made between two montane populations and these in turn were compared with three populations from lower elevations. In additions, several blood coefficients and electropherograms were compared between Sigmodon hispidus and T. bottae. Where appropriate, data collected for these species were compared with data for other rodents in the literature. The brief summaries of the results and the conclusions drawn from them are as follows:

(1) Based on the oxygen affinity versus body weight correlation of Schmidt-Nielsen and Larimer (1958), Thomomys bottae was found to have generally high affinities relative to body size. This may be characteristic of most strongly fossorial mammals.

(2) The higher oxygen affinities within T. bottae could not be consistently correlated with altitude. Nor could a correlation be made between differing affinities and hemoglobin electrophoretic migration patterns in this species.

(3) Two high altitude blood adaptations appeared to be operating in T. bottae--either of which appeared to be sufficient at about 2743 M: (1) consistently high oxygen affinities, and (2) consistently high

hemoglobin production--hence, high volume percent O_2 levels. Simultaneous operation of both mechanisms may not be necessary until altitudes well in excess of 2743 M are reached.

(4) Little interpopulation variability was found among the five subspecies of T. bottae with regard to the correlations between hematocrit, gram percent Hb and oxygen capacity. Also, no significant variability was observed in the hemoglobin electropherograms of seven subspecies of T. bottae and Cratogeomys castanops.

(5) Oxygen capacities and hemoglobin binding capacities for T. bottae were considerably higher than those of Sigmodon hispidus. The low ml O_2/gm Hb coefficients for this latter species suggests the presence of inactive hemoglobins and/or interference mechanisms.

(6) These previous data seriously question the accuracy of deriving oxygen capacity data by multiplying gram percent hemoglobin data by the theoretical constant of 1.36 ml O_2/gm Hb, since the frequency and percentages of methemoglobins may cause significant errors.

(7) The multiple hemoglobin bands of Sigmodon hispidus did not confer any special oxygen binding advantages over the single band found in Thomomys bottae. In fact, by comparison, the gopher hemoglobin appeared to be more efficient with regard to oxygen loading at saturation.

APPENDIX I

SPECIMENS EXAMINED

Listed below are the trapping localities of all animals used in the study along with sex ratios and numbers trapped at each locality. Trapping dates were July 1969 to February 1970, inclusive.

Geomyidae

Thomomys bottae albatus

ARIZONA: Yuma Co., 2 mi. W. Yuma along Colorado River. Elev. 38 m (4 males, 7 females)

Thomomys bottae alienus

ARIZONA: Graham Co., 1.8 mi. N. Thatcher along Gila River. Elev. 869 m (7 males, 10 females)

Thomomys bottae catalinae

ARIZONA: Pima Co., Santa Catalina Mtns. (Mt. Lemmon) 200 yds. SE. Air Force Station. Elev. 2713 M (7 males, 13 females)

Thomomys bottae extenuatus

NEW MEXICO: Hidalgo Co., 3 mi. N. Animas on SH 338. Elev. 1341 m (1 female)

Geomyidae--Continued

Thomomys bottae grahamensis

ARIZONA: Graham Co., Pinaleno Mtns. (Mt. Graham) Treasure Park. Elev. 2728 m (6 males, 11 females)

Thomomys bottae modicus

ARIZONA: Pima Co., Tucson, Univ. of Ariz. Farm on N. Campbell Ave. Elev. 823 m (6 males, 14 females)

Thomomys bottae proximus

ARIZONA: Santa Cruz Co., Santa Rita Mtns. Gardner Canyon 4.5 mi. W. of Hwy.83. Elev. 1646 m (1 male, 2 females)

Cratogeomys castanops lacrimalis

NEW MEXICO: Eddy Co., SE. Artesia. Elev. 1036 m (1 male, 1 female)

Cricetidae

Sigmodon hispidus confinus

ARIZONA: Graham Co., Artesia (on Hwy. 666). Elev. 1036 m (4 males, 4 females)

Sigmodon hispidus eremicus

ARIZONA: Yuma Co., 2 mi. W. Yuma along Colorado River. Elev. 38 m (5 males, 5 females)

APPENDIX II

A COMPARISON OF VARIOUS BLOOD VALUES AMONG FIVE SUBSPECIES OF THOMOMYS BOTTAE AND THE WHITE RAT

Species, Locality & Elevation	Body Weight (in Grams)	Hematocrit (Percent RBC)	Oxygen Capacity (Volume Percent)	Gm Hb/ 100 ml Blood	RBC Count (1×10^6/ mm^3 Blood
White Rat	(23)	(23)	(23)	(10)	(11)
U of A	314.7	43.6± 1.45	18.56± .859	13.9± .437	8.40± .593
762 M (2500')	(187.0-426.5)	(36.5-50.0)	(14.70-22.40)	(13.2-15.0)	(7.12-10.00)
T. b. albatus	(11)	(11)	(11)	(11)	(11)
Yuma	123.6	42.7± 1.13	18.43± .582	13.9± .457	6.86± .544
38 M (125')	(76.6-181.5)	(39.0-45.5)	(16.82-20.22)	(12.5-14.7)	(5.87-8.08)
T. b. modicus	(20)	(20)	(20)	(10)	(13)
Tucson	111.1	43.0± 1.35	17.98± .571	14.1± .509	7.50± .410
823 M (2700')	(67.4-157.0)	(38.0-49.5)	(15.80-20.21)	(12.7-15.1)	(6.40-8.88)
T. b. alienus	(19)	(20)	(19)	(8)	(12)
Thatcher	116.1	44.5± 1.57	19.05± .605	14.7± .485	7.61± .629
269 M (2850')	(65.8-172.0)	(34.5-48.5)	(16.65-21.03)	(13.7-15.4)	(6.16-9.92)
T. b. catalinae	(20)	(20)	(20)	(10)	(11)
Mt. Lemmon	101.9	47.7± 1.03	20.23± .576	15.1± .554	7.94± .524
2713 M (8900')	(56.5-135.0)	(45.0-53.0)	(18.65-22.50)	(14.3-16.2)	(6.59-9.12)
T. b. grahamensis	(17)	(17)	(17)	(7)	(13)
Mt. Graham	108.2	45.3± 1.31	19.30± .549	14.5± .626	8.33± .577
2728 M (8950')	(76.0-150.0)	(40.0-49.0)	(17.09-20.59)	(13.0-15.0)	(6.72-10.00)

Note: Upper figures in parentheses represent sample sizes. Middle figures represent $\bar{x} \pm t_{0.95}$ SE. Lower figures in parentheses represent the ranges.

APPENDIX III

A COMPARISON OF THREE BLOOD COEFFICIENTS AMONG THE GENERA RATTUS, THOMOMYS AND SIGMODON

Species	Locality and Elevation	ml O_2 per 100 ml RBC	ml O_2 per gm Hb	gm Hb per 100 ml RBC
White Rat	U of A	(23)	(10)	(10)
	762 M	42.6 ± .807	1.31 ± .027	32.3 ± .509
	(2500')	(39.2-47.0)	(1.26-1.36)	(31.4-33.7)
Thomomys	Yuma	(11)	(11)	(11)
bottae	38 M	43.2 ± .760	1.36 ± .016	31.9 ± .492
albatus	(125')	(42.0-45.5)	(1.30-1.38)	(30.9-33.0)
Thomomys	Tucson	(20)	(10)	(10)
bottae	823 M	41.9 ± .695	1.31 ± .022	32.2 ± .789
modicus	(2700')	(39.4-44.6)	(1.26-1.36)	(30.5-33.8)
Thomomys	Thatcher	(19)	(8)	(8)
bottae	869 M	42.4 ± .458	1.34 ± .019	32.0 ± .220
alienus	(2850')	(40.2-43.7)	(1.30-1.37)	(31.6-32.3)
Thomomys	Mt. Lemmon	(20)	(10)	(10)
bottae	2713 M	42.5 ± .653	1.35 ± .016	31.6 ± .491
catalinae	(8900')	(38.9-44.8)	(1.32-1.38)	(30.5-32.8)
Thomomys	Mt. Graham	(17)	(7)	(7)
bottae	2728 M	42.7 ± .475	1.34 ± .013	32.3 ± .497
grahamensis	(8950')	(41.0-44.3)	(1.32-1.35)	(31.3-32.9)
Sigmodon	Yuma	(9)	(10)	(9)
hispidus	38 M	36.7 ± 1.16	1.20 ± .038	30.7 ± .434
eremicus	(125')	(34.0-39.6)	(1.10-1.30)	(30.0-31.7)
Sigmodon	Artesia	(8)	(8)	(8)
hispidus	1036 M	37.1 ± .911	1.20 ± .022	30.9 ± .702
confinus	(3400')	(35.0-38.4)	(1.16-1.23)	(29.8-32.1)

Note: Upper figures in parentheses represent sample sizes. Middle figures represent $\bar{x} \pm t_{0.95}$ SE. Lower figures represent the ranges.

APPENDIX IV

PARTIAL PRESSURE VALUES OF OXYGEN AT 1/2 SATURATION OF HEMOGLOBIN FOR FIVE SUBSPECIES OF THOMOMYS BOTTAE

Species	Locality and Elevation	Sample Size	Partial Pressure of O_2 at 1/2 Sat. of Hb (mm Hg)
White Rat	U of A (762 M)	10	34.80 ± 1.298 (32.2-38.4)
T. b. albatus	Yuma (38 M)	8	24.81 ± 1.736 (22.7-29.6)
T. b. modicus	Tucson (823 M)	10	34.87 ± 0.810 (33.2-36.8)
T. b. alienus	Thatcher (869 M)	6	27.23 ± 1.990 (24.5-29.8)
T. b. catalinae	Mt. Lemmon (2713 M)	10	33.01 ± 1.737 (29.5-38.2)
T. b. grahamensis	Mt. Graham (2728 M)	10	24.54 ± 0.251 (24.2-25.3)

Note: All P_{50} values have been corrected to pH 7.40 by using the pH correction factors of Severinghaus (1965). Lower figures in parentheses represent the ranges. Upper figures represent $\bar{x} \pm t_{0.95}$ SE. Elevations are in meters.

APPENDIX V

THOMOMYS AND WHITE RAT P_{50} CORRECTIONS TO pH 7.40

Anim. #	pH	P_{50}	Corrected P_{50} Values to pH 7.40	Anim. #	pH	P_{50}	Corrected P_{50} Values to pH 7.40
White Rat				T. b. modicus			
11	7.80	22.0	34.1	13	7.86	20.2	33.8
16	7.92	19.7	34.9	7	7.75	25.0	36.8
18	7.91	20.1	35.3	11	7.93	19.8	35.4
12	7.95	17.6	32.2	4	7.87	20.6	34.8
13	7.90	19.3	33.4	5	7.90	19.5	33.7
14	8.01	17.5	33.0	2	7.88	20.2	34.3
10	7.75	26.1	38.4	3	7.85	20.5	33.2
15	7.82	22.4	35.6	9	7.98	19.1	36.1
17	7.94	20.2	36.7	1	7.83	21.7	35.0
19	7.92	19.4	34.4	12	8.05	18.3	35.6
Ave.	7.89	20.43	34.80	Ave.	7.89	20.49	34.87
T. b. albatus				T. b. alienus			
48	8.15	12.3	24.6	42	7.85	15.8	26.0
47	7.98	12.6	23.8	41	7.81	15.7	24.5
45	7.84	15.3	25.0	38	7.83	17.7	28.6
44	7.75	20.1	29.6	37	7.78	18.3	27.8
43	7.90	13.8	23.9	54	8.20	13.5	26.7
63	7.95	13.1	23.9	56	7.83	18.5	29.8
				Ave.	7.88	16.58	27.23

Anim. #	pH	P_{50}	Corrected P_{50} Values to pH 7.40	Anim. #	pH	P_{50}	Corrected P_{50} Values to pH 7.40
T. b. albatus--continued							
64	7.80	16.1	25.0				
46	7.86	13.6	22.7				
Ave.	7.90	14.61	24.81				
T. b. catalinae				T. b. grahamensis			
18	7.90	19.3	33.5	31	7.77	16.3	24.3
16	7.98	17.6	33.2	28	7.94	13.4	24.4
21	7.80	22.8	35.4	25	7.78	16.7	25.3
22	7.97	18.0	33.6	34	7.85	14.6	24.2
17	7.87	19.0	32.1	27	7.81	15.5	24.2
14	8.15	15.0	29.5	24	7.83	15.1	24.4
20	7.91	17.9	31.4	26	7.81	15.7	24.5
19	7.78	25.2	38.2	35	7.75	16.9	24.9
23	7.98	17.1	32.3	30	7.87	14.4	24.4
15	7.93	17.3	30.9	29	7.84	15.1	24.8
Ave.	7.93	18.9	33.01	Ave.	7.83	15.37	24.54

Note: Observed pH values and corresponding P_{50} values are given in the second and third columns, respectively. Corrected P_{50} values were determined by using the pH correction factors of Severinghaus (1965).

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