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A comparative Study of the Oxygen Dissociation Curves of Deer Mouse (\textit{Peromyscus leucopus}) and Meadow Vole (\textit{Microtus pennsylvanicus}) Hemoglobin

James Loeffelholz
\textit{College of Saint Benedict/Saint John's University}

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A COMPARATIVE STUDY OF THE OXYGEN DISSOCIATION CURVES
OF DEER MOUSE (PEROMYSCUS LEUCOPUS) AND MEADOW VOLE
(MICROTUS PENNSYLVANICUS) HEMOGLOBIN

A SENIOR PROJECT
The Honors Program
College of St. Benedict/St. John's University

In partial Fulfillment
of the Requirements for the Distinction "All College Honors"
and the Degree Bachelor of Arts
In the Department of Biology

by James Loeffelholz
A comparative study of the oxygen dissociation curves of deer mouse (*Peromyscus leucopus*) and meadow vole (*Microtus pennsylvanicus*) hemoglobin

Approved by:

[Signature]
Assistant Professor of Biology

[Signature]
Professor of Biology

[Signature]
Assistant Professor of Chemistry

[Signature]
Chair, Department of Biology

[Signature]
Director, Honors Program
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ABSTRACT

Oxygen equilibrium curves of dilute hemoglobin solutions from deer mice (*Peromyscus leucopus*) and meadow voles (*Microtus pennsylvanicus*) were determined by optical tonometry at pH 6.8 and pH 7.4. For deer mouse hemoglobin, \( P_{50\ 7.4} = 2.67 \) kilopascals (KPa), \( P_{50\ 6.8} = 4.54 \) KPa, \( n_{7.4} \) (cooperativity index at pH 7.4) = 1.13, \( n_{6.8} = 1.60 \) and Bohr shift = 1.91. For meadow vole hemoglobin, \( P_{50\ 7.4} = 2.74 \) KPa, \( P_{50\ 6.8} = 6.16 \) KPa, \( n_{7.4} = 1.11 \), \( n_{6.8} = 1.63 \), and Bohr shift = 2.35. The method was evaluated for use in the undergraduate laboratory.

INTRODUCTION

Gas exchange is of primary importance to animal life. Animals are aerobic, meaning that all cells in an organism require a supply of oxygen and removal of carbon dioxide metabolic waste. Gas transfer across cell membranes occurs by passive diffusion; for multicellular animals, gas transfer by passive exchange with the atmosphere is not adequate because cells inside the body have no exposure to the atmosphere. Tissues may be bathed by fluid carrying oxygen in simple solution, but in most cases the demand soon surpasses the carrying capacity of simple fluid, and a carrier molecule like hemoglobin (Hb) which reversibly bonds to oxygen becomes necessary (Hoar, 1975). For hemoglobin, this interaction with oxygen can be expressed as \( Hb + nO_2 \rightarrow Hb(O_2)_n \). Hemoglobin bonds to oxygen in the lungs and releases it at tissues throughout the body.

As an example of the significant effect hemoglobin has on oxygen transport, mammalian blood can carry 0.2 ml \( O_2 \) per 100 ml of blood in solution at standard atmospheric conditions. When
hemoglobin is present and functioning, however, 20 ml O₂ is present per 100 ml blood (Schmidt-Nielsen, 1983). The large oxygen capacity of hemoglobin results from its ability to carry four molecules of oxygen for each molecule of hemoglobin. Hemoglobin consists of four subunits, each possessing a heme group with a normally high affinity for bonding to oxygen (Cameron, 1989).

The metalloproteins such as hemoglobin that have evolved to fulfill this need for oxygen carriers typically exhibit some color change between their oxygenated and deoxygenated forms that is due to interaction between the porphyrin ring and the metal present (Prosser, 1973). For this reason they are termed respiratory pigments.

The function and properties of vertebrate hemoglobin discussed above are subjects of interest for numerous reasons. Mammalian hemoglobin is valuable to the biology and biochemistry instructor as an easily available example of a protein that bonds ligands in a fashion similar to enzymes sensitive to regulatory signals. Also, respiratory pigments permit the high degree of activity characteristic of the vertebrates by fulfilling the gas exchange demands such activity generates.

The four protein subunits comprising hemoglobin generally occur as two each of α and β types. Each subunit has a heme unit consisting of iron in the ferrous (II) form bound in the center of a porphyrin group (Cameron, 1989). It is the heme group that bonds to oxygen. Each molecule of hemoglobin, therefore, can carry four molecules of oxygen. The affinity of a heme group for oxygen is determined by the shape and charge of the subunits near the heme
bonding sites. The charge is affected by the ionization of certain amino acid residues (Cameron, 1989). Hemoglobin also exhibits the property of cooperativity, meaning that as heme groups bond to oxygen, the affinities of the remaining groups for oxygen increase. As the first molecule of oxygen bonds, a shift occurs in the structure of the other subunits from the "tight" (T) to the "relaxed" (R) state, increasing their affinities for oxygen (Hochachka, 1984).

The affinity of hemoglobin for oxygen decreases in increasingly acidic conditions, a characteristic termed the Bohr effect. The Bohr effect involves C-terminal histidines of β chains, N-terminal valines of α chains, and other histidyl residues in both α and β chains (Hochachka, 1984). These residues are collectively termed the Bohr groups. When oxygen bonds to hemoglobin, the Bohr groups change conformation and release protons (Hochachka, 1984). With increasing levels of proton activity, these groups become charged without bonding to oxygen and form salt bridges within the hemoglobin molecule, stabilizing it in the deoxygenated state (Kilmartin, 1976).

Because hemoglobin performance changes in predictable ways in different acid environments, by "controlling" blood pH the body can "control" hemoglobin action. This is also a characteristic of enzymes, as the body maintains control over their actions as well. This control of hemoglobin and enzymes means that both must possess the proper affinities for their respective substrates at the proper time. The Michaelis-Menten enzyme constant (Km), the substrate concentration at which half is bound to the enzyme, is equivalent to the hemoglobin P50, the oxygen pressure at which half
the hemoglobin is in its oxygenated state. Furthermore, the oxygen bonding action of hemoglobin is well-described and similar to the bonding of enzymes to their respective substrates (Hochachka, 1984). Hemoglobin is therefore an excellent example of some qualities of enzyme function.

The dynamics of hemoglobin action are best seen on an oxygen equilibrium curve (OEC) where the percent saturation of hemoglobin is plotted against oxygen partial pressure. The $P_{50}$ and $n$, the constant of cooperativity between tetramer subunits, are quantitative characteristics which can be used to compare hemoglobins from different species under different conditions. The first OEC's were published in 1878 by Paul Bert (Cameron, 1989) and appeared as hyperbolic curves. The true sigmoidal nature of hemoglobin OEC's (the result of cooperativity between tetramers) was not discovered until 1904 by Bohr (Cameron, 1989), and the first quarter of the 20th century saw OEC's for many species being determined under various conditions (review ed. by Prosser, 1973). Many techniques for the determination of OEC's are available but with the exception of optical tonometry, most require more specialized equipment than is commonly available in the undergraduate laboratory (Wells and Weber, 1989). Construction of such a curve and determination of $P_{50}$, $n$, and the Bohr shift through the technique of optical tonometry would provide hands-on learning suitable for an undergraduate physiology or biochemistry course if it could be completed in an afternoon. One object of this project was to determine the feasibility of such an idea.
A comparison of $P_{50}$, n, and the Bohr shift in different but similar species would potentially reveal similarities of hemoglobin action due to similar hemoglobin structure. Deer mice (*Peromyscus leucopus*) and meadow voles (*Microtus pennsylvanicus*) are plentiful and easily held in captivity, permitting the use of freshly drawn blood. Both are non-hibernating rodents of similar size and activity, although meadow voles frequently dig burrows and therefore lead a more fossorial existence than deer mice (Gunderson, 1953). OEC's for each species were expected to reveal the classic sigmoidal shape and Bohr shift between curves constructed at pH 6.8 and pH 7.4, although the meadow vole's fossorial habit suggests that its hemoglobin might maintain a higher affinity for oxygen at both pH levels. This is because mammal burrows commonly have higher concentrations of CO$_2$ and lower concentrations of O$_2$ in comparison to outside air. CO$_2$ may account for as much as 6% of the gas present and O$_2$ for as little as 14% in a rodent burrow (Boggs et al., 1984). Tunnels in snow, however, do not normally collect significant amounts of CO$_2$ (Korhonen, 1980). Maintaining a high hemoglobin-oxygen affinity would aid the meadow vole in loading sufficient oxygen at the lungs despite the oxygen-poor environment of the burrow. Hemoglobin with low $P_{50}$ is present in numerous species of fossorial mammals (Boggs et al., 1984).

**METHODS AND MATERIALS**

Optical tonometry was chosen as the method for constructing the OEC's. The system was based on that described by Hoar (1983). A water aspirator was connected to a five-gallon glass carboy immersed in a constant temperature water bath (Blue M Electric Co.,
Blue Island, IL). The carboy contained approximately two gallons of dH₂O to function as a wet compensation chamber for sudden pressure changes. This was in turn connected via a Y-connector to a one-meter mercury manometer and two tonometer-cuvettes via another Y-connector (Figure 1). A three-way stopcock was inserted in the line between a water aspirator and the carboy to allow the system to be opened to the atmosphere. Vacuum tubing and pipe clamps were used for all connections.

The tonometer-cuvettes were each constructed from a 60 ml glass separatory funnel and a 15 x 125 mm glass test tube (Figure 2). The test tube was wrapped on the outside at the open end with either teflon tape or parafilm and duct tape, and a light coating of vacuum grease applied. This end of the test tube was then inserted tightly into the top of the separatory funnel. Each tonometer-cuvette was clamped to a ring stand and positioned so that the bulb of the separatory funnel lay almost horizontal and just barely immersed in the water bath. The ring stands were connected to the water bath agitator by a length of wire, and with the agitator set at full speed and short duration, agitation of the hemoglobin suspension was provided.

Eight specimens each of meadow voles (M. pennsylvanicus) and deer mice (P. leucopus) were live-trapped in September and October 1991 on the campus of St. John's University, Collegeville MN. The animals were housed indoors in individual cages with food (Purina Lab Chow) and water ad libitum and a 10L:14D photoperiod.

Animals were vasodilated with a heat lamp, and blood was collected by nicking or cutting the tail or, if this was unsuccessful, by
Figure 1. Schematic of the experimental apparatus. a) to water aspirator, b) three-way stopcock, c) five-gallon glass carboy, d) mercury manometer, e) two-way stopcocks built in to tonometers, f) to tonometers.

Figure 2. Close-up diagram of tonometer apparatus. The clear portion represents the 60 ml separatory funnel, the lightly shaded area (a) the 12 x 125 mm test tube, and the dark shaded area (b) the junction between the two. (c) represents the bulb of the separatory funnel, where the Hb solution was equilibrated, (d) the two-way stopcock, (e) the vacuum tubing and pipe clamp, and (f) towards the rest of the apparatus.
cardiac puncture after ether anesthesia. One to three specimens were bled at any one time in order to obtain approximately 0.25 ml whole blood.

Preparation of the hemoglobin solution was adapted from techniques described by Foreman (1954) and Hoar (1983). Blood was collected into centrifuge bullet tubes containing 0.7 ml heparinized physiological saline (0.15 M NaCl, 3.40 units heparin/ml). Blood from individuals of the same species was pooled and washed three times by centrifugation for 5 minutes at 600 rpm (Safeguard Model CT 1004/D, Clay-Adams Inc., New York) in heparinized saline at a total volume of 5.0 ml per wash. After the supernatant was removed the final time, the packed erythrocytes were lysed in 1.5 ml cold distilled H₂O. After centrifugation for 5 minutes at 800 rpm, the clear, red supernatant was removed and passed through a 0.45 μm filter (Micron Separations Inc., Westboro, MA). The concentrated hemoglobin solution was diluted to a total volume of 4.0 ml with Sørenson buffer (see Hoar, 1983) pH-adjusted with HCl to either 6.8 or 7.4.

The buffered Hb solution was added to the tonometer which was clamped in a nearly horizontal position to increase the surface area of the solution. While being agitated in the separatory funnel as described above, the solution was allowed to equilibrate in the water bath for 7 to 10 minutes.

Absorbance at 625 nm was measured with a visible-UV spectrophotometer (Spec 20D, Milton Roy Co.). The room lights were turned off when the observations were made, as the size of the cuvette and its connection to the separatory funnel prevented the
hood of the spectrophotometer from being closed. Open- and closed-end readings from the manometer were recorded and air pressure recorded as the difference between them. The first observation, with the system open to the atmosphere, was assumed to represent fully oxygenated hemoglobin. With the three-way stopcock closed to the atmosphere and both two-way stopcocks in the separatory funnels open, pressure was lowered in 20mm Hg increments and data were recorded at each point after equilibration until the maximum evacuation was reached. The reading at maximum evacuation was assumed to represent fully deoxygenated hemoglobin. Atmospheric pressure was recorded at intervals during the procedure from a mercury barometer.

Often at lower pressures the test tube would begin to be drawn into the funnel by the vacuum. If this occurred, the stopcock in each separatory funnel was closed and the test tubes and funnels separated. The parafilm seal on each tube was reconstructed as quickly as possible to limit the exposure of the hemoglobin solution to the atmosphere and the system restored to its original condition and pressure.

Three trials were completed for each species at each pH treatment. Two tonometers were used at once, so deer mouse hemoglobin and meadow vole hemoglobin was paired and treated identically in any one trial. Seventeen data points were recorded for a sample during a trial over a maximum PO₂ range of 20.5 KPa. The total time for a trial including blood collection, hemoglobin preparation, and data collection was about six hours.
PO$_2$ and %Hb-O$_2$ were calculated by the method described by Hoar (1983). PO$_2$ = (barometer reading - overall manometer reading - water vapor pressure) x 0.2094. This gave a value in mm Hg which was converted to KPa. The percent saturation of hemoglobin with oxygen (S) was S/100 = ($Ar - As$)/($Ar - Ao$), where $Ar$ is the absorbance for fully deoxygenated Hb, $As$ is the absorbance for partially deoxygenated Hb, and $Ao$ is the absorbance of fully oxygenated hemoglobin. The data were divided into oxygen pressure categories and averaged between the three treatments. The mean for each PO$_2$ category was plotted as %Hb-O$_2$ vs. PO$_2$ in Figures 3 and 4. Hill plots were also determined for each treatment with unaveraged data as described by Cameron (1989) and used by Chappell (1988), Ingermann et al (1991), and Nicol (1991) and displayed in Figures 5 and 6. Limits for log S/(100-S) of 1 and -1 were used instead of 25-75% saturation. P$_{50}$ for each treatment was calculated from best-fit lines of regression for each Hill plot as the x-intercept at y=0 and 95% confidence intervals determined by the technique described by Sokal and Rohlf (1969). The Bohr shift of each species' hemoglobin was determined as $\Delta$log P$_{50}$/($2 \times \Delta$pH) as described by Foreman (1954).

RESULTS

The lowest partial pressure of oxygen reached by the system was 0.092 KPa, equating to a total pressure of 0.440 KPa. The hemoglobin solution at high partial pressures of oxygen was lightly red and clear with low values of absorption at 625 nm. At lower PO$_2$, the solution was darker, almost purple, and exhibited higher values of absorption. The standard OEC revealed a rapid increase in
oxygenated hemoglobin at oxygen pressures up to 7 KPa. The OEC slope decreased as the solution approached full oxygenation. This trend is seen as a gradual decline in the slope of the OEC's in Figures 3 and 4. There is no corresponding decline in slope as the solution approached full deoxygenation. The overall shape of the OEC, then, is hyperbolic as opposed to sigmoidal.

A general right shift of the curve towards higher PO₂ was seen at lower pH, particularly when the P₅₀ of each species was compared between treatments. The extent of this Bohr shift between pH 7.4 and pH 6.8 was greater with meadow voles than with deer mice. The Bohr shift of meadow vole hemoglobin was 2.35 while that of deer mouse hemoglobin was 1.91. A slight right shift was also observed in some preliminary trials when the hemoglobin solution was left to stand for as little as two hours.

Table 1. Calculations from Hill Plot regression lines

<table>
<thead>
<tr>
<th></th>
<th>P₅₀</th>
<th>95% Confidence Interval Limits</th>
<th>Cooperativity Index (n)</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deer Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8</td>
<td>4.54 KPa</td>
<td>3.05 KPa to 6.76 KPa</td>
<td>1.60</td>
<td>7</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>2.67 KPa</td>
<td>1.80 KPa to 3.94 KPa</td>
<td>1.13</td>
<td>6</td>
</tr>
<tr>
<td><strong>Meadow Vole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8</td>
<td>6.16 KPa</td>
<td>4.25 KPa to 8.95 KPa</td>
<td>1.63</td>
<td>3</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>2.74 KPa</td>
<td>1.42 KPa to 5.25 KPa</td>
<td>1.11</td>
<td>3</td>
</tr>
</tbody>
</table>

Hill plots constructed for log S/(100-S) values falling between 1 and -1 contained from 36 to 40 measurements for each treatment. All four Hill plots revealed best-fit regression lines with a minimum correlation coefficient of 0.909. Deviation from the line of regression tended to occur at either extreme of the Hill plot. Figures 5 and 6
Figure 3. Deer mouse oxygen equilibrium curves from averaged data. The PO2 was separated into 1 KPa divisions (0.0-0.9, 1.0-1.9, 2.0-2.9 KPa, etc.) and the mean of the data for percent saturation falling in each category was plotted at the midpoint of the same division (0.5, 1.5, 2.5 KPa, etc.). Each interval bracketed 1 to 3 points. Sample size at pH 6.8, \((N_{6.8}) = 7\). \(N_{7.4} = 6\).
Figure 4. Meadow vole oxygen equilibrium curves from averaged data as for mouse OEC in Figure 3. The deviation from the hyperbolic curve at 4.5 KPa PO₂ is most likely the result of the occurrence of only one datum in the PO₂ interval. Each interval bracketed 1 to 3 points. Sample size for both treatments was 3.
Figure 5. Hill plots of deer mouse hemoglobin. Values of log S/(100-S) falling between 1 and -1 were plotted from the three trials at each pH value and best-fit lines of regression assigned by computer. The equation for the line at pH 6.8 is y = 1.6037x - 1.0537, R = 0.959. That for the line at pH 7.4 is y = 1.1288x - 0.48140, R = 0.964. The slope of each line, n, is the Hill or cooperativity constant for mouse hemoglobin under those conditions. The number of included observations for pH 6.8 is 36, and for pH 7.4 is 40.
Figure 6. Hill plots of meadow vole hemoglobin. Lines were determined in same manner as those in Figure 5 for deer mouse hemoglobin. The line at pH 6.8 can be described by 
\[ y = 1.6344x - 1.2910, \text{ } R = 0.964. \] 
The line at pH 7.4 can be described by 
\[ y = 1.1053x - 0.48311, \text{ } R = 0.909. \] 
The number of observations included for pH 6.8 is 39, and for pH 7.4 is 36.
Figure 7. Meadow vole OEC at pH 6.8. The data is the same as that found in the Hill plot for meadow vole hemoglobin at pH 6.8, but the y axis represents %Hb-O₂, not log S/(100-S). The number of observations is 39. The figure is an example of the failure to reach a plateau characteristic of all the OEC from both species at both treatments, as expressed in this manner. According to Klotz (1986), this indicates that the hemoglobin was never fully saturated.
show the Hill plot regression lines for deer mouse and meadow vole hemoglobin, respectively. Figure 7, the OEC of meadow vole hemoglobin at pH 6.8 expressed as %Hb-O₂ vs. log PO₂, is included for analysis of the validity of the Hill Plots.

Table 2. Summary of P₅₀ values of deer mice and meadow voles

<table>
<thead>
<tr>
<th></th>
<th>This study</th>
<th>Foreman, 1954</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer Mouse P₅₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8</td>
<td>4.54 KPa</td>
<td>4.43 KPa</td>
</tr>
<tr>
<td>pH 7.2</td>
<td>2.67</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meadow Vole P₅₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8</td>
<td>6.16</td>
<td>4.51</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>2.74</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 reveals for each treatment the P₅₀ calculated from the regression line equations with the limits of its corresponding 95% confidence interval. Also shown is the cooperativity constant for each treatment. Sample size indicates the total number of rodents bled for either pH condition. Three trials were still run at each condition for both species. The P₅₀ confidence intervals between pH 6.8 and pH 7.4 overlap for both species. Once again, the greater Bohr shift in meadow voles can be seen from the difference in P₅₀ 6.8 values, especially since both species displayed similar P₅₀ 7.4 values. Hemoglobin of both species showed a higher degree of cooperativity at pH 6.8 than pH 7.4. Table 2 shows a comparison between the P₅₀ values of this study and those reported by Foreman (1954).

DISCUSSION

The color change of the hemoglobin solution and the change in absorbancy at different PO₂ indicate that deoxygenation of
hemoglobin did occur at low PO₂. The hyperbolic OEC's generated from averaged data in Figures 3 and 4 resemble the classic sigmoidal curves as a whole, because traditionally the sigmoidal portion of the curve at low PO₂ is not greatly pronounced. The end result is that sigmoidal OEC's often appear hyperbolic at first glance. Perhaps the recording of more data at low PO₂ would result in finer resolution of the low end of the curve, permitting the sigmoidal shape presumably present to be displayed.

It is also possible that the hyperbolic shape may have been caused by a failure to truly deoxygenate hemoglobin and reach the lower, sigmoidal portion of the curve. Calculation of S was dependent on the assumptions that the hemoglobin solution at air pressure represented fully oxygenated hemoglobin, and that the hemoglobin was fully deoxygenated at the lowest PO₂ reached by the system. Hoar (1983) stated that the system should be able to reach a pressure of 0.400 KPa to fully deoxygenate the hemoglobin. The lowest pressure attained by this system was 0.440 KPa, so the hemoglobin was most likely never fully deoxygenated.

The solution also might have never been fully oxygenated. This conclusion is supported by Figure 7, which exhibits a trend found among the OEC's of both species at both treatments. According to Klotz (1986), the failure of all such curves to reach any kind of plateau on a plot of fractional saturation vs. log PO₂ indicates that the effector molecule, in this case hemoglobin, was never saturated with its ligand. In this situation, fitting the data to a straight line in the Hill plots may yield a high correlation coefficient, but calculations
made from such a regression will not be wholly accurate (Klotz, 1986).

If the hemoglobin was indeed never fully deoxygenated or oxygenated, then my representations of the OEC's were missing some part of the curve at either end. Perhaps these endpoints could be better established by bubbling nitrogen and oxygen through the solution to produce fully deoxygenated and oxygenated hemoglobin, respectively.

The shape of the curve may also be due to the use of hemoglobin solutions instead of whole blood. Kilmartin (1976) reported OEC's of both stripped hemoglobin and whole blood, and that of the former was shifted to the left and had a less pronounced sigmoidal nature. Even recent observers such as Chappell et al (1988) reported hyperbolic OEC's. Barcroft and Roberts (1910) compared the OEC's of dialyzed and undialyzed hemoglobin and found that the dialyzed hemoglobin had a pronounced hyperbolic shape compared to the undialyzed hemoglobin. My filtered hemoglobin solutions were probably similar to Barcroft and Robert's dialyzed solutions. It would seem that use of a fresh hemoglobin solution reduces the intervening effects of naturally present compounds (2,3-diphosphoglycerate or 2,3-DPG, for example) which would normally cause the curve to be more sigmoidal (Kilmartin, 1976). This would also affect the cooperativity of the hemoglobin in question as discussed below (Wells and Weber, 1989). In some preliminary trials, however, addition of 2,3-DPG in excess of values reported by Prosser (1973) to the hemoglobin solution did not appear to affect oxygen bonding.
The fact that the Hill plots used for calculation of $P_{50}$ and other hemoglobin parameters were linear over such a wide range is somewhat contradictory to Cameron (1989), who reported that Hill plots were usually linear over the range of 25-75% saturation, but gave a sample Hill plot that clearly showed the line of regression extending from a log $S/(100-S)$ of 1 to -1. In a study of penguin hemoglobin, Nicol (1991) reported data which were linear over a broader range and used 1 and -1 as his limits for log $S/(100-S)$. The data reported here were linear over this same range, so the limits used by Nicol (1991) were used as cutoffs for data instead of saturations between 25% and 75%.

As shown in Table 2, the $P_{50}$ values for deer mouse hemoglobin are only roughly comparable to the values reported by Foreman (1954). My values for deer mouse hemoglobin are similar at pH 6.8, but the equality of values at pH 7.2 and pH 7.4 does not indicate similar results between studies; such a shift in proton activity should have a profound impact on $P_{50}$. The values for meadow vole $P_{50}$ 6.8 differ radically as well. No value for meadow vole $P_{50}$ 7.2 was given, but it seems that the values found here differ most from those of Foreman (1954) at levels of higher acidity. Such differences are perhaps not too surprising, as Barcroft and Camis (1910) reported that the OEC of both hemoglobin and whole blood show considerable variation among observers.

The shift of the OEC’s to the right at low pH was consistent with the Bohr effect. The lowered affinity of the hemoglobin for oxygen under increasingly acidic conditions requires a higher $PO_2$ to saturate half the hemoglobin. The fact that the 95% confidence intervals for
the P_{50} values overlap certainly indicates that the shift could also be
due to chance.

The greater Bohr shift displayed by meadow vole hemoglobin
is contrary to what was expected as an adaptation for a semi-
fossorial existence. Boggs et al (1984) reported that mammals use a
variety of adaptations to improve the capability for maintaining
proper oxygen uptake including an increase in respiratory rate,
greater blood volume, erythrocyte count, or hemoglobin levels. A
high hemoglobin affinity for oxygen is indeed used as a strategy for
many mammals, but no information was available for meadow voles.
Present data suggest that meadow voles may have a higher oxygen-
bonding capacity via one of the above adaptations instead of a high
oxygen affinity. This is because at the same pH levels, meadow vole
hemoglobin displayed an equal or lower affinity for oxygen than
der deer mouse hemoglobin.

However, maintaining proper oxygen uptake in the lungs by
increasing affinity or capacity is not the only strategy for
maintaining sufficient gas exchange in oxygen-poor environments, as
oxygen unloading in the tissues is aided by a low oxygen affinity
(Schmidt-Nielsen, 1983). A high affinity of hemoglobin for oxygen at
the lungs and a low affinity at the tissues would be ideal for gas
exchange. The Bohr shift makes this possible. As the waste CO_{2} from
the tissues enters the blood, it would associate with water in the
blood to form the weak acid HCO_{3}, making the blood more acidic. In
response, hemoglobin would experience a drop in its oxygen affinity
due to the Bohr effect, thereby easing the task of oxygen unloading.
The greater the Bohr effect, the greater the decrease in affinity and
the greater the ease of oxygen unloading. If the difference in Bohr shift between meadow voles and deer mice could be shown as significant, this might well be the adaptation used by meadow voles to cope with the oxygen-poor environment in their burrows.

There is some precedence for the use of strategies aimed at different ends of the gas exchange cycle in response to oxygen-poor environments. The hemoglobin of many animals adapted to high altitude have an unusually high affinity for oxygen, aiding the loading of scarce oxygen in the lungs. Humans acclimated to high altitude, however, experience a decrease in the affinity of their hemoglobin for oxygen by accumulating 2,3-DPG, aiding the unloading of oxygen at the tissues (Schmidt-Nielsen, 1983).

The decrease in oxygen affinity observed after the solution was left to sit for even two hours was slight but present nonetheless. Its cause is unclear to me. If hemoglobin was being analyzed in terms of an erythrocyte suspension, one might conclude that ATP or 2,3-DPG accumulation was occurring for some reason, but these factors should have little effect on a hemoglobin solution. Another possibility is that hemoglobin did not equilibrate during the normal time allowed, and the amount of oxyhemoglobin present after sitting for two hours is the correct value. Yet another possibility is the dissociation of the hemoglobin molecule itself into its subunits, which could lower the affinity. However, this normally occurs at only at either very high or low pH (Harper, 1975). Finally, perhaps some of the heme iron molecules oxidized from the $2^+$ to the $3^+$ state. This methemoglobin would not react with oxygen (Eckert, 1988). Whatever the cause of
the right shift, the overall shape of the curve did not change in this instance and this trial was not included in the final analysis.

The index of cooperativity for both species was lower in both treatments than the normally reported value of 2-3 in mammals. According to Wells and Weber (1989), this may not be unexpected, as hemoglobin solutions lack much of the phosphate-hemoglobin interaction found in whole blood, thereby reducing the effects of cooperativity.

Overall, the system worked well and could be used in a classroom laboratory setting with few modifications. The equipment is relatively inexpensive and obtainable, the only exceptions being the large water bath, manometer, and glass carboy. A vacuum pump might generate lower pressures, but the water aspirator functions well and is very inexpensive. If an entire afternoon was not available, it would be beneficial to have the hemoglobin solution prepared immediately beforehand by the instructor or an assistant. Optical tonometry, if not the most sophisticated method for generating an OEC, is certainly well-tested and true and does not require an expensive and fragile oxygen electrode. As described by Foreman (1954), it permits the use of dilute hemoglobin solutions which are valuable when species with a low blood volume are being studied. Also, as the whole blood or erythrocytes of different species possess many different characteristics that may affect the OEC, the use of a hemoglobin solution prevents these factors from influencing the comparison of hemoglobin between species. Wells and Weber (1989) recommend it highly for its precision and simplicity. The simplicity would also make it easier for students to understand the
mechanics of the system. The drawbacks include the length of time required and the necessity of using a hemoglobin solution, although this latter condition can be circumvented by using a dual-beam spectrophotometer to compensate for the characteristic light-scattering of erythrocyte suspensions. The use of dilute hemoglobin solutions also makes it difficult to draw connections to the true physiology of the organism in question, but the mechanics of hemoglobin action can be displayed quite well.

REFERENCES


