The Function of the Cellular Hemoglobin in the Arcid Clam Noetia ponderosa (Say)

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THE FUNCTION OF THE CELLULAR HEMOGLOBIN
IN THE ARCID CLAM NOETIA PONDEROSA (SAY)

A Thesis
Presented to
The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Marvin Alan Freadman
1974
APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Arts

Author

Approved, May 1974

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I wish to express my sincere gratitude and appreciation to Dr. Charlotte P. Mangum for her guidance, enthusiasm, and inspiration throughout this study. I am also grateful to Drs. Martin C. Mathes and Stewart A. Ware for their careful reading and critical review of the manuscript. Special thanks are also extended to the Virginia Institute of Marine Science Eastern Shore Laboratory staff for assistance in the collection of animals, to Dr. Carl W. Vermeulen for aid in writing computer programs used in this study, to Dr. Norman J. Fashing for statistical advice, and to Dr. Robert E. L. Black for willing and helpful discussion. I also wish to thank several fellow graduate students whose efforts facilitated various aspects of this study: Lewis Deaton, Alan Weiland, and Larry Hirsch.
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Oxygen equilibrium properties of the cellular hemoglobin of the arcid clam *Noetia ponderosa* were studied in buffered suspensions of intact cells in the pH range 6.6-7.4 at 10 and 23°C. This pH range encompasses the *in vivo* pH measured in intact animals. The oxygen affinity of hemoglobin in intact cells differs appreciably from the value reported for extracts of the pigment at 23°C. The oxygen equilibrium curves are not completely linearized by the Hill transformation. Two phases can be detected, each with a significantly different Hill coefficient (n). The slope of the phase at lower oxygenation levels is less than that of the phase at higher oxygenation levels.

Curves of total oxygen content and internal partial pressures of oxygen of animals exposed to a wide range of oxygen conditions suggest that the cellular hemoglobin functions in oxygen transport at intermediate and high oxygen levels. The same measurements indicate that the blood from clams exposed to very low oxygen conditions contains appreciable amounts of oxygen.

Hemoglobin concentration in the blood is 3.49 g/m/100 ml. The molecular weight of the pigment estimated on a calibrated gel column appears to be 34,500, suggesting a dimeric hemoglobin.
THE FUNCTION OF THE CELLULAR HEMOGLOBIN
IN THE ARCID CLAM NOETIA PONDEROSA (SAY)
Protoheme proteins are found in all classes of molluscs except for the cephalopods and monoplacophorans. In the Lamellibranchia, the pigment occurs in solution or in cells of the blood, and in some cases in muscle tissues as myoglobin. Although at least 21 species of bivalves possess the circulating hemoprotein, the function of bivalve hemoglobins is poorly known (Read, 1966). Previous work has resulted in many biochemical data but very little physiological data are currently available. Oxygen binding of extracts containing hemoglobin from 6 species has been studied and some extrapolations from these data to physiological function have been made (Manwell, 1963). Oxygen equilibrium studies by Kawamoto (1928) on hemoglobin extracts from the *Anadara inflata* cells indicated an oxygen affinity (P$_{50}$) of 10 mm Hg and a Hill coefficient (n) of 1.15 at 20°C at an unstated pH. Manwell (1963) reported a P$_{50}$ of 11 mm Hg at 22-24°C in the pH range 7.03-7.50 for the dissolved hemoglobin of *Cardita floridana*. The Hill coefficient was slightly less than 1. Manwell (1963) also investigated oxygen equilibrium properties of hemoglobin extracts from *Noetia ponderosa* cells and reported an oxygen affinity of 13.5 mm Hg at 22-24°C with no change in P$_{50}$ in the pH range 6.68-8.24. The Hill coefficient was 1.1. On the basis of the high oxygen affinity of the cellular hemoglobin of this clam, he postulated an oxygen storage function under conditions when ventilation of the gills ceases at low tide. However, the PO$_2$ of the blood under these conditions and
also those when ventilation is maximal is not known. It is possible that in vivo operating conditions are suitable for an oxygen transport function of arcid hemoglobin.

More recently, Collett and O'Gower (1972) studied oxygen equilibrium properties of hemoglobin extracts from cells of three other species of arcids, Anadara maculosa, A. trapezia, and A. granosa. These investigators reported that, in the temperature interval 15-20°C, the $P_{50}$ values for the hemoglobin of all three species decrease with increasing temperature, which is the expected result, but at 20-30°C, the curves shift to the left resulting in higher oxygen affinities. The authors offer no suggestions as to the physiological function of the pigment although they suggest that the properties of the pigment may represent a molecular adaptation to the available oxygen in the warm, marine environment of these animals.

The occurrence of the ponderous ark, Noetia ponderosa (Say), on the Eastern Shore of Virginia offered an excellent opportunity to investigate the role of the cellular hemoglobins in molluscs. The animal is a member of the primitive family Arcidae, many members of which are common along the Atlantic Coast. Noetia ponderosa is found from Virginia south to the Florida Keys (Abbott, 1966). It either burrows in sand, clay or mud in 2-3 meters of water, or occasionally it occurs intertidally. The objectives of the present study are to investigate oxygen equilibrium properties of Noetia ponderosa cellular hemoglobin in intact cells and to determine how in vivo oxygen content of the blood changes with internal and external $P_{O_2}$. 
MATERIALS AND METHODS

Animal collection

Specimens of Noetia ponderosa (Say) were collected from the waters near Wachapreague, Virginia, with an oyster dredge. Animals were returned to the laboratory in iced coolers and maintained at 17-19°C in natural seawater (29-32 o/oo salinity) which was vigorously aerated and recirculated through spun glass and charcoal filters. Clams were maintained in the laboratory for at least two weeks prior to use. During this period they were exposed to one of the experimental temperatures (10, 19, or 23°C) for at least two weeks prior to experimentation. Mortality shortly after collection was about 15% and surviving clams lived for several months without apparent deleterious effects.

Gel filtration

Hemoglobin containing cells were removed from the heart and washed in seawater three times; no lysis was detected during the washing. The hemoglobin was extracted directly from the cells into KH$_2$PO$_4$ : Na$_2$HPO$_4$ buffer (pH 7.4; ionic strength 0.2). The approximate concentration of hemoglobin in 5 preparations ranged from 0.5-3.0 gm/100 ml. The resulting solution was centrifuged until clear and an aliquot (1.0 ml) was applied to a column of Sephadex G-100 (0.9 x 60 cm) equilibrated at 5°C with buffer. Void volume was determined with Blue Dextran 2000 (Pharmacia Chemicals) and the column calibrated with 1 ml volumes of 0.37% cytochrome a (Sigma
Chemical Co.), 0.15% sperm whale myoglobin (Sigma Chemical Co.), and 0.15% recrystallized bovine hemoglobin (Sigma Chemical Co.). In addition, coelomic cell hemoglobin from the annelid bloodworm *Glycera dibranchiata* was extracted in the same manner as above and applied to the column.

**Absorption bands**

A Beckman DK-2A spectrophotometer was used to determine the absorption spectra of hemoglobin solutions. Oxyhemoglobin, deoxyhemoglobin and carbon monoxyhemoglobin bands were also located with a Hartridge Reversion Spectroscope.

**Hematocrit and hemoglobin concentration**

Undiluted samples of blood were withdrawn from the heart of clams and the sample centrifuged until no further compaction took place, according to the method of Wintrobe (Miller, 1952). The percent of the total volume of blood composed of hemoglobin containing cells was calculated.

Hemoglobin concentration was estimated by converting the substance present in whole blood to the cyanomethemoglobin derivative according to Tietz (1970). The oxygen combining capacity was calculated on the assumption that 1.36 ml of oxygen combine with 1 gm of hemoglobin (Miller, 1952).

**Total oxygen content in intact animals and oxygen carrying capacity**

The total oxygen content of the blood of animals exposed to various external PO$_2$'s was measured using a method adapted from Scholander et al. (1955) and Roughton and Scholander (1943). Since the shell of an animal harbors sufficient microbiota to decrease the
oxygen content of 4 l of the medium by more than 10% during the equilibrium period of 11-12 hours, animals were induced to close, quickly dipped in low melting point paraffin wax and immediately placed in iced seawater (4°C), hardening the wax and cooling the animal. Like two other species of shelled molluscs (Kushins and Magnum, 1971; Magnum and Burnett, 1974), none of the 4 l animals subjected to this treatment suffered mortality during 21 days of observation.

Each clam was placed in a magnetically stirred, 4 l vessel of known oxygen content and allowed to equilibrate in the dark at 19°C for 11-12 hours. No appreciable decrease (less than 10%) in oxygen content occurred as long as the wax did not slough off the shell. A Yellow Springs Instrument Co. Model 5420 oxygen probe was used at the beginning and end of the equilibration period to measure the oxygen content of the seawater prepared by flushing previously air-saturated water with washed nitrogen gas (Matheson Gas Co.).

At the end of the equilibration period, the animal was placed under mineral oil and forced open at the umbo region to expose the heart. A 0.2 ml blood sample was removed from the heart with a No. 23 gauge needle attached to a Scholander et al. (1955) extractor syringe which had been previously flushed and filled with fresh potassium ferricyanide. The oxygen content of the gas bubble thus elicited was analyzed according to Scholander et al. (1955).

To determine the oxygen carrying capacity of the blood, samples were withdrawn from the hearts of 11 clams and equilibrated with the atmosphere in magnetically stirred, parafilm covered flasks for 30-45 minutes. The total oxygen content was determined volumetrically as
above.

**Blood PO$_2$ and pH**

The PO$_2$ of blood samples procured anaerobically from the hearts of intact clams exposed at 23°C to a wide range of external PO$_2$'s was measured with a Radiometer PO$_2$ electrode. For blood pH, animals were exposed to either 10 or 23°C for at least two weeks and the pH of blood samples procured anaerobically from the heart was measured with a Radiometer BMS 1 blood gas system equipped with Acid-Base Analyzer PHM71.

**Cell respiration**

VO$_2$ or the rate of oxygen consumption of the cell suspensions used in oxygen release experiments was calculated from that part of the recorder trace where oxygen depletion remains constant (above 70 mm Hg).

**Oxygen release by cell suspensions**

Hemoglobin containing cells were obtained from clams exposed to the experimental temperature for at least two weeks. Cells were prepared by washing three times in seawater, centrifuged and the pellet was then resuspended in 0.05 M Bis-Tris buffer (Sigma Co.). The pH of the buffer was measured at the experimental temperature with a Severinghaus liquid junction electrode (Radiometer Model G298A).

The Hill constants $P_{50}$ and $n$ for hemoglobin in intact cells were estimated from the release of hemoglobin bound oxygen in a buffered suspension of aerobically respiring cells (Colman and Longmuir, 1963). Measurements were made at 10 and 23°C in the pH range of 6.6-7.4. Cell suspensions (4.66-8.66 ml cells/100 ml buffer) were emptied into a magnetically stirred vessel and the rate of oxygen
depletion monitored at 10 and 23°C with a Yellow Springs oxygen probe. Mechanical agitation of the cells did not result in any detectable hemolysis. The pH of the cell suspension was measured before and after a determination. No change occurred unless the experiment was allowed to continue 15-30 minutes after the PO2 reached the expected endpoint. Data from replicate experiments were pooled and the results analyzed in terms of the Hill equation. A log-log regression line was constructed for the pooled data at a particular pH and temperature as well as pooled data from all measurements at a particular temperature.
RESULTS

Gel filtration

The elution of *Noetia ponderosa* hemoglobin on Sephadex G-100 is shown in Fig. 1. The hemoprotein was eluted shortly after bovine hemoglobin and before sperm whale myoglobin, suggesting a dimeric hemoglobin with a molecular weight of 34,500. When coelomic cell hemoglobin from the bloodworm, *Glycera dibranchiata*, is treated in the same manner, two bands resulted and the molecular weights obtained resemble those previously reported by Mangum and Carhart (1972). Manwell (1963) found two bands for the hemoglobin of *N. ponderosa* using starch gel electrophoresis. More recently however, L. J. Parkhurst (personal communication to C. P. Magnum) also found two electrophoretic components; he also determined that the minimum molecular weight is 28,000 with values as high as 32,000. While Yagi et al. (1957) and Ui (1957) reported a molecular weight of 73,000 for the cellular hemoglobin of *Anadara inflata*, Svedberg and Hedenius (1934) and Svedberg and Pederson (1940) found a molecular weight of 33,600 for the hemoprotein of *Anadara ovalis*, which is similar to the present result.

During the course of various experiments in the present study, two cell types were observed but the very low rate of occurrence (6 of 70 preparations) suggests that the smaller cells observed were developmental stages of the larger cells.
Fig. 1. The elution of *N. ponderosa* hemoglobin on a column of Sephadex G-100.
Absorption spectra and bands

Spectrophotometric examination of hemoglobin solutions indicated that oxyhemoglobin absorbs at 541 m\(\mu\) and 578 m\(\mu\) as well as the Soret band. Carbon monoxyhemoglobin absorbs at 540 m\(\mu\) and 570 m\(\mu\) while deoxyhemoglobin absorbs at 560 m\(\mu\). Spectroscopic examination (Hartridge Reversion Spectroscope) indicated oxyhemoglobin absorbs at 538.7 m\(\mu\) and 572.2 m\(\mu\) in addition to the Soret band. Carbon monoxyhemoglobin absorbs at 535 m\(\mu\) and 571.3 m\(\mu\) while deoxyhemoglobin absorbs at 556 m\(\mu\). It is very interesting to note that intact cells invariably appear dark red and the various states of the hemoglobin (oxy, deoxy) are very difficult to discern from visual observation. When the cells are lysed, the states of ligand combination in the resulting hemoglobin solution are easily differentiated.

Hematocrit and hemoglobin concentration

Hematocrit for thirteen animals is 8.11 (± 0.51 S.E.) ml cells/100 ml blood while the range is 6.30-11.9 vol per cent. The mean value is higher than that reported for Anadara inflata, which is 6.5 vol per cent (Nicol, 1966).

Hemoglobin concentration is more variable; the mean is 3.49 (± 0.23 S.E.) gm/100 ml (N = 12) while the range is 2.42-5.59. The predicted oxygen carrying capacity of the blood is 5.21 (± 0.29 S.E.) ml O\(_2\)/100 ml blood (N = 12), and the range is 3.78-7.55. While the hematocrit is low, the high concentration of hemoglobin indicates that there is a high hemoglobin concentration in each cell, relative to other invertebrate red cells (Hoffman and Mangum, 1970; Mangum et.al., 1974).
Total oxygen content of blood in whole animals

The total oxygen content of blood withdrawn from the hearts of intact animals equilibrated to various external PO$_2$'s is shown in Fig. 2. When animals are equilibrated to 155 mm Hg, the total oxygen content in vivo is 3.54 (± 0.096 S.E.) ml O$_2$/100 ml blood, which is significantly lower (P < 0.05) than the oxygen carrying capacity of blood equilibrated directly to the atmosphere. This result indicates that either the hemoglobin is never completely oxygenated in vivo at 19°C, or that afferent and efferent fluids are mixed in the heart. This data may also have been influenced by the fact that the procedure for total oxygen content analysis required about 5 minutes from entry of sample into the syringe until the measurement was completed.

The total oxygen content of the blood declines fairly rapidly at high and intermediate external PO$_2$'s. At oxygen partial pressures below 70 mm Hg, total oxygen content does not change appreciably. Blood taken from 7 clams exposed to very low oxygen tensions, 6-8 mm Hg, still contains 0.244 (± 0.040 S.E.) vol per cent which is significantly (P < 0.05) higher than zero.

Oxygen carrying capacity

The oxygen carrying capacity of undiluted blood equilibrated to the atmosphere is 4.53 (± 0.16 S.E.) ml O$_2$/100 ml blood, including both hemoglobin bound and dissolved oxygen (N = 11). This figure is not significantly different (P > 0.05) from that predicted by hemoglobin concentration plus dissolved O$_2$.

Blood PO$_2$

The PO$_2$ of blood from the hearts of animals exposed to a wide
Fig. 2. The change (± S.E.) in total oxygen content of blood from the heart (circles, solid line) at 19°C, and the change (± S.E.) in heart blood P0₂ (squares, dashed line) at 23°C in clams equilibrated to various external P0₂'s.
range of external PO\textsubscript{2}'s is shown in Fig. 2. The curve describing the change in blood PO\textsubscript{2} with external PO\textsubscript{2} has the same shape as the curve for total oxygen content, despite the small (4°C) temperature difference in the equilibration medium. The same result, which is expected from the causal relationship between the two parameters, was reported for annelids and a sipunculid (Mangum and Van Winkle, 1973; Mangum and Kondon, 1974; Mangum et al., 1974). Total oxygen content and internal PO\textsubscript{2} decrease most rapidly at intermediate and high external oxygen levels, and lower oxygen levels cause little further alteration.

**Blood pH**

The pH of anaerobically procured samples of blood from the heart at 10°C is 7.321 (+ 0.021 S.E.) (N = 20) while the value at 23°C is 7.019 (+ 0.025 S.E.) (N = 20). These results show the predicted physical effects of temperature on ionization in that ΔpH/°C does not differ significantly (P > 0.05) from the slope of the curve describing the change in pH with temperature (Howell et al., 1973). At each temperature however, the values do not differ significantly from neutrality. Therefore they do not fit the model of relative alkalinity. The values are less alkaline than those obtained previously (Mangum, 1973) on animals measured only one week after collection in the summer.

**Cell respiration**

Cell respiration at 23°C, pH 7.4 and above 70 mm Hg is 233.17 (+ 24.8 S.E.) (N = 20) μl/hr dry wt. and at 10°C the value at pH 7.4 and above 70 mm Hg is 108.07 (+ 4.16 S.E.) (N = 20) μl/hr dry wt. The value at 23°C is nearly twice as great as that for mammalian erythrocytes and slightly lower than coelomic cells of G. dibranchiata.
Cell characterization

The hemoglobin containing cells (observed with a Zeiss II Photomicroscope) are fairly uniform in shape, size and color (Fig. 3). The cells are biconvex discs about 17 μm in diameter, and they are invariably mononucleate. There are occasional granular inclusions in the cytoplasm which appear yellow-brown. These observations resemble those made by Dawson (1933; cited by Read, 1966) on the hemoglobin containing cells of *Anadara transversa*.

Oxygen release by hemoglobin cell suspensions

When the Hill transformation is used to describe the data for oxygen release by cell suspensions (Fig. 4, Table 1), the data are not linearized by this procedure. Two phases can be distinguished, each having a significantly different slope with the slope of the first phase less than that of the second phase. At all pH's and both temperatures, the very high coefficients of determination ($r^2$) reflect the close fit of the points to the regression lines. Therefore, the slopes are most accurately estimated by the regression coefficient ($b$) for each phase.

At 10°C and in the pH range 6.6-7.4, values for $n_2$ and $n_1$ are homogeneous ($P > 0.05$, F test). For the combined data, the change in slope occurs at 46% oxygenation. There is no significant change in $P_{50}$ with pH; for the combined data at 10°C, the oxygen affinity is 5.30 mm Hg (Table 1), which indicates an intermediate oxygen affinity relative to other invertebrate hemoglobins.

At 23°C and in the pH range 6.6-7.4, values for $n_2$ and $n_1$ are homogeneous. The change in slope occurs at 50% oxygenation. The values
Fig. 3. The hemoglobin containing cells of *N. ponderosa*.

X 2000.
Fig. 4. Regression lines describing oxygen release for buffered suspensions of hemoglobin containing cells from *N. ponderosa* at 10 and 23°C. \( Y = \) per cent saturation with oxygen. Shaded areas are 95% confidence belts.
Table 1. Regression analysis of oxygen release by cell suspensions from Noetia ponderosa at 10 and 23°C.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>No.</th>
<th>PH</th>
<th>Hill constant</th>
<th>95% Confid. Interval</th>
<th>L₂</th>
<th>95% Confid. Interval</th>
<th>L₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>27</td>
<td>6.61-7.39</td>
<td>1.36</td>
<td>1.169, 1.604</td>
<td>5.05</td>
<td>0.905</td>
<td>0.892</td>
</tr>
<tr>
<td>23</td>
<td>32</td>
<td>6.61-7.39</td>
<td>1.43</td>
<td>1.091, 1.734</td>
<td>6.25</td>
<td>0.929</td>
<td>0.869</td>
</tr>
</tbody>
</table>
for $P_{50}$ in the pH range 6.6-7.4 are also homogeneous and the combined data at 23°C indicate an oxygen affinity of 6.25 mm Hg, only 1 mm Hg greater ($P < 0.05$) than the value at 10°C. Collett and O'Gower (1972) found that the $P_{50}$ of hemoglobin extracts from *Anadara granosa* at 25°C and pH 7.5 is 4.37 mm Hg while the value at 10°C is 3.42 mm Hg and even at 35°C, is 2.85 mm Hg. Their results for extracts from *A. maculosa* and *A. trapezia* also show the same trend; very similar oxygen affinities at different temperatures. While the $P_{50}$ values decrease at lower temperatures, they also decrease at higher temperatures, a result which has not been found for other hemoglobins. Using the values of $P_{50}$ at 10 and 23°C, the heat of oxygenation of hemoglobin in cells of *N. ponderosa* is -2.12 kcal/mole. Collett and O'Gower (1972) found that for extracts from *A. granosa*, $\Delta H$ between 10 and 20°C is -8 kcal/mole while the value between 25 and 35°C is +10 kcal/mole. These authors note that the hemoglobin may denature at higher temperatures, which would explain the anomalous result; in their description of methods however, they state that denaturation and methemoglobin formation were limited to less than 6%.

Table 2 gives the values for $P_{100}$, or the lowest $P_{O_2}$ at which the hemoglobin is 100% oxygenated. At 23°C, $P_{100}$ is 55.0 ($\pm$ 1.3 S.E.) mm Hg ($N = 21$) while the value at 10°C is significantly ($P < 0.05$) reduced to 31.0 ($\pm$ 1.2 S.E.) mm Hg ($N = 23$).
Table 2. PO$_2$ of $P_{100}$ or the lowest PO$_2$ at which the hemoglobin is 100% oxygenated.

<table>
<thead>
<tr>
<th>Temp. ($^\circ$C)</th>
<th>$P_{100}$ (mm Hg)</th>
<th>$\pm$ S.E. of $\bar{X}$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.0</td>
<td>1.2</td>
<td>23</td>
</tr>
<tr>
<td>23</td>
<td>55.0</td>
<td>1.3</td>
<td>21</td>
</tr>
</tbody>
</table>
DISCUSSION

The ponderous ark, *Noetia ponderosa*, contains large amounts of circulating cellular hemoglobin, 3.49 gm/100 ml. The high concentration suggests a role of considerable importance to the animal. Other values for hemoglobin concentration in bivalves are 1.9 gm/100 ml for *Anadara inflata* (Sato, 1931) and 1-2 gm/100 ml for *Cardita floridana* (Manwell, 1963).

When *N. ponderosa* is exposed to various external oxygen tensions, the curves relating external P02 with internal P02 and total oxygen content have two components: a rapid decrease at high and intermediate oxygen tensions and very little change at lower oxygen tensions below 70 mm Hg. Clearly the largest gradient between external and internal P02 exists at high and intermediate oxygen tensions. Interestingly, the measurements indicate that appreciable amounts of oxygen remain in animals exposed to very low oxygen conditions. This result, also true of several annelid species with cellular hemoglobins (Mangum and Carhart, 1972; Mangum et al., 1974), may reflect an adaptation that maintains the integrity of the cells under lower oxygen conditions. When exposed to anoxia *in vitro* for periods longer than 15-30 minutes, the cells lyse. When the intact animal is exposed to low external oxygen levels, a compensatory response must result in the maintenance of small volumes of oxygen in body fluids. While we have no data on the effects of low oxygen conditions on circulation, a circulatory response such as slowing down of heart rate might be a likely possibility.
Also, the possibility exists that the animal may switch off aerobic metabolism. Below external PO$_2$'s of 79 mm Hg the magnitude of the PO$_2$ gradient is very small and it seems unlikely that the hemoglobin functions in oxygen transport. Fig. 2 indicates most of the pigment is oxygenated at intermediate and high oxygen tensions and the magnitude of the PO$_2$ gradient at these oxygen tensions suggests that the hemoglobin functions in oxygen transport at these PO$_2$'s.

The transport function suggested by Fig. 2 is consistent with unpublished data on oxygen uptake with intact _N. ponderosa_ with and without functional hemoglobin (Deaton and Mangum, unpublished data). Also, the internal PO$_2$'s found in the present study indicate that the hemoglobin can be fully oxygenated _in vivo_ when ventilation is maximal even though the total oxygen content of animals exposed to near air-saturated oxygen levels is significantly lower than the oxygen carrying capacity of the blood. While we have no data on the oxygen content of afferent blood, blood in the heart may be as oxygenated as the tissues ever experience since it consists largely of oxygenated blood returning from the gills. However it is mixed with small amounts of blood returning from the excretory organ (Robb, 1965).

The occurrence of phase shifts in oxygen equilibrium curves has been noted previously for various respiratory pigments (Antonini et.al., 1962; Mangum et.al., 1974; Weber, 1970) and the phenomena has recently been shown to occur in non-heme pigments also (Mangum and Kondon, 1974). While an explanation of the phenomena is beyond the scope of the present study, it may reflect only simple molecular properties of arcid hemoglobin; a similar change is shown by Collett and O'Gower (1972). No data were obtained for internal PO$_2$ and total oxygen content at
$10^\circ C$, but it seems likely that the hemoglobin does become fully oxygenated at this temperature since the $P_{100}$ is much lower than at $23^\circ C$.

Although most investigations of gas exchange in lamellibranchs assume that oxygen uptake is essentially restricted to the gills (Ghiretti, 1966), gas exchange in *N. ponderosa* may also be accomplished by the mantle and foot since these organs contain appreciable amounts of blood. These organs pulsate and blood cells flow rapidly through them. When the shell gapes slightly to permit water movement across the gills, the mantle and foot are also exposed to the current.

The relation between the oxygen affinity of *N. ponderosa* hemoglobin in solution and in intact cells can be portrayed from recent data. L. J. Parkhurst (personal communication to C. P. Mangum) found that the $P_{50}$ of the major electrophoretic component of *N. ponderosa* hemoglobin is 6.6 mm Hg while the oxygen affinity of a minor component is 4.2 mm Hg. Both results were obtained at pH 7.0 and $20^\circ C$. These results suggest that the oxygen equilibrium properties of *N. ponderosa* hemoglobin in cells and solution do not differ.

The major function of the cellular hemoglobin of *N. ponderosa* appears to be in oxygen transport at intermediate and high oxygen tensions that occur in the subtidal habitat of the animal. Although a subtidal population may be exposed to very low oxygen levels in the summer in estuaries, animals that are subtidal usually extract oxygen from oxygen levels near air-saturation.
SUMMARY

1. Oxygen equilibrium properties of the cellular hemoglobin of Noetia ponderosa were studied in buffered cell suspensions. Changes in total oxygen content of blood with internal and external PO$_2$ were measured in intact animals. Molecular weight of the hemoprotein was also estimated.

2. Oxygen equilibrium curves are not completely linearized by the Hill transformation in the pH range 6.6-7.4 at 10 and 23°C; two phases can be detected, each with significantly different slopes. Oxygen affinity at 10°C is 5.30 mm Hg while at 23°C the value is 6.25 mm Hg.

3. The curves describing changes in total oxygen content and internal PO$_2$, measured over a wide range of oxygen conditions, have two components. At intermediate and high PO$_2$'s, the pigment is believed to function in oxygen transport. Animals exposed to very low oxygen conditions contain appreciable amounts of oxygen in their blood.

4. The molecular weight of the hemoprotein estimated by elution on a calibrated gel column, appears to be 34,500, suggesting a dimeric hemoprotein.
REFERENCES


VITA

Marvin A. Freadman

Born in Pittsfield, Massachusetts on 18 August 1949. Graduated from Pittsfield High School in that city, June, 1967. Received B.A. from the University of Colorado, Boulder, Colorado, in December, 1971. Entered graduate school at William and Mary in September, 1972 and served as a Graduate Teaching Assistant. Currently a candidate for the degree Master of Arts in Biology.