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# Effects of CO<sub>2</sub> pH and temperature on Hb-O<sub>2</sub> affinity of muskrat blood

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EFFECTS OF CO<sub>2</sub>, pH, AND TEMPERATURE ON Hb-O<sub>2</sub> AFFINITY  
OF MUSKRAT BLOOD


By

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B. A., University of Washington, 1982

Presented in partial fulfillment of the requirements  
for the degree of  
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Approved by

  
Chairman, Board of Examiners

  
Dean, Graduate School

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Henwood, Daniel K., M.A., April 1986

Zoology

Effects of CO<sub>2</sub>, pH, and Temperature on Hb-O<sub>2</sub> Affinity of Muskrat Blood (27 pp.)

Director: Delbert L. Kilgore, Jr. *DWK*

The muskrat (Ondatra zibethicus), as a burrower and diver, naturally encounters extreme respiratory environments of O<sub>2</sub> and CO<sub>2</sub>. O<sub>2</sub> transport properties of the blood were examined to determine (1) if there is a specific CO<sub>2</sub> effect on blood O<sub>2</sub> affinity and (2) if the CO<sub>2</sub> ( $\phi_{CO_2}$ ) and fixed-acid ( $\phi_{AH}$ ) Bohr effects are saturation dependent. Six muskrats were used to produce in vitro blood O<sub>2</sub> equilibrium curves at varying levels of CO<sub>2</sub>, H<sup>+</sup>, or temperature. Hill coefficients ( $n_H$ ) between 15 and 85% saturation were highly linear with a mean  $n_H$  of 2.81 at 37°C.  $n_H$  at 35°C was significantly different from that at 37°C and 39°C with a value of 3.31. The mean  $\phi_{CO_2}$  and  $\phi_{AH}$  slopes at P<sub>50</sub> were -0.625 and -0.453, respectively. Neither varied significantly with O<sub>2</sub> saturation, although  $\phi_{CO_2}$  decreased with increasing saturation. Both the specific CO<sub>2</sub> effect and temperature coefficient (d log P<sub>O<sub>2</sub></sub>/d T) were saturation dependent, with values at P<sub>50</sub> of 0.190 and 0.0088, respectively. It is concluded that the high CO<sub>2</sub> Bohr factor and large specific CO<sub>2</sub> effect do not allow the muskrat to utilize it's lungs as an O<sub>2</sub> store during a dive but facilitates the unloading of O<sub>2</sub> at the tissues under these same conditions.

## PREFACE

I would like to thank all who helped me during this project. Special thanks go to Dr. Delbert L. Kilgore who provided much of his time, encouragement, and advise as my major advisor.

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## INTRODUCTION

THERE ARE A NUMBER OF ALLOSTERIC MODIFIERS of hemoglobin oxygen affinity. Included among these is  $\text{CO}_2$ , which reduces  $\text{O}_2$  binding to hemoglobin (7). This modification of blood  $\text{O}_2$  affinity by  $\text{CO}_2$  is known as the  $\text{CO}_2$  Bohr effect and has been shown to result from the combined effects of  $\text{CO}_2$  hydration on intraerythrocytic pH and the direct binding of  $\text{CO}_2$  to hemoglobin (31). Recently, attention has been focused on not only the  $\text{CO}_2$  Bohr effect, but also on the independent effects of pH on the oxygen affinity of hemoglobin (14, 15, 19, 29, 37). This fixed-acid Bohr effect is an indicator of the sensitivity of Hb- $\text{O}_2$  affinity to non-respiratory pH changes. From the  $\text{CO}_2$  and fixed-acid Bohr effects, the specific influence of molecular  $\text{CO}_2$  via carbamino formation on oxygen affinity can be determined (14). While this specific  $\text{CO}_2$  effect is negligible in some mammals [e.g., dogs (37)] it is somewhat more important in the grey seal, a diving species (20) and the burrow dwelling echidna (22). A reduced affinity due to carbamino formation would favor utilization of blood oxygen stores during a dive or during exposure to hypercapnic hypoxic burrow gas environments, while maintaining a substantial diffusion gradient for oxygen from the blood to the tissues.

The  $\text{CO}_2$  and fixed-acid Bohr effects have also been

shown to be saturation-dependent in some instances. In human blood, the CO<sub>2</sub> Bohr effect varies considerably with percent saturation of the hemoglobin (14). Lutz et al. (22) recently found that at elevated PCO<sub>2</sub> the fixed-acid Bohr effect in platypus blood declined markedly with decreasing O<sub>2</sub> saturation at values below 60%. This decrease in the pH dependent effect of CO<sub>2</sub> on Hb-O<sub>2</sub> affinity would most likely help in extraction of O<sub>2</sub> from the lungs when alveolar PO<sub>2</sub> and blood pH is reduced, conditions that might be expected during a dive. The fixed-acid and CO<sub>2</sub> Bohr effects and their saturation dependence may, therefore, profoundly influence oxygen transport.

I undertook the present study on the O<sub>2</sub> transport properties of the blood of muskrats (Ondatra zibethicus), a species that naturally encounters extreme respiratory environments and is also a diver, to determine (1) if there is a specific CO<sub>2</sub> effect on blood O<sub>2</sub> affinity and (2) if the CO<sub>2</sub> and fixed-acid Bohr effects are saturation dependent. During the winter, muskrats congregate in winter lodges where CO<sub>2</sub> levels routinely equal or exceed 3%, reaching a potential maximum of 10%, and where oxygen levels may decline, approaching 18% (26). Muskrats also regularly dive beneath the ice during the winter to forage on submerged vegetation, and distances to feeding shelters may exceed 100 m (23). During dives under these

conditions, body temperature may decline by 2°C (25). Cooling of the blood of these mammals may also potentially effect O<sub>2</sub> affinity of the blood. Because these mammals are exposed to extreme gaseous conditions in lodges, are divers, and experience fluctuations in body temperature, we might reasonably expect them to show adaptive variations in blood O<sub>2</sub> affinity characteristics.

## METHODS

Experimental animals. A total of 12 muskrats of both sexes (mean body mass of 1.058 ± 0.086 kg) were livetrapped during September and October 1984 along the Bitterroot River two kilometers south of Lolo, Missoula Co., Montana. Each muskrat was individually housed in a wire bottomed cage (40 x 50 x 30 cm) at 20 ± 2°C and under a fixed photoperiod (14L:10D) for a period of 30-60 days prior to the start of experiments. All muskrats were fed commercial rat chow supplemented daily with fresh carrots and provided water ad libitum. Adjustment to captivity was excellent; muskrats maintained their body mass and were active.

Blood collection and hematology. Six to 10 ml of whole blood were obtained by cardiac puncture from intact individual muskrats lightly anesthetized with ether. In all cases blood was drawn into heparinized syringes and put on ice until used.

Hemoglobin (Hb) concentrations in the blood samples were measured spectrophotometrically at 540 nm after conversion to cyanmethemoglobin (Sigma kit No. 525-A). Packed cell volumes (Hct) were obtained by the microhematocrit method (12,500 g for 10 minutes). Erythrocyte counts (RBC) were obtained from a Neubauer hemocytometer using a 1:200 dilution of blood in Hayem's solution (Unopette). Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were then calculated from the Hb, Hct, and RBC data. Oxygen carrying capacity of the blood was also calculated from hemoglobin concentration, assuming 1.0 g Hb binds with 1.34 ml of oxygen (10).

Blood gas analysis and oxygen equilibrium curves. Whole blood  $O_2$  affinity and acid-base status were evaluated using an open-circuit tonometry system similar to that described by Bjork and Hilty (5). Two 2.0 ml aliquots of whole blood were transferred to tonometers and equilibrated with humidified gas mixtures at 37°C for a period of 30 minutes. Preliminary tests indicated that at a gas flow rate of  $200 \text{ ml} \cdot \text{min}^{-1}$ , 30 minutes was sufficient for complete oxygenation or deoxygenation of the samples. The tonometers were constructed according to the design of Hall (13) but with smaller, 25 ml chambers. The gas flowing through one tonometer was composed of a pre-determined percentage of  $CO_2$  with the balance  $N_2$ ; gas

flowing through the second tonometer was composed of the same percentage CO<sub>2</sub>, 30% O<sub>2</sub>, and balance N<sub>2</sub>. The three-gas mixture was obtained from a gas mixing pump (Wosthoff, model 301 a/F). Following equilibration, aliquots of fully oxygenated and fully deoxygenated blood were mixed anaerobically following the method of Scheid and Meyer (39) to obtain various levels of oxygen saturation (S) needed to construct the multiple-point static O<sub>2</sub> equilibrium curves (O<sub>2</sub>EC). Accuracy of estimating blood volumes with this mixing technique was improved by weighing the glass syringes to within  $\pm 0.1$  mg before and after aspirating blood from the tonometers (39). These mixtures of oxygenated and deoxygenated blood were then analyzed for pH, PO<sub>2</sub>, and PCO<sub>2</sub> (Radiometer BMS3-Mk2). The PO<sub>2</sub> electrode of the blood gas analyzer was calibrated with liquid solutions prior to the determination of an O<sub>2</sub>EC. The calibration of the PO<sub>2</sub> and PCO<sub>2</sub> electrode was checked with certified gases before each blood sample was analyzed. The pH electrode was also calibrated with precision buffers (Radiometer). Unless otherwise noted, all blood gas measurements were made at 37°C, a temperature that conforms closely to the mean abdominal temperature of muskrats (24).

Each O<sub>2</sub>EC was derived from the PO<sub>2</sub> of seven oxy/deoxy mixtures. Percent saturations of these mixtures ranged from 0 to near 100% ("saturated"), inclusive, and were

evenly spaced. The Hill equation, relating  $\log (S/100-S)$  to  $\log PO_2$ , was then used to interpolate  $PO_2$  values at intermediate saturation levels between 15 and 85% (only those data points between 15 and 85% saturation were used in calculation of the Hill slope). Maginniss et al. (30) and Reeves et al. (37) have shown that the Hill relationship approximates  $O_2EC$  data of homozygous sheep and dog blood, respectively, between 15 and 85% saturation with a maximum error in  $PO_2$  of 2.0 Torr. The error is generally less than 0.5 Torr. Both homozygous sheep and dog blood exhibit one hemoglobin fraction, as does the muskrat (38).

Mean ( $\pm$  SD) barometric pressure was  $676.9 \pm 4.2$  Torr during these experiments.

Bohr factors. To obtain  $CO_2$  ( $\phi_{CO_2}$ ) and fixed-acid ( $\phi_{A_H}$ ) Bohr factors blood pH was varied by (1) addition of isotonic (0.15 N) lactic acid or NaOH to effect a shift in pH of 0.12 to 0.34 units from normal base excess (fixed-acid titration) or (2) varying  $CO_2$  concentrations in the equilibrating gas ( $CO_2$  titration). Lactic acid or NaOH was added to the plasma fraction of 4.0 ml of whole blood. The cells were resuspended before aliquots were added to the tonometers. Centrifugation of small samples of the titrated blood revealed no discernable signs of lysing.  $O_2ECs$  were determined at three different pHs at a constant  $CO_2$  of 5.5%.  $CO_2$  concentrations of 0.0, 3.0,



5.5, 8.0, and 12.0% in the equilibrating gases were used to produce a family of isocapnic  $O_2$ ECs at a constant base excess. In both experiments,  $\log PO_2$  taken from each  $O_2$ EC for a given saturation was then regressed on pH. The slope of the resulting regression line ( $d \log PO_2 / d \text{pH}$ ) was the  $\phi_{CO_2}$  or  $\phi_{AH}$ .

The specific  $CO_2$  effect on  $O_2$  affinity was calculated as the difference between the  $\phi_{CO_2}$  and  $\phi_{AH}$  divided by the buffer slope ( $d \log PCO_2 / d \text{pH}$ ) (14).

Temperature effects on Hb- $O_2$  affinity. To determine the effects of temperature on the oxygen affinity of muskrat blood,  $O_2$ ECs were measured under constant  $CO_2$  (5.5%) at 35 and 39°C. This 4°C thermal range approximates the body temperature fluctuations recorded in free-ranging muskrats (25). The temperature coefficient ( $d \log PO_2 / d T$ ) was then calculated for the blood of each muskrat.

Data analysis. Reported values are means  $\pm$  1 SEM, unless otherwise indicated. Regression lines were determined by the least squares method (44) and tested using analysis of variance. The saturation dependence of, and difference between Bohr factors were tested using a two-way fixed factor ANOVA. Means were compared using the appropriate t-test (44). A  $P \leq 0.05$  was considered significant in all statistical tests.

## RESULTS

The hematological characteristics, respiratory properties, and buffer values of muskrat blood appear in Tables 1 and 2. The  $P_{50}$ s of muskrat blood at a  $PCO_2$  of 40 Torr and at normal body temperature were consistently lower than those predicted on the basis of mass indicating a higher than expected Hb- $O_2$  affinity. The differences between predicted and observed  $P_{50}$  (at  $PCO_2$  equal 40) values ranged from 4.8 to 6.8 Torr. The mean  $P_{50}$ s at 35 and 39°C at  $PCO_2$  equal 40 Torr were 28.5 and 31.6 Torr, respectively.

For all  $O_2$ ECs the relationship between  $\log (S/100-S)$  and  $\log PO_2$  was highly linear ( $P < 0.01$ ;  $r^2 = 0.92$  to  $0.99$ ) over a saturation range of 15 to 85%. The mean slope of these relationships, the Hill coefficient ( $n_H$ ), was 2.81 for all  $CO_2$  and fixed-acid titration  $O_2$ ECs (Table 2). Since  $n_H$  did not vary with pH or  $PCO_2$  ( $P > 0.10$  and  $P > 0.25$ , respectively), nor was there a significant difference between the mean  $n_H$  values of  $O_2$ ECs at all levels of  $CO_2$  and pH ( $P > 0.50$ ),  $n_H$  values from all  $O_2$ ECs were combined. The Hill relationship at a blood temperature of 39°C was also linear ( $P < 0.005$ ;  $r^2 = 0.98$  to  $0.99$ ) with a mean slope of 2.84. The mean  $n_H$  of these  $O_2$ ECs were not significantly different from the combined mean at 37°C ( $P > 0.50$ ). The Hill relationships of  $O_2$ ECs

TABLE 1  
Hematological characteristics of muskrat blood

| n | Mass<br>(g)         | Hct<br>(%) | Hb<br>(g/100 ml) | RBC<br>( $10^6/\text{mm}^3$ ) | MCV<br>( $\mu\text{m}^3$ ) | MCH<br>(pg) | MCHC<br>(%) | Oxygen capacity<br>(vol %) |
|---|---------------------|------------|------------------|-------------------------------|----------------------------|-------------|-------------|----------------------------|
| 6 | 1058.2 <sup>*</sup> | 39.9       | 16.06            | 6.405                         | 62.25                      | 25.09       | 40.31       | 21.53                      |
|   | $\pm 86.1$          | $\pm 0.4$  | $\pm 0.21$       | $\pm 0.071$                   | $\pm 0.57$                 | $\pm 0.35$  | $\pm 0.35$  | $\pm 0.28$                 |
|   | (33) <sup>^</sup>   | (36)       | (24)             | (22)                          |                            |             |             |                            |

<sup>\*</sup> Mean  $\pm$  SEM

<sup>^</sup> Numbers in parentheses are total number of determinations for all six muskrats.

TABLE 2

Respiratory properties and buffer values of muskrat blood<sup>a</sup>

|   |                         |
|---|-------------------------|
| $P_{s_0}$ (7.4) <sup>b</sup> (Torr)                       | 33.7 ± 0.4 <sup>c</sup> |
| $P_{s_0}$ (40) <sup>d</sup> (Torr)                        | 28.6 ± 0.3              |
| Predicted $P_{s_0}$ <sup>e</sup> (Torr)                   | 34.6 ± 0.15             |
| Hill Coefficient ( $n_H$ )                                | 2.81 ± 0.03             |
| Åstrup slope <sup>f</sup> (d log PCO <sub>2</sub> /d pH)  | -1.308 ± 0.053          |
| Buffer capacity (d [HCO <sub>3</sub> <sup>-</sup> ]/d pH) | -26.0 ± 3.3             |
| Standard bicarbonate <sup>g</sup> (mM/L)                  | 33.1 ± 1.0              |

<sup>a</sup> All values are for blood at 37 C.

<sup>b</sup>  $P_{s_0}$  adjusted to a pH of 7.4 using appropriate  $\phi_{CO_2}$  factors.

<sup>c</sup> Mean ± SEM

<sup>d</sup>  $P_{s_0}$  adjusted to a PCO<sub>2</sub> of 40 Torr using  $\phi_{CO_2}$  and relationship between log PCO<sub>2</sub> and pH.

<sup>e</sup> Predicted for individual muskrats using allometric equation of Schmidt-Nielsen and Larimer (40).

<sup>f</sup> Åstrup slope of saturated (S<sub>1.0</sub>) blood.

<sup>g</sup> Calculated or determined at pH of 7.4.

determined at 35°C were also highly linear ( $P < 0.005$ ;  $r^2 = 0.98$  to  $0.99$ ) with a mean slope of 3.31. This latter mean is significantly greater than the mean Hill coefficient of  $O_2$ ECs determined at 39°C ( $P < 0.001$ ), and at 37°C ( $P < 0.001$ ).

The regression of  $\log PCO_2$  on pH from all  $O_2$ ECs obtained by  $CO_2$  titration over a pH range of 7.25 to 8.40 was highly linear ( $P < 0.005$ ;  $r^2 = 0.97$  to  $0.99$ ) yielding a mean slope (Åstrup buffer slope) for saturated blood of -1.308. The Åstrup slope for deoxygenated blood (-1.119) was significantly lower ( $P < 0.05$ ). The calculated buffer capacity ( $d [HCO_3^-]/d \text{pH}$ ), based on all  $O_2$ ECs obtained by  $CO_2$  titration, and the mean  $HCO_3^-$  concentration at a pH of 7.400 are reported in Table 2.

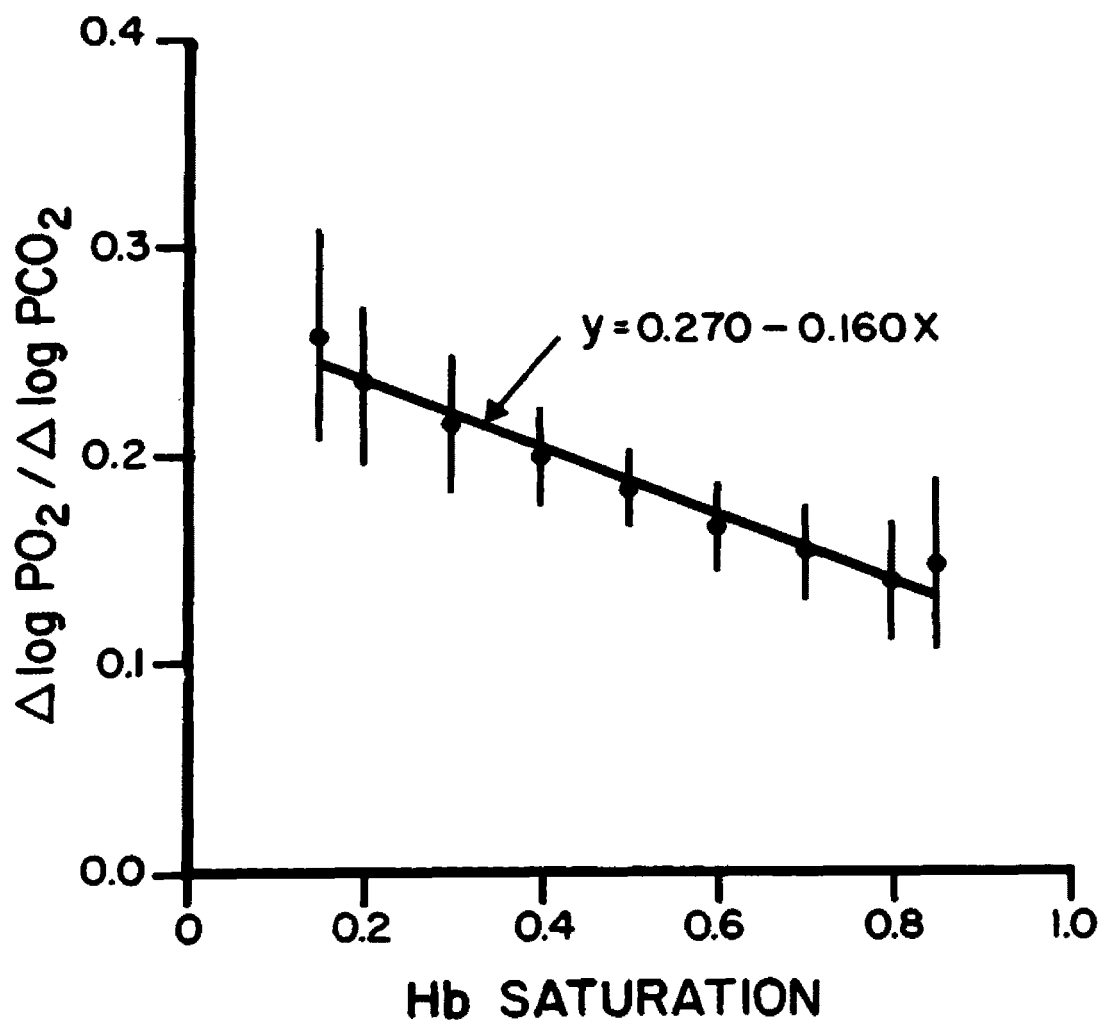
The mean  $\phi_{CO_2}$  and  $\phi_{AH}$  slopes at half saturation ( $d \log P_{50}/d \text{pH}$ ) were -0.625 and -0.453, respectively. Neither of these  $\phi_{CO_2}$  nor  $\phi_{AH}$  coefficients varied significantly with the level of oxygen saturation ( $p > 0.05$ ), although the  $\phi_{CO_2}$  slopes decreased with increasing saturation (Table 3). The fixed-acid Bohr factor was less than the  $\phi_{CO_2}$  at all levels of saturation, suggesting that there was a significant specific  $CO_2$  effect on Hb- $O_2$  affinity. These differences between the Bohr factors were statistically significant ( $P < 0.001$ ) at all levels of saturation and were saturation dependent (Fig. 1). The slope (-0.160) of the regression line relating the

TABLE 3  
Bohr and temperature coefficients of muskrat blood  
as a function of Hb saturation

| S    | $\phi_{CO_2}$       | $\phi_{AH}$        | TEMPERATURE<br>COEFFICIENT |
|------|---------------------|--------------------|----------------------------|
| 0.15 | -0.661 $\pm$ 0.054* | -0.477 $\pm$ 0.054 | 0.0014 $\pm$ 0.0009        |
| 0.20 | -0.635 $\pm$ 0.048  | -0.448 $\pm$ 0.049 | 0.0022 $\pm$ 0.0013        |
| 0.30 | -0.642 $\pm$ 0.039  | -0.450 $\pm$ 0.042 | 0.0044 $\pm$ 0.0017        |
| 0.40 | -0.634 $\pm$ 0.032  | -0.452 $\pm$ 0.037 | 0.0065 $\pm$ 0.0021        |
| 0.50 | -0.625 $\pm$ 0.028  | -0.453 $\pm$ 0.034 | 0.0088 $\pm$ 0.0022        |
| 0.60 | -0.617 $\pm$ 0.024  | -0.455 $\pm$ 0.033 | 0.0111 $\pm$ 0.0024        |
| 0.70 | -0.608 $\pm$ 0.024  | -0.456 $\pm$ 0.034 | 0.0136 $\pm$ 0.0027        |
| 0.80 | -0.597 $\pm$ 0.028  | -0.458 $\pm$ 0.039 | 0.0166 $\pm$ 0.0030        |
| 0.85 | -0.590 $\pm$ 0.032  | -0.442 $\pm$ 0.056 | 0.0186 $\pm$ 0.0033        |

\* Mean  $\pm$  SEM

Figure 1. Relationship between the specific CO<sub>2</sub> effect ( $d \log PO_2 / d \log PCO_2$ ) on Hb-O<sub>2</sub> affinity and hemoglobin saturation of muskrat blood. In the least squares regression equation included in this figure,  $y = d \log PO_2 / d \log PCO_2$  and  $x = S$ .





specific  $\text{CO}_2$  effect to  $S$  is significantly different from zero ( $P < 0.001$ ).

The temperature effects on  $\text{O}_2$  affinity ( $d \log \text{PO}_2 / d T$ ) over the  $4^\circ\text{C}$  change in blood temperature increased significantly ( $P < 0.001$ ) with increasing hemoglobin saturation. The temperature coefficient at  $P_{50}$  was 0.0088 and ranged from 0.0014 at 15% saturation to 0.0186 at 85% saturation (Table 3).

## DISCUSSION

Hematological and respiratory characteristics. The hematological characteristics of muskrat blood are within the normal range of values for other mammals, including divers and fossorial mammals (6, 27, 45) and are similar to those reported in other studies of muskrats (27, 38, 43).

The respiratory properties of muskrat blood reported herein are also generally within the range of such values reported by MacArthur (27), Rothstein (38), and Snyder and Binkley (43). However, the  $P_{50}$  of the blood of muskrats used in this study, both at pH 7.4 (33.7 Torr) and adjusted to a  $\text{PCO}_2$  of 40 (28.6 Torr) are somewhat higher than those values previously reported for this species. These within species differences may be attributable to the different times of year that experiments were performed or perhaps to the difference in methods used for

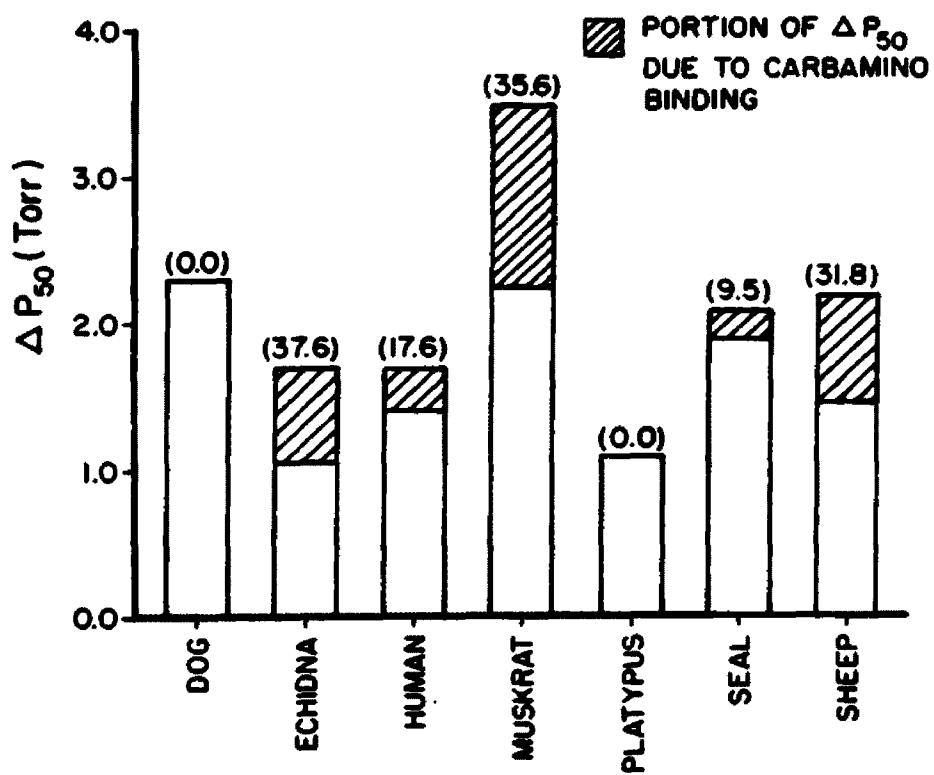
determination of specific parameters. MacArthur (27) has shown that both hematological and respiratory characteristics of muskrats vary seasonally. Nevertheless, muskrats do display a higher than predicted Hb-O<sub>2</sub> affinity. This higher O<sub>2</sub> affinity is due in part to a reduced level as well as reduced interaction of muskrat hemoglobin with 2,3-DPG (38, 41). Muskrats have a CO<sub>2</sub> Bohr effect at S<sub>0.5</sub> (Table 3; 27, 38, 43) that is close to the upper limit of the typical mammalian range of -0.390 to -0.620 (18). The buffer capacity and non-carbonic buffer strength (Åstrup slope) of muskrat blood compare well with those previously reported for muskrats (27, 38, 43). Standard bicarbonate of the blood of muskrats used in this study was higher than in previous studies on muskrats. Elevated HCO<sub>3</sub><sup>-</sup> values at a pH of 7.4 have been reported for other burrowing and diving species (8, 34, 35).

Saturation dependency of  $\phi_{CO_2}$  and  $\phi_{AH}$ . In muskrat blood, the CO<sub>2</sub> Bohr effect is oxylabile, decreasing at higher levels of Hb saturation (Table 3). The fixed-acid Bohr effect, however, is independent of Hb saturation. A saturation dependent  $\phi_{CO_2}$  has also been reported in some mammals, notably in pregnant sheep (15) and in humans (14) and in other vertebrates, for instance, frogs (28) and green and loggerhead turtles (19). However, both  $\phi_{CO_2}$  and  $\phi_{AH}$  have been found to be reasonably saturation

independent in the grey seal (20), the dog (37), and fetal sheep (15). Physiologically, a saturation dependent Bohr effect could have significant effects on  $O_2$  transport in muskrats as it does in other divers. In sea turtles, for instance, both  $\phi_{CO_2}$  and  $\phi_{AH}$  are saturation dependent, being very low at low levels of Hb-saturation and increasing markedly at elevated saturations (19). During sustained dives, when  $H^+$  and  $PCO_2$  rise and Hb saturation declines the low Bohr factors would act to facilitate oxygen loading at the lung by keeping oxygen affinity high.

Oxylabile carbamino  $CO_2$  binding. There is a substantial specific  $CO_2$  (carbamino) effect on blood  $O_2$  affinity in muskrats that is also saturation dependent (Fig. 1). Among mammals, adult sheep (15) and echidna (22) both exhibit a substantial carbamino  $CO_2$  effect (Fig. 2), while carbamate formation has only a modest effect on  $O_2$  affinity of human blood (14) or that of grey seals (20). Several other mammals (Fig. 2) show no specific  $CO_2$  effect, including the dog (37) and duck-billed platypus (22). Within other vertebrate groups, there is likewise considerable variation in the specific effect of  $CO_2$  on Hb- $O_2$  affinity. For instance, in the blood of the house sparrow there is a modest carbamino effect on Hb- $O_2$  binding (29), while the muscovy duck (32), domestic chicken (21), and burrowing owl (Maginniss, Kilgore, and

Figure 2. Change in the affinity ( $P_{50}$ ) of the blood of various mammals that would result from a 10 Torr increase in  $PCO_2$ . Cross hatched area is that portion of the total  $PO_2$  shift due to carbamino  $CO_2$  formation (i.e., the specific  $CO_2$  effect). The total change in  $P_{50}$  was determined by first calculating the change in pH that would result from a 10 Torr increase in the reported  $PCO_2$  at  $P_{50}$  using the Åstrup equation. The change in pH was then used to calculate  $d \log PO_2$  using the appropriate  $\phi_{CO_2}$  factor. The portion of the total change in  $P_{50}$  due to the specific  $CO_2$  effect was determined from the following equation:  $d \log PO_2 = d \log PCO_2 * (d \log PO_2 / d \log PCO_2)$ . Calculations for each species are based on data from the following sources: Dog (37); echidna (22, 35); human (14); muskrat (this study); platypus (22, 34); seal (20); and sheep (15).



Szewczak, unpubl. data) display little or no specific  $\text{CO}_2$  effect. The  $\phi_{\text{CO}_2}$  and  $\phi_{\text{AH}}$  factors are different in the blood of some reptiles (19), but not in fishes (12) and frogs (47). In human, sheep, muskrat, and sparrow blood carbamino  $\text{CO}_2$  binding is saturation dependent; in all cases the specific  $\text{CO}_2$  effect is greater at lower saturations and decreases at higher levels of  $\text{Hb-O}_2$  saturation.

The concentrations and binding properties of organic phosphates present in an animal's red blood cells are at least partially responsible for the above differences between animals in the magnitude of the carbamino  $\text{CO}_2$  effect. Organic phosphates and  $\text{CO}_2$  compete for the N-terminal residues of the beta chains on the hemoglobin molecule (4, 16, 17). In mammals, for instance, the magnitude of the carbamino  $\text{CO}_2$  effect is inversely proportional to the [2,3-DPG] (e.g., dog, man and sheep). However, muskrats have a [2,3-DPG] that is comparable to man (41), yet display a specific  $\text{CO}_2$  effect twice as large. This may be due to the reduced interaction between 2,3-DPG and Hb in muskrat blood (38).

The specific  $\text{CO}_2$  effect may be physiologically important, especially to divers or burrowers. Fossorial mammals encounter elevated ambient  $\text{CO}_2$  concentrations in their burrows (45), while in divers there is an increased production of  $\text{CO}_2$  in the tissues during a dive, leading to

an elevated blood  $PCO_2$ . A large specific  $CO_2$  effect in both groups would therefore significantly affect  $O_2$  transport by decreasing  $O_2$  uptake at the lungs and facilitating unloading of  $O_2$  at the tissues. In species like the muskrat, where the carbamino effect is also saturation dependent, this direct effect of  $CO_2$  on  $O_2$  transport is even more pronounced at the low levels of Hb-saturation that exist with hypoxemia during a dive or when they are also exposed to hypoxic burrow conditions. It is not known why the platypus, a diver, has no carbamino effect, however, it may be due to a change in the primary Hb structure or perhaps to the way in which 2,3-DPG interacts with its hemoglobin.

Effects of temperature on Hb- $O_2$  affinity. The temperature coefficient of 0.0088 (at  $P_{50}$ ) reported here is exceedingly low compared to those reported for other mammals (Table 4) and is about one-half the value of that for the European hedgehog and mole rat, both of which also display a low coefficient. In muskrats, this temperature coefficient is also saturation dependent, ranging from 0.0014 at  $S_{0.15}$  to 0.0186 at  $S_{0.85}$  (Table 3). It has been shown by MacArthur (24), based on abdominal cooling data, that muskrats swimming under laboratory conditions are in a negative energy balance at all water temperatures below and including  $30^\circ C$  in summer and  $25^\circ C$  in winter, with net mean abdominal temperature changes of up to  $4^\circ C$  in summer

TABLE 4  
Temperature coefficients in whole blood of mammals

| Species              | $d \log P_{50} / d T$ | Range ( $^{\circ}C$ ) |
|----------------------|-----------------------|-----------------------|
| Man (48)*            | 0.0240                | 22-42                 |
| Marmot (11)          | 0.0229                | 7-38                  |
| Dog (37)             | 0.0220                | 25-39                 |
| Ground squirrel (33) | 0.0215                | 6-38                  |
| Hamster (46)         | 0.0210                | 6-38                  |
| Hedgehog (8)         | 0.0167                | 5-38                  |
| Mole rat (1)         | 0.0152                | 30-37                 |
| Muskrat              | 0.0088                | 35-39                 |

\* Reference



and 2°C in winter. Data from free-ranging muskrats show that abdominal temperature declines rarely exceeded 2°C and are relatively independent of foraging time for excursions exceeding 40 minutes duration (25). Since a decline in blood temperature increases the oxygen affinity of the hemoglobin (2, 3) a decrease in body temperature during a dive would favor loading of O<sub>2</sub> from the lungs. This effect of temperature on Hb-O<sub>2</sub> affinity in the muskrat, however, is relatively small compared to that of other mammals. For example, a 4°C decrease in blood temperature in man, from 37 to 33°C, decreases the P<sub>50</sub> at a PCO<sub>2</sub> of 40 by 5.2 Torr, while an identical decline in blood temperature of muskrats under comparable conditions would decrease the P<sub>50</sub> by only 2.2 Torr. The O<sub>2</sub> affinity of muskrat Hb is, then, relatively independent of temperature during a dive, when body temperature is declining.

Do muskrats use the lung as an O<sub>2</sub> store during a dive? Muskrats have lung volumes comparable to those of similar sized terrestrial mammals, are thought to dive with their lungs at least partially inflated (42) and thus may utilize their lungs as a potential oxygen store. However, the results of my study have demonstrated that muskrats display a large  $\phi_{CO_2}$  factor that increases with a decrease in Hb-O<sub>2</sub> saturation and a substantial specific CO<sub>2</sub> effect that also is greater at lower levels of Hb-O<sub>2</sub> saturation,

which would inhibit  $O_2$  unloading from the lungs during a dive, when  $PaO_2$  is falling, and  $H^+$  and  $PaCO_2$  are increasing. It has been shown in beavers that during a dive,  $PaCO_2$  increases throughout submersion due to non-respiratory acidosis. However, the  $CO_2$  content of mixed venous plasma remained nearly constant, indicating that  $CO_2$  was retained in the tissues and trapped in the lungs (9). If this were also true for muskrats, the alveolar  $CO_2$  concentration during a dive would increase and additionally inhibit utilization of  $O_2$  stores in the lungs due to the large carbamino  $CO_2$  effect.

From my data it appears that muskrats have not developed adaptations to allow a more complete utilization of the lung  $O_2$  stores during a dive, and in fact seem to be adapted to unloading of  $O_2$  at the tissues.

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