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Effects of CO2 pH and temperature on Hb-O 2 affinity of muskrat blood

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EFFECTS OF CO_2, pH, AND TEMPERATURE ON Hb-O_2 AFFINITY OF MUSKRAT BLOOD

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Effects of CO_2, pH, and Temperature on Hb-O_2 Affinity of Muskrat Blood (27 pp.)

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The muskrat (Ondatra zibethicus), as a burrower and diver, naturally encounters extreme respiratory environments of O_2 and CO_2. O_2 transport properties of the blood were examined to determine (1) if there is a specific CO_2 effect on blood O_2 affinity and (2) if the CO_2 (ϕ_{CO_2}) and fixed-acid (ϕ_{AH}) Bohr effects are saturation dependent. Six muskrats were used to produce in vitro blood O_2 equilibrium curves at varying levels of CO_2, H^+, 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 ϕ_{CO_2} and ϕ_{AH} slopes at P_{50} were -0.625 and -0.453, respectively. Neither varied significantly with O_2 saturation, although ϕ_{CO_2} decreased with increasing saturation. Both the specific CO_2 effect and temperature coefficient (d log PO_2/d T) were saturation dependent, with values at P_{50} of 0.190 and 0.0088, respectively. It is concluded that the high CO_2 Bohr factor and large specific CO_2 effect do not allow the muskrat to utilize it's lungs as an O_2 store during a dive but facilitates the unloading of O_2 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.

Thanks go to Mr. David Maclay for allowing me to trap muskrats on land under his control.

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INTRODUCTION

THERE ARE A NUMBER OF ALLOSTERIC MODIFIERS of hemoglobin oxygen affinity. Included among these is CO_2, which reduces O_2 binding to hemoglobin (7). This modification of blood O_2 affinity by CO_2 is known as the CO_2 Bohr effect and has been shown to result from the combined effects of CO_2 hydration on intraerythrocytic pH and the direct binding of CO_2 to hemoglobin (31). Recently, attention has been focused on not only the 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-O_2 affinity to non-respiratory pH changes. From the CO_2 and fixed-acid Bohr effects, the specific influence of molecular CO_2 via carbamino formation on oxygen affinity can be determined (14). While this specific 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 CO_2 and fixed-acid Bohr effects have also been

shown to be saturation-dependent in some instances. In human blood, the CO_2 Bohr effect varies considerably with percent saturation of the hemoglobin (14). Lutz et al. (22) recently found that at elevated PCO_2 the fixed-acid Bohr effect in platypus blood declined markedly with decreasing O_2 saturation at values below 60%. This decrease in the pH dependent effect of CO_2 on Hb-O_2 affinity would most likely help in extraction of O_2 from the lungs when alveolar PO_2 and blood pH is reduced, conditions that might be expected during a dive. The fixed-acid and CO_2 Bohr effects and their saturation dependence may, therefore, profoundly influence oxygen transport.

I undertook the present study on the O_2 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_2 effect on blood O_2 affinity and (2) if the CO_2 and fixed-acid Bohr effects are saturation dependent. During the winter, muskrats congregate in winter lodges where CO_2 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_2 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_2 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 $ml \cdot min^{-1}$, 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_2, 30% O_2, and balance N_2. 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_2 equilibrium curves (O_2EC). 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_2 and PCO_2 (Radiometer BMS3-Mk2). The PO_2 electrode of the blood gas analyzer was calibrated with liquid solutions prior to the determination of an O_2EC. The calibration of the PO_2 and PCO_2 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_2EC was derived from the PO_2 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 (± SD) barometric pressure was 676.9 ± 4.2 Torr during these experiments.

Bohr factors. To obtain CO_2 (ϕ_{CO_2}) and fixed-acid (ϕ_{AH}) 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_2ECs at a constant base excess. In both experiments, log PO_2 taken from each O_2EC for a given saturation was then regressed on pH. The slope of the resulting regression line (d log PO_2/d pH) was the ϕ_{CO_2} or ϕ_{AM}.

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

Temperature effects on Hb-O_2 affinity. To determine the effects of temperature on the oxygen affinity of muskrat blood, O_2ECs 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_2ECs 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_2ECs (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_2ECs at all levels of CO_2 and pH ($P > 0.50$), n_H values from all O_2ECs 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_2ECs were not significantly different from the combined mean at 37°C ($P > 0.50$). The Hill relationships of O_2ECs

TABLE 1

Hematological characteristics of muskrat blood

n	Mass (g)	Hct (%)	Hb (g/100 ml)	RBC (10^6/mm^3)	MCV (um^3)	MCH (pg)	MCHC (%)	Oxygen capacity (vol %)
6	1058.2*	39.9	16.06	6.405	62.25	25.09	40.31	21.53
	±86.1	±0.4	±0.21	±0.071	±0.57	±0.35	±0.35	±0.28
	(33)^	(36)	(24)	(22)				

* Mean ± SEM
^ Numbers in parentheses are total number of determinations for all six muskrats.

TABLE 2

Respiratory properties and buffer values of muskrat blood[A]

P_{50} (7.4)[B] (Torr)	33.7 ± 0.4[C]
P_{50} (40)[D] (Torr)	28.6 ± 0.3
Predicted P_{50}[E] (Torr)	34.6 ± 0.15
Hill Coefficient (n_H)	2.81 ± 0.03
Åstrup slope[F] (d log PCO_2/d pH)	-1.308 ± 0.053
Buffer capacity (d [HCO_3^-]/d pH)	-26.0 ± 3.3
Standard bicarbonate[G] (mM/L)	33.1 ± 1.0

[A] All values are for blood at 37 C.
[B] P_{50} adjusted to a pH of 7.4 using appropriate ϕ_{CO_2} factors.
[C] Mean ± SEM
[D] P_{50} adjusted to a PCO_2 of 40 Torr using ϕ_{CO_2} and relationship between log PCO_2 and pH.
[E] Predicted for individual muskrats using allometric equation of Schmidt-Nielsen and Larimer (40).
[F] Åstrup slope of saturated ($S_{1.0}$) blood.
[G] 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_2ECs 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_2ECs 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 pH), based on all O_2ECs obtained by CO_2 titration, and the mean HCO_3^- concentration at a pH of 7.400 are reported in Table 2.

The mean ϕ_{CO_2} and ϕ_{AH} slopes at half saturation (d log P_{50}/d pH) were -0.625 and -0.453, respectively. Neither of these ϕ_{CO_2} nor ϕ_{AH} coefficients varied significantly with the level of oxygen saturation ($p > 0.05$), although the ϕ_{CO_2} slopes decreased with increasing saturation (Table 3). The fixed-acid Bohr factor was less than the ϕ_{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

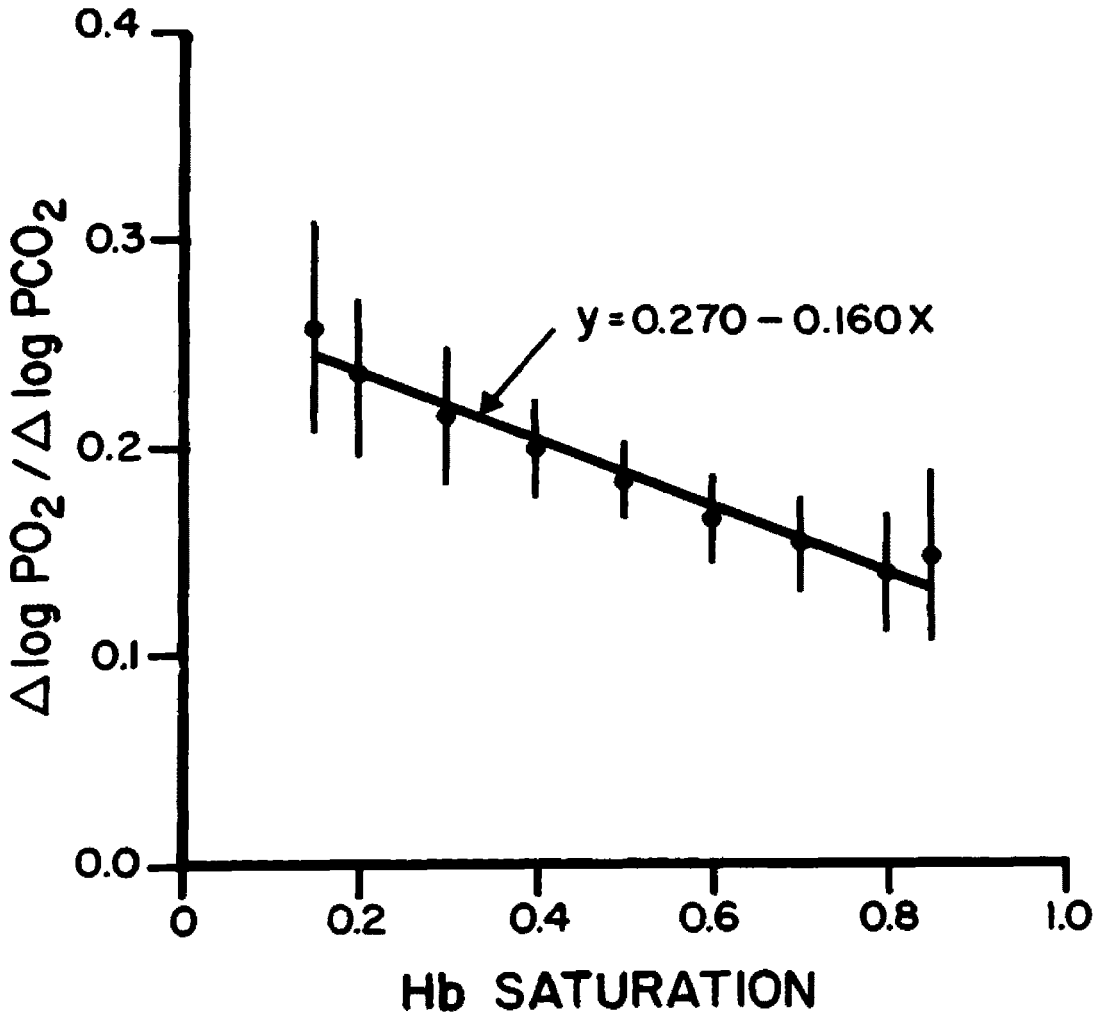
TABLE 3

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

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

* Mean ± SEM

Figure 1. Relationship between the specific CO_2 effect (d log PO_2/d log PCO_2) on Hb-O_2 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 CO_2 effect to S is significantly different from zero ($P < 0.001$).

The temperature effects on O_2 affinity (d log PO_2/d T) over the 4°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 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

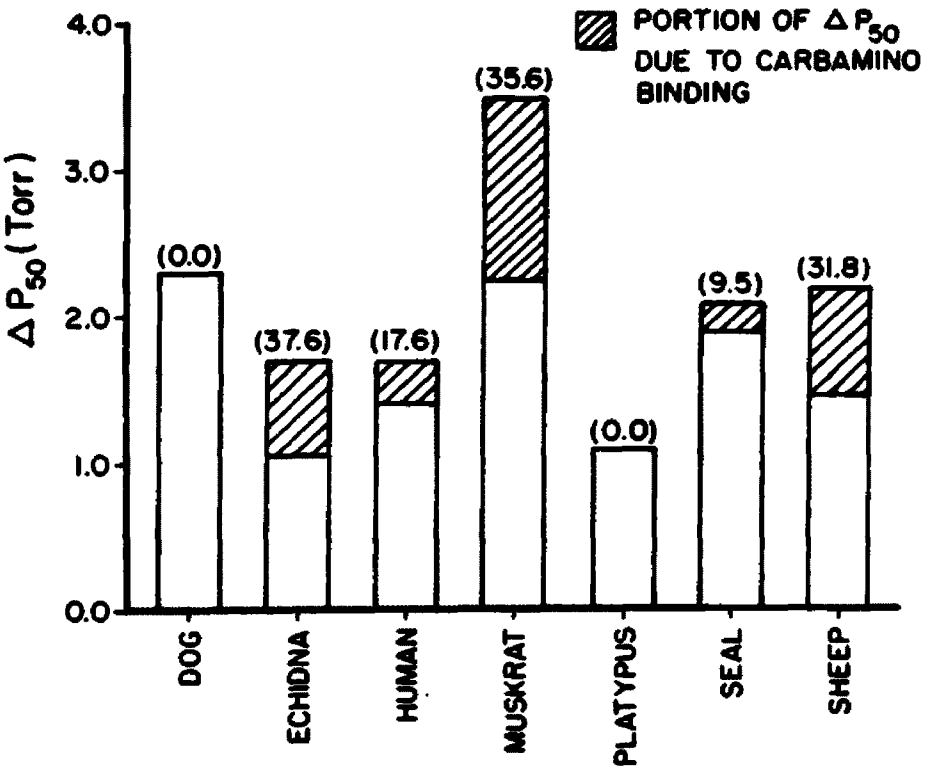
determination of specific parameters. MacArthur (27) has shown that both hematological and respiratory characteristics of muskrats vary seasonally. Nevertheless, muskrats do display a higher than predicted Hb-O_2 affinity. This higher O_2 affinity is due in part to a reduced level as well as reduced interaction of muskrat hemoglobin with 2,3-DPG (38, 41). Muskrats have a CO_2 Bohr effect at $S_{0.5}$ (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_3^- values at a pH of 7.4 have been reported for other burrowing and diving species (8, 34, 35).

Saturation dependency of ϕ_{CO_2} and ϕ_{AN}. In muskrat blood, the CO_2 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 ϕ_{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 ϕ_{CO_2} and ϕ_{AN} 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 ϕ_{CO_2} and ϕ_{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 ϕ_{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 CO_2 effect. The ϕ_{CO_2} and ϕ_{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 CO_2 binding is saturation dependent; in all cases the specific CO_2 effect is greater at lower saturations and decreases at higher levels of 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 CO_2 effect. Organic phosphates and 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 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 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 CO_2 effect may be physiologically important, especially to divers or burrowers. Fossorial mammals encounter elevated ambient CO_2 concentrations in their burrows (45), while in divers there is an increased production of 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 it's 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°C in summer and 25°C in winter, with net mean abdominal temperature changes of up to 4°C in summer

TABLE 4

Temperature coefficients in whole blood of mammals

Species	d log P_{50}/d T	Range (°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_2 from the lungs. This effect of temperature on Hb-O_2 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_{50} at a PCO_2 of 40 by 5.2 Torr, while an identical decline in blood temperature of muskrats under comparable conditions would decrease the P_{50} by only 2.2 Torr. The O_2 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_2 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 ϕ_{CO_2} factor that increases with a decrease in Hb-O_2 saturation and a substantial specific CO_2 effect that also is greater at lower levels of Hb-O_2 saturation,

which would inhibit O_2 unloading from the lungs during a dive, when PaO_2 is falling, and H^+a 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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