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A Cephalopod Approach to Rethinking about the Importance of the Bohr and Haldane Effects[1]

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ABSTRACT: This study concerns the physiological implications of the Bohr and Haldane effects and the buffer values in the blood from the cephalopods *Nautilus pompilius*, *Octopus macropus*, *Sepia latimanus*, *Nototodarus sloani philippinensis*, and *Sepioteuthis lessoniana*. All species studied except one (*Nautilus*) have Bohr and Haldane coefficients numerically higher than unity, and the two effects were found to be nearly identical in all cases, in accord with the theoretical prediction of Wyman (1964). However, the functional Haldane coefficient was significantly lower than the Haldane coefficient in two cases (*Sepia* and *Sepioteuthis*). Buffer values were highest in the two species with the lowest oxygen requirement (*Nautilus* and *Octopus*), whereas the three fast swimmers studied (*Nototodarus*, *Sepia*, and *Sepioteuthis*) display comparatively low buffer values. It is concluded that the large Bohr effects seen in four of the five species may have their primary effect on oxygen loading in the gills.

THE OXYGEN AFFINITY of cephalopod blood is pH-sensitive in all reported cases. However, for no other group of animals does the pH-sensitivity of the O_2 binding, expressed by the Bohr coefficient ($\Delta \log P_{50}/\Delta pH$), show such a large variability between species (P_{50} denotes the oxygen tension at half O_2 saturation of the blood).

For *Loligo pealei* the Bohr coefficient is −1.80 (Redfield and Goodkind 1929); for *Sepia officinalis*, −1.50 (Wolvekamp et al. 1942); for *Octopus dofleini*, −0.80 (Lenfant and Johansen 1965); and for *Nautilus pompilius*, −0.20 (Johansen, Redmond, and Bourne 1978). This wide range far exceeds what is known for mammals (Riggs 1960), amphibians (Johansen and Lenfant 1972), or major invertebrate taxa for which information is available (Weber 1980).

Since the Bohr effect is generally acredited physiological significance in respiratory blood gas transport, variations in it that are related to behavior, habitat, or systemic factors should be easily discernible in cephalopods.

The present study compares blood respiratory properties and discusses their possible physiological significance in five species of tropical cephalopods.

THEORETICAL BACKGROUND

Oxygen Transport

The Bohr effect causes metabolically produced CO_2 to promote the dissociation of oxygen from the respiratory pigment and thus facilitate unloading of oxygen to the tissues. This facilitation should be proportional to the size of the Bohr coefficient (Riggs 1960). However, the Bohr effect is tantamount to an oxygen-linked binding of protons to the respiratory pigment, and the quantitative expression for this binding of protons, the Haldane coefficient, should theoretically be identical with the Bohr coefficient (Wyman 1964). Thus, the higher the Bohr effect, the smaller should

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be the effect of metabolically produced protons on blood pH. In fact, with Bohr coefficients less than -1.0, a pH increase, rather than a decrease, should accompany unloading of O_2 from the respiratory pigment. Recent investigations have suggested that this may well be the case for *Sepia latimanus* (Lykkeboe, Brix, and Johansen 1980), *Sepia officinalis*, and *Loligo forbesii* (Brix, Lykkeboe, and Johansen 1981). If this is true, the large Bohr coefficient may be a detriment rather than an asset for O_2 unloading (Brix et al. 1981).

However, the physiological significance of these large Bohr effects ($\phi < -1.0$) may instead be related to their effect on loading of oxygen in the gills when O_2 affinity increases in response to a pH increase caused by a hyperventilatory response to hypoxic water. Hyperventilation during burst swimming also may improve gill O_2 loading by an affinity increase if O_2 uptake becomes diffusion-limited at the high rate of gill perfusion attending vigorous exercise.

A factor influencing the importance of the large Bohr coefficients for facilitation of O_2 unloading from the blood of some cephalopods may be related to a form of blood CO_2 binding described recently. Lykkeboe et al. (1980) demonstrated the presence of an O_2-linked CO_2 component independent of pH in the blood of *Sepia latimanus*. When CO_2 bound in this way is released at deoxygenation, it may form the basis for the pH decrease with O_2 unloading needed for further O_2 release via the Bohr shift. Such an arterio–venous pH decrease was demonstrated for the squid *Loligo pealei* by Redfield and Goodkind (1929), who reasoned that this is the basis for the phenomenally high O_2 utilizations from circulating blood in many cephalopods. However, Redfield and Goodkind (1929) did not recognize the need for a CO_2 component such as described by Lykkeboe et al. (1980) as a requirement for giving the large Bohr coefficients physiological importance in O_2 unloading from cephalopod blood.

Carbon Dioxide Transport

The relationship between the CO_2 tension (P_{CO_2}) and the total CO_2 content ($ctCO_2$) for oxygenated and deoxygenated blood is illustrated in Figure 1*A*. The higher $ctCO_2$ seen in the deoxygenated blood at constant P_{CO_2} indicates that oxygenated blood is more acid than deoxygenated blood. The vertical displacement between the two curves is referred to as the *Haldane effect*. A quantitative expression for this effect is represented by a plot of $ctCO_2$ against corresponding pH values (Figure 1*B*). The curved relationship in Figure 1*A* changes to a linear relationship by this transformation. We will define the change in $ctCO_2$ upon deoxygenation at constant pH (the vertical distance between the two lines in Figure 1*B*) as the *functional Haldane effect* of the blood. Its numerical expression, the functional Haldane coefficient, is thus represented by the ratio $(\Delta ctCO_2/\Delta ctO_2)_{pH}$, that is, the change in $ctCO_2$ over the change in O_2 content between fully oxygenated and fully deoxygenated blood, which in turn numerically corresponds to the blood O_2 binding capacity. Expressed differently, the functional Haldane coefficient shows how much CO_2 the blood can load or unload per unit of O_2 unloaded or loaded at constant blood pH in a closed system.

The functional Haldane coefficient differs in one respect from the traditionally defined Haldane coefficient (i.e., the change in the amount of protons bound to the hemocyanin per unit O_2 unloaded or loaded at constant pH) in that the functional Haldane coefficient, based on total CO_2 content, also includes a significant, but small, fraction of physically dissolved CO_2. This difference may in many cases be the only one between the two coefficients. Figure 1*C* illustrates this by showing total CO_2 (solid lines) and the calculated bicarbonate concentrations (dashed lines) of oxygenated and deoxygenated blood as a function of pH. The increase in bicarbonate concentration upon deoxygenation at constant pH, $\Delta[HCO_3^-]_{pH}$, reflects the increase in the amount of protons bound to the hemocyanin upon complete deoxygenation, and the ratio between $\Delta[HCO_3^-]_{pH}$ and the oxygen-binding capacity (both in millimoles) should thus be identical with the numerical value of the Haldane coefficient. The negative slope of the broken lines in Figure 1*C* is also a measure of the blood buffer value, $meq \cdot liter^{-1} \cdot pH^{-1}$,

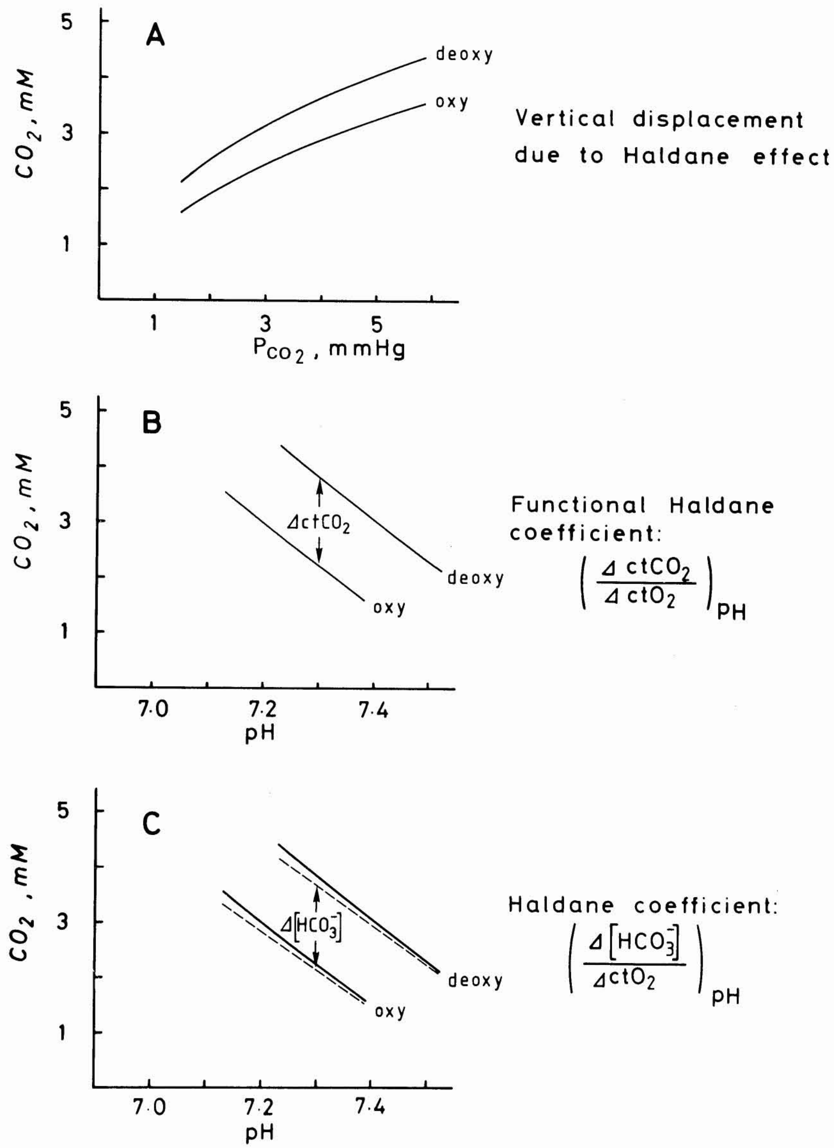

FIGURE 1. Constructed carbon dioxide dissociation curves. The blood was ascribed a buffer capacity of 6.7 meq · liter^{-1} · pH^{-1}. A pK' value of 5.94 and a solubility coefficient for CO_2 of 0.039 mmole · liter^{-1} · mm Hg^{-1} were used in the calculations. The vertical displacement due to the Haldane effect was arbitrarily chosen. (See text for definitions of functional Haldane coefficient and Haldane coefficient.)

of the nonbicarbonate blood buffers (Burton 1973). The slope of the lines expressing $ctCO_2$ as a function of pH will be referred to as the *functional buffer value* for CO_2, i.e., mmole CO_2 · liter^{-1} · pH^{-1}, at a constant level of oxygenation.

The functional Haldane coefficient and the functional buffer value for CO_2 are required to describe fully the acid–base changes associated with oxygenation or deoxygenation of the blood under aerobic conditions. If the exchange ratio, $R_E = \dot{V}_{CO_2}/\dot{V}_{O_2}$, exceeds the numerical value of the functional Haldane coefficient, a pH decrease may occur in blood during passage of the tissues, and the size of this pH decrease will be inversely related to the numerical value of the functional buffer value for CO_2. Conversely, a pH increase can be predicted when R_E is smaller than the numerical value of the functional Haldane coefficient.

MATERIALS AND METHODS

The cephalopods studied were caught in the Tanon Straits near Negros Island in the Philippines in October 1979. The animals were kept in running seawater in holding tanks at 25–30°C on board the research vessel *Alpha Helix*. Blood from the following species was studied: *Nautilus pompilius*, *Octopus macropus*, *Sepia latimanus*, *Nototodarus sloani*, and *Sepioteuthis lessoniana*.

Blood was sampled percutaneously from the vena cava cephalica or from indwelling catheters implanted in the vena cava under ethyl alcohol anesthetic. The blood was immediately centrifuged for sedimentation of cells and debris and kept stored in iced water.

Carbon dioxide dissociation curves and acid–base properties of the blood were performed on board *Alpha Helix*, and blood aliquots were brought frozen to our laboratory in Denmark for determinations of O_2 dissociation curves and O_2 capacity.

For the CO_2 binding studies, blood samples were equilibrated at 25°C for 20 min against either 0.2 or 0.8% CO_2, rest N_2 (deoxy); or 0.2 and 0.8% CO_2 in 50% O_2 and rest N_2 (oxy). The pH was measured using a microcapillary glass electrode system associated with the tonometry equipment (Radiometer BMS-2) and read on a Radiometer precision pH meter (PHM 64). Wösthoff gas mixing pumps delivered the gas mixtures from stock gases of N_2, O_2, and CO_2. Total CO_2 content of the blood was measured using a micro Van Slyke

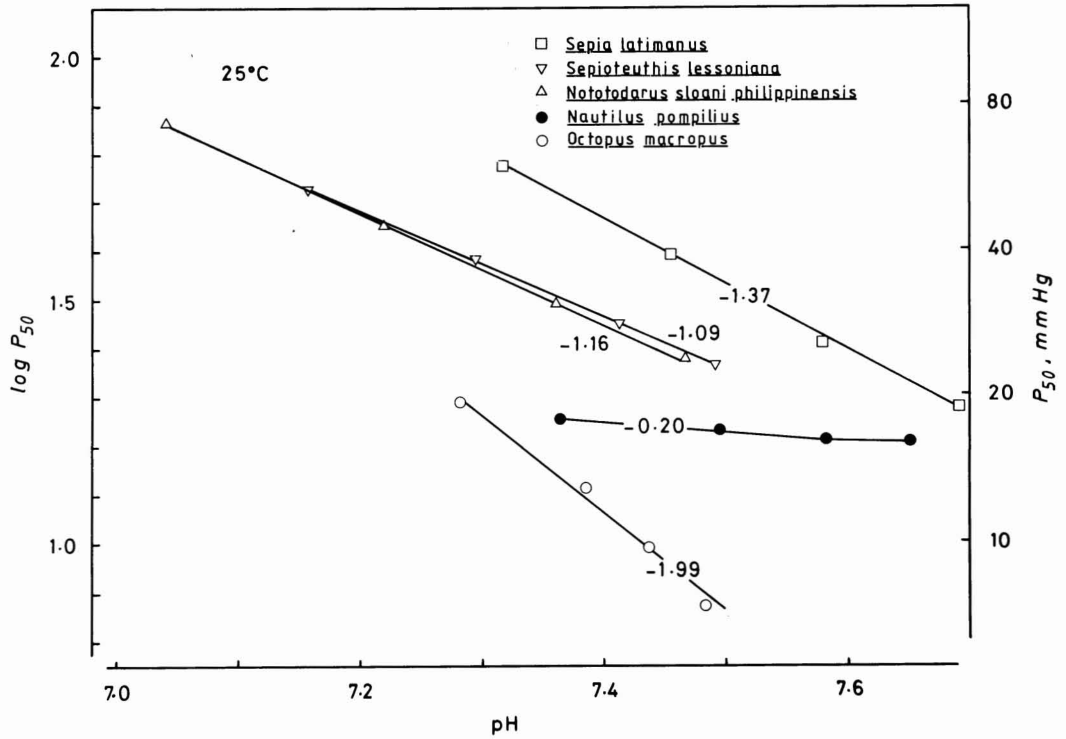

FIGURE 2. Bohr shift curves for *Sepia latimanus*, *Sepioteuthis lessoniana*, *Nototodarus sloani philippinensis*, *Nautilus pompilius*, and *Octopus macropus* illustrating the relationship between O_2 affinity (log P_{50}) and pH at 25°C. Each data point represents a single determination.

TABLE 1

BOHR COEFFICIENTS, HALDANE COEFFICIENTS, AND FUNCTIONAL HALDANE COEFFICIENTS FOR FIVE CEPHALOPOD SPECIES

SPECIES	BOHR COEFFICIENT $(\Delta \log P_{50}/\Delta \mathrm{pH})$	HALDANE COEFFICIENT $(\Delta[\mathrm{HCO_3^-}]/\Delta \mathrm{ctO_2})\mathrm{pH}$	FUNCTIONAL HALDANE COEFFICIENT $(\Delta \mathrm{ctCO_2}/\Delta \mathrm{ctO_2})\mathrm{pH}$
Nautilus pompilius	-0.20	$-0.20_{7.5}$	$-0.20_{7.5}$
Octopus macropus	-1.99	$-1.88_{7.3}$	$-1.81_{7.3}$
Sepia latimanus	-1.37	$-1.49_{7.4}$	$-1.09_{7.4}$
Nototodarus sloani philippinensis	-1.16	$-1.07_{7.3}$	$-1.07_{7.3}$
Sepioteuthis lessoniana	-1.09	$-1.11_{7.3}$	$-0.86_{7.3}$

NOTE: See the text for definitions of the Haldane coefficient and the functional Haldane coefficient. The Bohr coefficients were obtained from Figure 2, and the Haldane and functional Haldane coefficients are mean values of two determinations.

apparatus (Arthur Thomas, Magna matic model; Van Slyke and Plazin 1961). For calculations of the apparent bicarbonate concentrations pK' values were estimated according to Lykkeboe et al. (1980).

Oxygen dissociation curves were determined spectrophotometrically at 366 nm (Lykkeboe, Johansen, and Maloiy 1975). Curves were measured at four CO_2 levels, 0.1, 0.2, 0.4, and 0.8% for determination of the CO_2 Bohr factor. Oxygen capacities were calculated as half of the blood copper content (Ghiretti 1966) measured on a Perkin Elmer 503 atomic absorption spectrophotometer.

RESULTS

Figure 2 shows the CO_2 Bohr effects, log P_{50} as a function of pH, for blood from the five species at 25°C. The CO_2 Bohr coefficients, ϕ, expressed by the slopes of the relationships between log P_{50} and pH, are all negative in the pH intervals concerned. The range spans from -0.20 in *Nautilus pompilius* to -1.99 in *Octopus macropus*, with the remaining three species, *Sepioteuthis lessoniana*, *Nototodarus sloani*, and *Sepia latimanus*, having values of -1.09, -1.16, and -1.37, respectively. Table 1 compares these Bohr coefficients with both the Haldane and functional Haldane coefficients. It is evident from Table 1 that the Bohr and Haldane coefficients in all cases correspond closely, a correspondence that conforms with the linkage theory (Wyman 1964). In the case of *Nautilus*, *Octopus*, and *Nototodarus*, this general correspondence is also present for the functional Haldane coefficient, whereas *Sepia* and *Sepioteuthis* show somewhat lower values for this coefficient compared with the Bohr and Haldane coefficients. Figure 3 further illustrates this difference between the species. For each species, the figure shows the CO_2 content for oxygenated and deoxygenated blood plotted against the corresponding pH values (solid lines) together with calculated values for apparent bicarbonate (dashed lines). The difference between the Haldane coefficients and the functional Haldane coefficients for *Sepia* and *Sepioteuthis* can be ascribed to the fact that the total CO_2 content of the oxygenated blood far exceeds the corresponding apparent bicarbonate concentration.

Figure 3 also illustrates that there are substantial differences in the buffering capacities of the five blood samples. These differences are due to differences in both the hemocyanin concentration (O_2 capacity) and the inherent buffer value of the hemocyanins.

Table 2 gives the buffer values and the functional buffer values for CO_2 of blood from the five species as a function of the oxygen capacity. *Nautilus* displays the highest buffer value, 9.80 $\mathrm{meq \cdot pH^{-1} \cdot (mmole\ O_2)^{-1}}$, and *Octopus* an intermediate 6.61 $\mathrm{meq \cdot pH^{-1} \cdot (mmole\ O_2)^{-1}}$. *Nototodarus* has the lowest buffering power, 3.25 $\mathrm{meq \cdot pH^{-1} \cdot (mmole\ O_2)^{-1}}$. The functional buffer values for CO_2 given in Table 2 are in all cases about 0.5 higher than the buffer values. This reflects the fact that the former values include changes in physically dissolved CO_2.

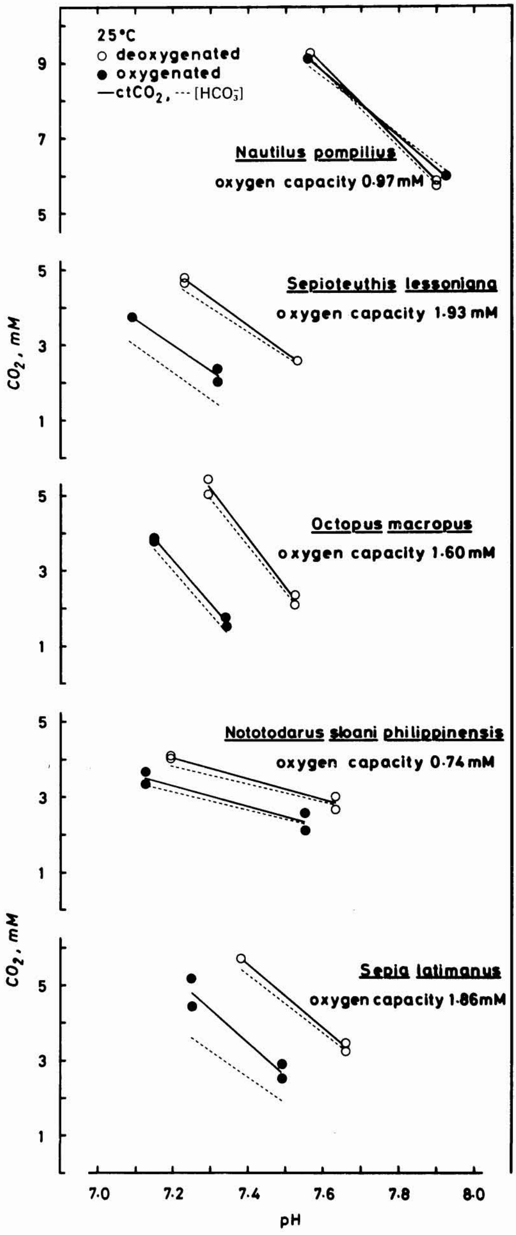

FIGURE 3. Single determinations of the CO_2 dissociation curves of oxygenated and deoxygenated blood from five cephalopod species. The data for total CO_2 ($ctCO_2$, double determinations) and calculated apparent bicarbonate $[HCO_3^-]$ are plotted against the corresponding pH values. The actual oxygen capacity (in millimoles) is referred to for each species.

DISCUSSION

The physiological significance of the Bohr and Haldane effects can be evaluated from three different, but not mutually exclusive, viewpoints. First, the traditional viewpoint is that the metabolically produced CO_2 promotes the dissociation of O_2 bound to the respiratory pigment and thus facilitates the transport of O_2 to the tissues (Bohr, Hasselbalch, and Krogh 1904). Second and concurrently, the dissociation of O_2 from the carrier will facilitate the removal of CO_2 from the tissues and aid CO_2 elimination in the gas exchange organs with a minimal change in hydrogen ion concentration (isohydric CO_2 transport; Christiansen, Douglas, and Haldane 1914). From a third point of view, the magnitude of the Bohr effect is decisive for the influence of pH changes on the oxygen affinity. The pH changes in response to hyperventilation during hypoxia would be a case in point. Table 3 weighs the relative importance of the Bohr and Haldane effects against these three stated viewpoints for the cephalopods studied presently. The data in Tables 1 and 2 were used for this evaluation.

If we assume that the hydration of CO_2 is complete when blood leaves the tissues, a promotion of O_2 unloading via the Bohr and Haldane shift interaction is predictable only for *Nautilus* and *Sepioteuthis*, because among the species studied they alone have a functional Haldane coefficient near or below an expected gas exchange ratio (Table 1). For *Nautilus*, the effect can become effective only marginally because of its very low Bohr coefficient (giving a relatively small effect of free protons) and also because its functional buffer capacity for CO_2 has such a high value (reducing the probability of a net proton change in the blood). This prediction corresponds well with directly measured arterio–venous pH differences of about 0.030 in *Nautilus* (Johansen et al. 1978). For *Sepioteuthis*, we must predict a gas exchange ratio close to unity (which is unlikely for a carnivorous animal) in order for an effect to become apparent. The effect on O_2 unloading can at best be small in spite of the high Bohr effect and the low numerical value of the functional

TABLE 2

BUFFER VALUES AND FUNCTIONAL BUFFER VALUES OF BLOOD FROM FIVE CEPHALOPOD SPECIES

SPECIES	BUFFER VALUE* $[\text{meq} \cdot \text{pH}^{-1} \cdot (\text{mmole } O_2)^{-1}]$	FUNCTIONAL BUFFER VALUE FOR CO_2* $[\text{mmole } CO_2 \cdot \text{pH}^{-1} \cdot (\text{mmole } O_2)^{-1}]$
Nautilus pompilius	9.80 ± 1.00	−10.33 ± 1.04
Octopus macropus	6.61 ± 1.05	−7.16 ± 0.70
Sepia latimanus	4.00 ± 0.59	−4.59 ± 0.09
Nototodarus sloani philippinensis	3.25 ± 0.09	−3.75 ± 0.04
Sepioteuthis lessoniana	4.28 ± 0.96	−4.72 ± 1.05

NOTE: Values are mean ± 1 SD; $N = 4$.

* Multiplication of these values by the blood oxygen capacity (in millimoles) gives the buffer value of the blood.

TABLE 3

RELATIVE IMPORTANCE OF THE BOHR AND HALDANE EFFECTS IN FIVE CEPHALOPOD SPECIES

	POSSIBLE INFLUENCE OF BOHR AND HALDANE EFFECTS ON BLOOD GAS TRANSPORT		
SPECIES	FACILITATION OF O_2 UNLOADING	ISOHYDRIC CO_2 TRANSPORT	P_{50} DEPENDENCE ON BLOOD PH
Nautilus pompilius	(+)	+	(+)
Octopus macropus	−	−	+++
Sepia latimanus	−	+	++
Nototodarus sloani philippinensis	−	+	+
Sepioteuthis lessoniana	+	+	+

NOTE: − denotes absence and (+), +, ++, and +++ denote presence graded from weak, (+), to very strong +++ of a given property.

buffer capacity for CO_2. For the other three species, we must predict that the large Bohr coefficients will exert no facilitating effect on O_2 unloading from hemocyanin in the tissues.

If we next address the influence of CO_2 transport on blood pH, as indicated in Table 3, all species except *Octopus* will have a near isohydric CO_2 transport because their functional Haldane coefficients have values near unity (−1.0), or, as is the case for *Nautilus*, have a very high functional buffer value (Table 2).

The isohydric transport of CO_2 may be of utmost importance in a fast-swimming animal powered mostly by aerobic metabolism. The O_2 affinity is notably very low in all three fast swimmers among the species studied, *Nototodarus*, *Sepia*, and *Sepioteuthis* (Figure 2). An acidification of the blood in their tissues may compromise O_2 loading in the gills and increase their requirement for blood perfusion if arterial blood becomes less than fully saturated. We may argue that for these species the facilitation of O_2 unloading by tissue acidification of the blood has a reduced importance due to their inherently low blood O_2 affinity and the pronounced sigmoidicity of the oxygen-binding curve expressed by P_{50} and the Hill coefficient (Table 4). But their mode of life, particularly that of the very fast swimming *Nototodarus*, seems best served by a gas exchange system in which the respiratory pigment acts in pH stabilization. The very large Bohr and Haldane shifts contribute significantly to such pH stability. It merits interest that the only affinity change likely to have physiological consequence is that affecting O_2 loading in the gills.

Octopus is the only species studied with a functional Haldane coefficient much higher than unity (−1.81), and it will predictably exhibit much higher pH values in venous than

TABLE 4

OXYGEN AFFINITY, P_{50}, AND HILL COEFFICIENT, n_H, OF BLOOD FROM FIVE CEPHALOPOD SPECIES

SPECIES	P_{50} (mm Hg, at 25°C, pH = 7.4)	HILL COEFFICIENT (n_H)
Sepia latimanus	46.4	3.1 ± 0.3
Sepioteuthis lessoniana	29.1	3.1 ± 0.3
Nototodarus sloani philippinensis	27.9	2.9 ± 0.2
Nautilus pompilius	17.7	2.7 ± 0.2
Octopus macropus	11.6	2.1 ± 0.3

NOTE: The P_{50} values are from Figure 2; the Hill coefficients are mean ± 1 SD; $N = 4$.

in arterial blood. While this large increase in venous pH is incompatible with facilitation of O_2 unloading (see earlier discussion), it is bound to exert a marked effect on O_2 loading (Table 3). *Octopus macropus* is often found near the shore along soft substrates and, on occasion, trapped in tidepools. Here, the water temperature may be very high and the water O_2 levels low. Safeguarding O_2 loading in the gills seems important in this habitat—a purpose well served by a large Bohr effect and venous blood pH higher than arterial. The hyperventilatory response to acute hypoxia will also elevate blood pH and further contribute to improved O_2 loading in the gills. Oxygen loading must always be compromised with O_2 unloading from a respiratory pigment. The reason why hemocyanin in *O. macropus* appears structured more for compensatory improvement of O_2 loading than unloading may rest with a much lower O_2 requirement and a very high tolerance to hypoxic water compared to the other species (*Nautilus* excepted) presently studied.

We conclude that the Bohr and Haldane shifts in cephalopods involve the respiratory pigment in pH stabilization and that variations in these effects reflect differences in behavior and habitat factors of the species.

The traditional view that a large Bohr shift will facilitate O_2 unloading in an obligatory way is untenable, since the data obtained demonstrate the opposite. Rather, the magnitudes of the Bohr and Haldane effects and the general acid–base status of the animals studied lead us to believe that the Bohr and Haldane effects may have their primary effect on O_2 loading in the gills.

LITERATURE CITED

BOHR, C., K. HASSELBALCH, and A. S. KROGH. 1904. Ueber einen in biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung Übt. Skand. Arch. Physiol. 16:402–412.

BRIX, O., G. LYKKEBOE, and K. JOHANSEN. 1981. The significance of the linkage between the Bohr and Haldane effects in cephalopod bloods. Respir. Physiol. 44: 177–186.

BURTON, R. F. 1973. The roles of buffers in body fluids: Mathematical analysis. Respir. Physiol. 18:34–42.

CHRISTIANSEN, J., C. G. DOUGLAS, and J. S. HALDANE. 1914. The absorption and dissociation of carbon dioxide by human blood. J. Physiol. (Lond.) 48:244–271.

GHIRETTI, F. 1966. Molluscan hemocyanins. Pages 175–208 *in* K. M. Wilbur and C. M. Yonge, eds. Physiology of Mollusca. Vol. 2. Academic Press, New York.

JOHANSEN, K., and C. LENFANT. 1972. A comparative approach to the adaptability of O_2-Hb affinity. Proc. Alfred Benzon Symp. IV, Copenhagen, Munksgaard, 750–780.

JOHANSEN, K., J. R. REDMOND, and G. B. BOURNE. 1978. Respiratory exchange and transport of oxygen in *Nautilus pompilius*. J. Exp. Zool. 205:27–36.

LENFANT, C., and K. JOHANSEN. 1965. Gas transport by hemocyanin-containing blood of the cephalopod *Octopus dofleini*. Amer. J. Physiol. 209:991–997.

LYKKEBOE, G., O. BRIX, and K. JOHANSEN. 1980. Oxygen-linked CO_2 binding independent of pH in cephalopod blood. Nature 287:330–331.

LYKKEBOE, G., K. JOHANSEN, and G. M. O. MALOIY. 1975. Functional properties of hemoglobins in the teleost *Tilapia grahami*. J. Comp. Physiol. 104:1–11.

REDFIELD, A. C., and R. GOODKIND. 1929. The significance of the Bohr effect in the respiration and asphyxiation of the squid, *Loligo pealei*. J. Exp. Biol. 6:340–349.

RIGGS, A. 1960. The nature and significance of the Bohr effect in mammalian hemoglobins. J. Gen. Physiol. 43:737–752.

VAN SLYKE, D. D., and J. PLAZIN. 1961. Micromanometric analysis. Williams and Wilkins, Baltimore.

WEBER, R. E. 1980. Functions of invertebrate hemoglobins with special reference to adaptations to environmental hypoxia. Amer. Zool. 20:79–101.

WOLVEKAMP, H. P., G. P. BAERENDS, B. KOK, and W. F. H. M. MOMMAERTS. 1942. O_2- and CO_2-binding properties of the blood of the cuttlefish (*Sepia officinalis* L.) and the common squid (*Loligo vulgaris* Lam.). Arch. Neerl. Physiol. 26:203–211.

WYMAN, J., JR. 1964. Linked functions and reciprocal effects in hemoglobin: A second look. Adv. Prot. Chem. 19:224–286.