

Integrated Function of the Respiratory Pigment Hemocyanin in Crabs¹

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SYNOPSIS. A large number of factors are now known to influence the oxygen affinity of hemocyanins in crabs. In this article I review several case studies in which a number of different variables, including pH, CO₂, calcium ions, lactate, urate, dopamine, and temperature, act together on hemocyanin oxygen affinity to bring about adaptive changes in oxygen transport. These changes act in tandem with ventilatory and cardiovascular adjustments to stabilize oxygen uptake during hypoxia or to increase oxygen uptake during exercise in a swimming crab. It is important, however, that the effects of the different modulators be examined at very low concentrations, where their effects are large, to assess their importance in resting crabs where their concentrations may be low. Temperature effects among hemocyanins in crabs are variable and the magnitude may be inversely related to the magnitude of the Bohr effect. Several examples are presented of acclimation effects where oxygen affinity increases significantly at cold temperatures. The acclimation response may be important in partially regulating the metabolic responses of crabs to seasonal changes in temperature.

INTRODUCTION

The number of papers published on the hemocyanins has greatly increased in recent years. Interest in the crustacean hemocyanins has risen dramatically since the publication of a short paper by Truchot in 1980 that demonstrated that L-lactate at physiological concentrations increased oxygen affinity in the crab *Carcinus maenas*. This exciting discovery indicated that the oxygen binding characteristics of the hemocyanins, much like the hemoglobins, were affected by organic molecules. Furthermore, analysis of dialyzed samples of hemocyanin made it clear that other factors undescribed were controlling oxygen affinity. These findings led investigators to search for the mechanisms by which L-lactate interacted with and controlled oxygen affinity (Graham *et al.*, 1983) and to search for the identity of the "unknown factors" controlling oxygen

affinity (*e.g.*, Morris *et al.*, 1985). The functional importance of these findings and their placement in a historical context have been reviewed recently by Morris (1990).

In this paper I will focus on some of the variables that allow hemocyanin to function as part of a set of integrated cardiovascular and ventilatory responses of crabs to environmental perturbations. I will do this by examining a number of situations that occur in crabs where our information is fairly complete. These "case studies" will illustrate how a single factor can affect the oxygen affinity of hemocyanin in a way that either opposes or supports the effect of one or more other factors. Mention will also be made of how the oxygen binding properties of hemocyanin act in tandem with adjustments in cardiac output to achieve an adaptive result.

I will ignore the effects of these variables on the cooperativity of oxygen binding, not because they are unimportant, but because the effects, where they occur, are complex and their function less well understood (*e.g.*, Morris and Bridges, 1989).

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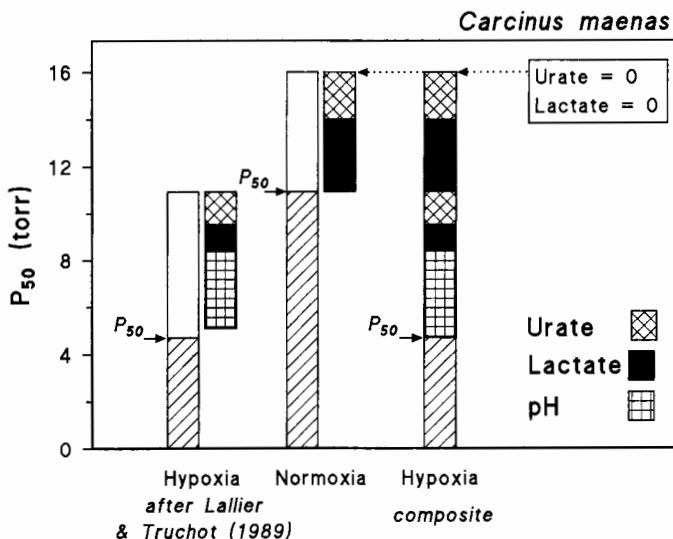


FIG. 1. Adaptive increases in oxygen affinity occur when the crab, *Carcinus maenas* is exposed to hypoxia (ambient $P_{O_2} = 30$ torr at 15°C) for three hours as a result of increases in hemolymph pH, urate, and lactate. The hypoxia data set on the left is redrawn after Lallier and Truchot (1989). However, urate and lactate also play significant roles in determining the "resting" oxygen affinity in normoxic crabs (middle bars). Measured P_{50} is indicated in all cases by the striped bars. The P_{50} of the hemocyanin in the absence of the effects of urate, lactate, and pH is approximated using data presented by Lallier and Truchot (1989) and indicated by the striped bar plus the open bar in the middle data set. The magnitude of the effects of the individual modulators is also indicated with a composite set of bars drawn for the situation during hypoxia.

ESTABLISHING OXYGEN AFFINITY IN "RESTING" CRABS

Organic factors such as lactate and urate may or may not affect oxygen affinity (Morris, 1990). When oxygen affinity is sensitive to a variable, however, it is important to appreciate the magnitude of this sensitivity as well as the distribution of the sensitivity as a function of concentration. In most cases where this has been documented, the greatest sensitivities, *i.e.*, the largest changes in oxygen affinity, occur at the lowest "factor" concentrations. Thus, the oxygen affinity of crab hemocyanin in the "normal" or "resting" animal is indeed established by the presence of a factor that is present at a low concentration. For example, Lallier and Truchot (1989) present a bar graph showing the contribution of lactate, urate and pH in modulating oxygen affinity in crabs exposed to hypoxia. The bar graph shown in Figure 1 is adapted from Lallier and Truchot (1989) and shows clearly that all three variables are important in bringing about an adaptive increase in oxygen affinity during hypoxia.

However, it should also be recognized that lactate and urate play an important role in establishing the oxygen affinity of the hemocyanin in normoxic animals. This situation is represented for crabs held in normoxia in Figure 1 by showing that low levels of lactate and urate serve to raise oxygen affinity to its measured value. It is entirely possible that minor changes in the concentrations of these factors in the normoxic crab may have a quantitatively greater effect than the environmentally stimulated change.

VARIABLES AFFECTING OXYGEN AFFINITY

Even a cursory survey of the literature reveals that a large number of factors affect hemocyanin oxygen affinity in crabs. In this article I will focus on an important subset of these factors to illustrate the integrated effects of modulators of hemocyanin to bring about important and adaptive changes to oxygen affinity when the oxygen transport system is stressed.

I will present three different case studies to illustrate the functional importance of

TABLE 1. A number of important variables affect the oxygen affinity of crab hemocyanin.*

Variable	Oxygen affinity
↑ pH	↑
↑ CO ₂	—↑
↑ Calcium	—↑
↑ Lactate	—↑
↑ Urate	—↑
↑ Temperature	—↓
↑ Dopamine	—↑

* When the magnitude of the variable increases (↑), the oxygen affinity may either remain the same (—), increase (↑), or decrease (↓).

some of the variables listed in Table 1. The case studies will focus on the blue crab *Callinectes sapidus* for which there exists a large body of information. To illustrate the integrative properties of hemocyanin in *C. sapidus* I have chosen three situations that challenge the oxygen transport system: 1) chronic hypoxia, 2) air exposure, and 3) exercise.

I will then discuss the different patterns of responses of hemocyanin to both acute and long-term effects of temperature. Finally, I will explore briefly the importance of dopamine, recently found to modulate oxygen affinity, on oxygen transport.

CHRONIC HYPOXIA

Crabs respond typically to short-term hypoxia by increasing the ventilation of water over their gills and this has been shown to induce an adaptive alkalosis in the hemolymph (Truchot, 1975a; Burnett and Johansen, 1981; Lallier and Truchot, 1989). The blue crab *C. sapidus* is no exception to this pattern. Pease and deFur (1986) demonstrated that *C. sapidus* underwent a sustained respiratory alkalosis for 5 days when exposed to moderate hypoxia. The alkalosis is helpful to crabs with normal Bohr shifts in maintaining the oxygenation of post-branchial hemolymph when PO₂ is falling.

A separate study (deFur *et al.*, 1990) on the same species showed no evidence of an alkalosis after 7 and 25 days of moderate hypoxia. Reasons for the different results obtained in the two studies (Pease and deFur, 1986; deFur *et al.*, 1990) are unclear. However, a number of other important changes occurred in the 7 and 25 day study that illustrate adaptive adjustments in the prop-

erties of the oxygen transport system. There was a significant increase in the concentration of hemocyanin circulating in the hemolymph after 25 days from $3.11 \text{ g} \times 100 \text{ ml}^{-1}$ to $4.40 \text{ g} \times 100 \text{ ml}^{-1}$. In addition, oxygen affinity was increased by several factors. Hemolymph lactate and urate concentrations increased slightly resulting in a small increase in oxygen affinity of less than 1 torr for each modulator. Hemolymph calcium concentration also increased, and deFur *et al.* (1990) maintained that this also results in an increase in oxygen affinity.

The most interesting result of this study, however, is the intrinsic change in oxygen affinity due to a change in the subunit composition of the hemocyanin. Hemocyanin in *C. sapidus* can be separated electrophoretically into 6 distinct monomers (Mangum and Rainer, 1988). The contribution of each subunit to the total number of subunits can therefore be quantified. Shifts in the concentrations of individual subunits can result in changes in the overall oxygen affinity of the pigment. deFur *et al.* (1990) report that the levels of subunits 3, 5, and 6 decrease, bringing about an increase in oxygen affinity of about 5 torr.

The results of all of these changes is a net movement of the oxygen equilibrium curve upward as a result of the increase in hemocyanin concentration and to the left as a result of intrinsic and extrinsic factors (Fig. 2). While measurements of prebranchial PO₂ during chronic hypoxia are unavailable, the values are likely to be low. All these factors act in concert to maintain levels of oxygen uptake similar to those found in the normoxic animal as shown by the results of the product of cardiac output and the post-branchial-prebranchial oxygen concentration difference (Fig. 2). Thus, during sustained moderate hypoxia oxygen uptake is regulated by a combination of adjustments in convection, *i.e.*, circulation and ventilation, and in the chemical properties of the oxygen transporter.

AIR EXPOSURE

Many crabs are exposed to bouts of air exposure whether they are uncovered by an outgoing tide, actively seek to move into the air, or both (Burnett and McMahon,

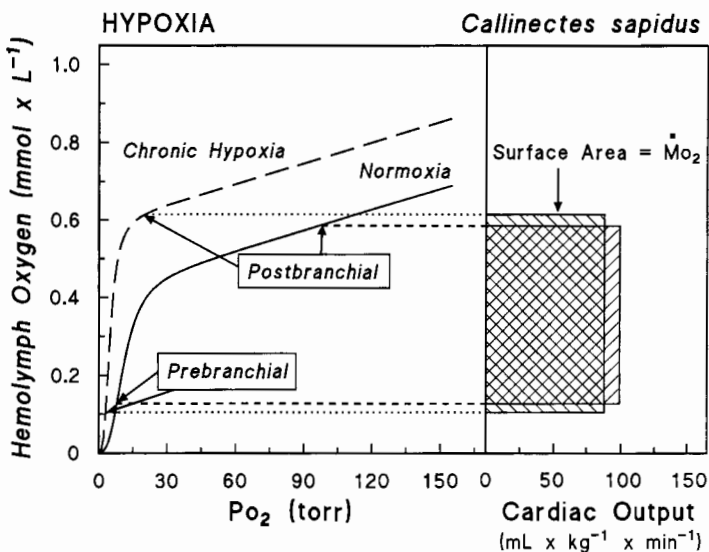


FIG. 2. Chronic hypoxia (50–55 torr) in *Callinectes sapidus* results in an increase in the hemocyanin concentration and an increase in hemocyanin oxygen affinity due to intrinsic and extrinsic factors (dashed curve). Oxygen uptake ($\dot{M}O_2$) is shown in normoxic and hypoxic conditions as the product of cardiac output and the oxygen concentration difference between postbranchial and prebranchial hemolymph. Data are taken from Booth *et al.* (1982) and deFur *et al.* (1990). Values for prebranchial hemolymph during chronic hypoxia are unknown and assumed to be slightly lower than values for normoxia.

1987). The responses of crabs and a variety of other organisms are similar in that CO_2 tends to accumulate in the hemolymph (Burnett and McMahon, 1987; Burnett, 1988). In crabs the accumulation of CO_2 leads to an acidosis which is almost always compensated to some degree. Recently, Mangum and Burnett (1986) surveyed a number of hemocyanins, including crab hemocyanins, and determined that some pigments had a specific sensitivity to CO_2 while others were insensitive. The possibility exists, therefore, that a CO_2 -sensitive pigment plays a role in preserving the respiratory function of hemocyanin during air exposure when PCO_2 rises.

I will next present another case study that is somewhat atypical to illustrate how a CO_2 sensitive hemocyanin may be adaptive to a crab that is air exposed. In my atypical example I again use the blue crab, *C. sapidus*. *C. sapidus* is not noted for its ability to venture onto land. However, as a commercial commodity the blue crab is well known to be able to survive hours and days when air exposed on its way to the market. Survival is especially high when the animals

are kept cool. I have observed another species of *Callinectes*, *Callinectes bellicosus* in the Gulf of California dash out of shallow tide-pools onto land in a seconds-long pursuit of small fiddler crabs. While it is possible that specimens can be isolated on dry land for longer periods, such situations probably represent the exception and not the rule. Nevertheless, the case study of *C. sapidus* is instructive.

When *C. sapidus* is exposed to air for 4 hr, hemolymph lactate increases and pH decreases (deFur *et al.*, 1988). The increase in lactate does not usually accompany air exposure in crabs (Burnett, 1988). Postbranchial hemolymph PO_2 decreases significantly from about 100 torr to 24.4 torr and while PCO_2 was not measured in this study, it is safe to assume that it increases in a pattern similar to the other crabs studied (Fig. 3). The large change in hemolymph pH from 7.7 to 7.5 upon emersion would, in isolation, result in a large right shift of the oxygen equilibrium curve (dotted line in Fig. 3). However, a pronounced lactate effect shifts the curve back to the left. In addition, the oxygen affinity increases in the presence

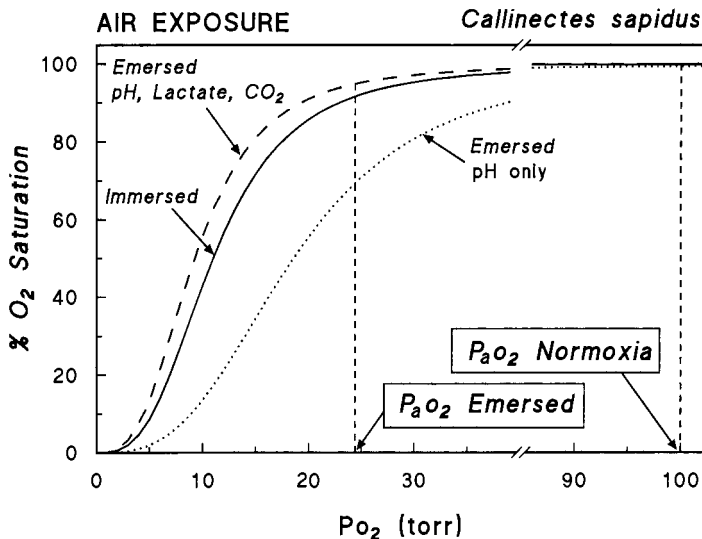


FIG. 3. Exposing *Callinectes sapidus* to air for four hours results in an acidosis, which in isolation would decrease the oxygen affinity (dotted curve). However, the modulating effects of lactate and CO_2 oppose this shift (dashed curve) resulting in little or no net change in oxygen affinity. Values for postbranchial hemolymph P_{aO_2} (P_{aO_2}) in immersed and emersed crabs are indicated. Data are taken from deFur *et al.* (1988).

of molecular CO_2 in this species (Mangum and Burnett, 1986). Assuming that hemolymph PCO_2 increases to about 10 torr (see examples in Burnett, 1988), then P_{50} decreases (oxygen affinity increases) by about 3 torr. Therefore, the modulating effect of both CO_2 and lactate return the oxygen equilibrium curve (dashed line in Fig. 3) to approximately the pre-emersion position.

In the case of the blue crab, resisting a decrease in oxygen affinity allows the circulating hemolymph to continue to be well oxygenated despite the decline in postbranchial hemolymph P_{O_2} . Clearly, in the blue crab, there is some disruption of oxygen supply to the tissues as evidenced by the small increase in hemolymph lactate. deFur *et al.* (1988) have shown that gill lamellae collapse onto one another during air exposure removing them as effective gas exchange organs and perhaps altering or disrupting the flow of hemolymph through the gills. The disruption of access to oxygen of the normally well-ventilated and well-oxygenated gills may result in the production of small amounts of lactate. On the other hand, if the gills are an important site for the oxidation of lactate (Morris and Greenaway, 1989), the reduction of circulation through

the collapsed gills during emersion might easily result in the accumulation of small amounts of lactate in the hemolymph. It would be interesting to measure oxygen uptake in emersed blue crabs.

EXERCISE

One of the best examples of the integrated response of the oxygen transport system to a challenge is in swimming blue crabs. *C. sapidus* is known for its ability to migrate long distances (*e.g.*, Judy and Dudley, 1970). When *C. sapidus* is stimulated to swim in the laboratory, a severe acidosis in the hemolymph is observed where the prebranchial pH changes from 7.6 to 7.1 (Booth *et al.*, 1982). This acidosis is thought to arise largely from the metabolic production of lactic acid, which increases from 0.7 to 9.8 $\text{mmol} \times \text{liter}^{-1}$ after 30 minutes of sustained swimming. The effect of the acidosis on decreasing the hemocyanin oxygen affinity (dotted curve in Fig. 4) is offset largely by the significant increase in hemolymph lactate, which increases the oxygen affinity. The lactate effect greatly attenuates the otherwise large right shift in the oxygen equilibrium curve (dashed curve in Fig. 4). Hemocyanin continues to be highly satu-

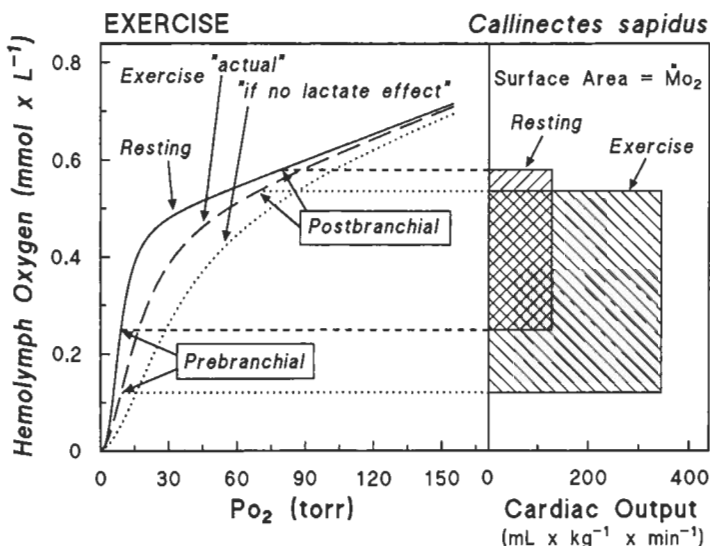


FIG. 4. When *Callinectes sapidus* swims for 30 min, its hemolymph becomes acidotic. The acidosis, if it occurred in isolation, would result in a significant decrease in oxygen affinity (dotted curve). However, the large increase in hemolymph lactate mitigates the effect of the acidosis (dashed curve). The result is an oxygen equilibrium curve that is slightly right-shifted, allowing significant oxygenation to occur at the gills and facilitating the unloading of oxygen at the tissues. These changes, in combination with a large increase in cardiac output, result in a 2.6-fold increase in oxygen uptake (\dot{M}_{O_2}). Data are from Booth *et al.* (1982).

rated with oxygen during exercise. The large venous reserve is tapped effectively, and this, in combination with an increase in cardiac output, results in a large increase in oxygen delivery. The integrated response of the oxygen transporting system can best be seen in Figure 4 by comparing the product of cardiac output and the postbranchial-prebranchial oxygen concentration difference, *i.e.*, the surface areas (=oxygen uptake) before and immediately after exercise. This illustration depicts the mechanisms by which the large increase in oxygen uptake during swimming is accomplished. While, postbranchial PO_2 decreases slightly, the hemocyanin remains 84% oxygenated. Prebranchial PO_2 , on the other hand, remains the same. In this case, the small net shift of the oxygen equilibrium curve to the right functions to tap the venous oxygen reserve. The larger postbranchial-prebranchial oxygen concentration difference during exercise coupled with a significant increase in cardiac output, serves to increase oxygen uptake by 2.6 times.

TEMPERATURE

Nearly all crabs encounter temperature changes in their environment. The responses of the oxygen transport system to temperature changes can occur easily in each of the three case studies mentioned above. And yet few studies have dealt directly with the effects of temperature integrated with other changes in environmental variables. There are enough studies available on the effects of temperature on hemocyanin oxygen affinity that some generalities of temperature effects independent of other effects can be made.

The influence of temperature on hemocyanin oxygen affinity is somewhat variable in crabs. However, when the phenomenon is measured in crabs over a sufficiently large temperature range, some patterns are discernable. Four general responses to acute temperature changes have been documented: 1) temperature insensitivity, *e.g.*, Burnett *et al.* (1988), 2) temperature sensitivity with decreasing sensitivity as temperature increases (Fig. 5A), *e.g.*, Burnett *et*

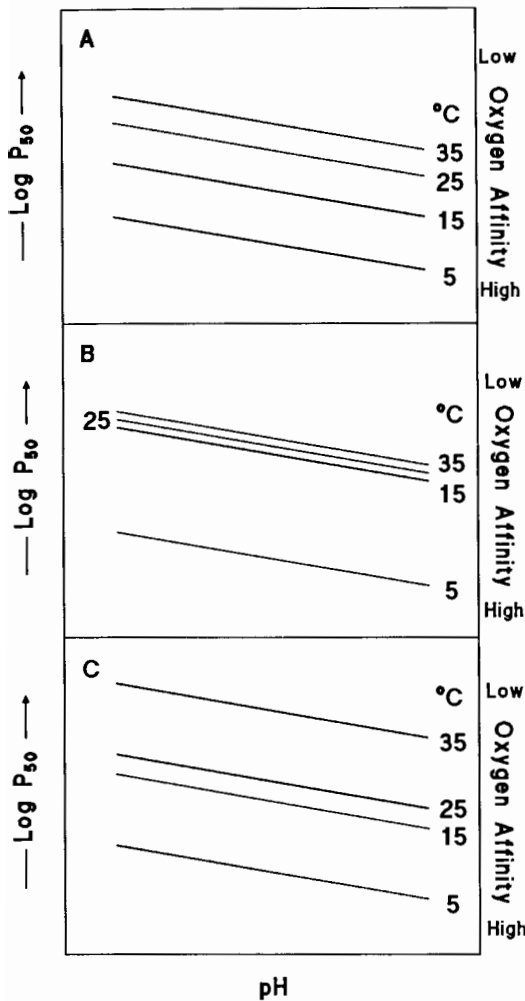


FIG. 5. When large temperature ranges are tested, four general patterns of acute responses are observed. The hemocyanin may be temperature insensitive (not shown); sensitivity decreases as temperature increases (A); maximum sensitivity occurs at low temperature while being greatly depressed at high temperatures (B); or minimum sensitivity occurs in the physiological range (C).

al. (1988), 3) maximum temperature sensitivity at low temperature and much lower sensitivity at high temperature (Fig. 5B), *e.g.*, Burnett *et al.* (1988), and 4) temperature sensitivity with a minimum sensitivity in the physiological temperature range of the crab (Fig. 5C), *e.g.*, Morris and Bridges (1986). Other crab hemocyanins, however, exhibit more complex responses to temperature and some of these responses may be

related to changes in cooperativity (Morris and Bridges, 1989).

The functional meaning of the different patterns is not yet clear. There may be an inverse relationship between the magnitude of temperature sensitivity and the magnitude of the Bohr effect (Burnett *et al.*, 1988). These authors suggest that this may be important to minimize the effect of temperature due to the thermal sensitivity of hemolymph pH. The result of this interaction is to minimize the change in oxygen affinity due to temperature.

The oxygen binding properties of hemocyanin are also influenced by acclimation temperature. Mauro and Mangum (1982) have shown that the oxygen affinity of *C. sapidus* hemocyanin varies seasonally. The oxygen affinity of "winter" crabs is significantly higher than the oxygen affinity of "summer" animals at a common temperature. These authors speculated that the lower oxygen affinity of the summer animals plays an important role in the natural history of the animal in downshifting its overall metabolism to the "hibernating" state with the onset of winter temperatures (5°C). They argued that in the absence of the ability of the crab to make cardiovascular and ventilatory adjustments to change hemolymph PO_2 , less and less oxygen is transported by hemocyanin as temperatures begin to fall resulting in lower rates of oxygen uptake.

With the shift to the higher oxygen affinity "winter" hemocyanin and the lower hemolymph PO_2 one would predict that oxygen uptake could be maintained. But, as Mauro and Mangum (1982) state, "the oxygen affinity of the carrier becomes so high that very little oxygen can be extracted by the tissue" and oxygen uptake at 5°C is only 9% of what it is at 25°C.

During the spring, the high affinity "winter" hemocyanin becomes quantitatively important as temperature and hemolymph PO_2 rises (Mauro and Mangum, 1982). As temperatures continue to rise, however, there is a gradual decrease in the intrinsic oxygen affinity to "summer" hemocyanin. This change assures a functional oxygen equilibrium curve with an adequate venous reserve of oxygen at 25°C.

This seasonal change in the intrinsic properties of hemocyanin was not duplicated in the laboratory within three weeks (Mauro and Mangum, 1982); however, other studies have shown an acclimation phenomenon within days (Truchot, 1975*b*) or weeks (Rutledge, 1981).

DOPAMINE

Finally, the recent discovery by Morris and McMahon (1989) that dopamine can be an important modulator of hemocyanin oxygen affinity has broadened further our definition of an integrated response of an oxygen transport system in crabs. Morris and McMahon (1989) demonstrated that dopamine increases oxygen affinity in the hemocyanin of the Dungeness crab *Cancer magister* at concentrations that are presumably physiological. This effect adds to the lactate effect in this species and presents the possibility of an additional method of physiological control of hemocyanin oxygen affinity. The importance of dopamine as a modulator will become clear with the documentation of its precise physiological levels in the hemolymph and how these levels change in response to stresses. Morris and McMahon (1989) speculate that dopamine, in addition to its interaction with hemocyanin, may stimulate ventilation and circulation. Thus, the importance of dopamine may lie in its great potential to enhance the chemical properties of the oxygen transport system and also affect directly the dynamics of the hemolymph and water convection systems.

SUMMARY

We now know of many variables that are important in rendering the oxygen binding properties of crab hemocyanins labile and responsive to changes in the environment and the behavior of crabs. Complete studies of the oxygen transport systems of crabs must take into account not only the interactions between the ventilatory and cardiovascular systems of crabs, but also the thermal acclimation history and the chemical properties of hemocyanin, which may respond to a variety of extrinsic factors. In addition, environmentally induced structural modifications of hemocyanin can ren-

der interesting and adaptive changes in its intrinsic properties.

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