The role of blood pigments in the delivery of oxygen to tissues

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KURZFASSUNG: **Die Rolle der Blutpigmente für die Sauerstoffversorgung der Gewebe.** Die Art und Weise, in welcher die Hämoglobine als Sauerstoffüberträger im Wirbeltierblut funktionieren, ist wohlbekannt. Die spezifischen Eigenschaften der Wirbeltier-Hämoglobine sind wohl abgestimmt auf die physiologischen Ansprüche der betreffenden Arten und auf deren Umweltsituationen. Studien über die Hämocyanine der Arthropoden und Mollusken dagegen deuten darauf hin, daß das Ausmaß vergleichbarer Korrelationen bei den bisher untersuchten Arten erheblich variiert. Häufig zeigen der Verlauf der Sauerstoffdissoziationskurve und dessen Veränderungen in Abhängigkeit vom pH keine augenfälligen Beziehungen zur Stoffwechselintensität, Umgebungstemperatur oder zum Sauerstoffangebot. Es wird angenommen, daß die Hämocyanine und andere Blutatmungspigmente der Wirbellosen variable Stadien in der funktionellen Evolution dieser Substanzen darstellen, wobei die Primärfunktion bei vielen Wirbellosen die Erhaltung starker Sauerstoffdiffusionsgradienten über die respiratorischen Oberflächen ist. Ladungsspannungen sowie Art und Ausmaß des Bohreffekts sind möglicherweise korreliert mit dem Wirkungsgrad und der Zugänglichkeit der Kiemen, wie auch der Natur des Kreislaufsystems.

INTRODUCTION

The role of hemoglobin in the respiration of the vertebrates is fairly well understood. With a few exceptions (RUUD 1954), this blood respiratory pigment is essential in increasing the oxygen carrying capacity of the blood and in transporting oxygen and carbon dioxide between the tissues and respiratory surface. In addition, the specific properties of the particular hemoglobins are closely related to the metabolic needs of the individual species and to aspects of their environments. This is well illustrated among the fishes, which occupy a variety of habitats and which span a wide range of activity. For example the mackeral, *Scomber* (ROOT 1931), a very active fish living in well aerated marine waters, has hemoglobin which becomes half-saturated at a pressure of 16 mm oxygen, while the bowfin, *Amia* (BLACK 1940), which is relatively inactive and lives in muddy situations, has hemoglobin which becomes half-saturated at 4 mm oxygen. An increase of 10 mm carbon dioxide shifts the half-saturation of mackeral hemoglobin to 52 mm, but that of the bowfin to only 9 mm. Among the invertebrates, however, the relationships of oxygen equilibrium curves and Bohr effects to the physiology and ecology of the organisms is not so clear. I would like to illustrate this with studies on molluscan hemocyanins and suggest that perhaps in many invertebrates the primary function of the blood respiratory pigment may not necessarily be that of increasing the oxygen carrying capacity of the blood.

Among the first quantitative studies on the *in vivo* transport of oxygen by hemocyanin were those of Winterstein in 1909 and Redfield and co-workers (Redfield et al. 1926, Redfield & Goodkind 1929) on the cephalopods. Working with *Octopus vulgaris,* Winterstein found the the post-branchial blood contained about 4.5 volumes per cent oxygen, the pre-branchial blood about 0.4 volumes per cent oxygen. In passing through the gills the hemocyanin became essentially saturated with oxygen and in passing through the tissues delivered about 90 % of this oxygen to the cells of the body. Redfield found very similar quantitative relationships for the blood of the squid, *Loligo pealii.* In addition, he found the hemocyanin of *Loligo* to have a high half-saturation pressure, 36 mm Hg of oxygen at 23° C, and to be very sensitive to pH change. He reported this extreme Bohr effect to be responsible for the release of $^1/_3$–$^1/_4$ of the oxygen carried by the hemocyanin as carbon dioxide diffused into the blood from the tissues. Although the Bohr effect is unusually great, the properties of squid hemocyanin are those that would be expected of an active animal living in open sea water. The results of these investigations indicated that the hemocyanins of the cephalopods served in essentially the same manner as do the hemoglobins of the vertebrates.

As information accumulated, however, certain puzzling facts came to light. The blood of *Limulus,* the horseshoe crab, and certain decapod crustacea had rather low oxygen capacities; ranging from less than twice to about five times the amount that could be dissolved in sea water (Prosser & Brown 1961). The actual oxygen content of the blood of these forms was usually low, sometimes less than could be carried in physical solution (Redfield et al. 1926, Winterstein 1909). Three instances of a reverse Bohr effect were reported. In *Limulus* (Redfield & Ingalls 1933), the horseshoe crab, *Busycon* (Redfield et al. 1926), a marine snail, and *Helix* (Wolvekamp 1932), the garden snail, increasing concentrations of carbon dioxide shifted the oxygen equilibrium curves of their hemocyanins to the left, increasing the oxygen affinity of their hemocyanins rather than decreasing it.

Recent studies on the hemocyanins of the decapod crustacea have shown them normally to be responsible for the transport of most of the oxygen consumed by these organisms, and to have characteristics of a fairly uniform nature (Redmond 1955, 1962; Spoek 1962). The normal Bohr effect, which has been found in all crustacean hemocyanins investigated, appears not to be important in total oxygen exchange but may play a role in local regions of high metabolic activity (Redmond 1955). The characteristics of the hemocyanins of the mollusca, however, vary considerably, often in ways which are difficult to interpret. In a previous study (Redmond 1962) it was found that the hemocyanins of four species of chitons *Acanthopleura granulata, Chiton tuberculatus, Mopalia mucosa,* and *Katherina tunicata,* had unexpectedly high half-saturation pressures. Yet the chitons are not by any means active animals. The behavior of the hemocyanins of these species toward changing pH varied considerably. Although all were rather insensitive to small pH changes, two showed a normal Bohr effect and two a reverse Bohr effect. Manwell (1958) reported the hemocyanin of the giant chiton, *Amicula stelleri* (= *Cryptochiton*), to show no Bohr effect within a physiological range of pH. Like the other species, the hemocyanin of *Amicula* has a relatively high loading pressure.

RESULTS AND DISCUSSION

Figures 1 and 2 show representative oxygen equilibrium curves for the hemocyanins of two species of marine snails, *Fasciolaria tulipa* from the west coast of Florida, and *Fusitriton oregonensis* from the Northwest coast of the United States.

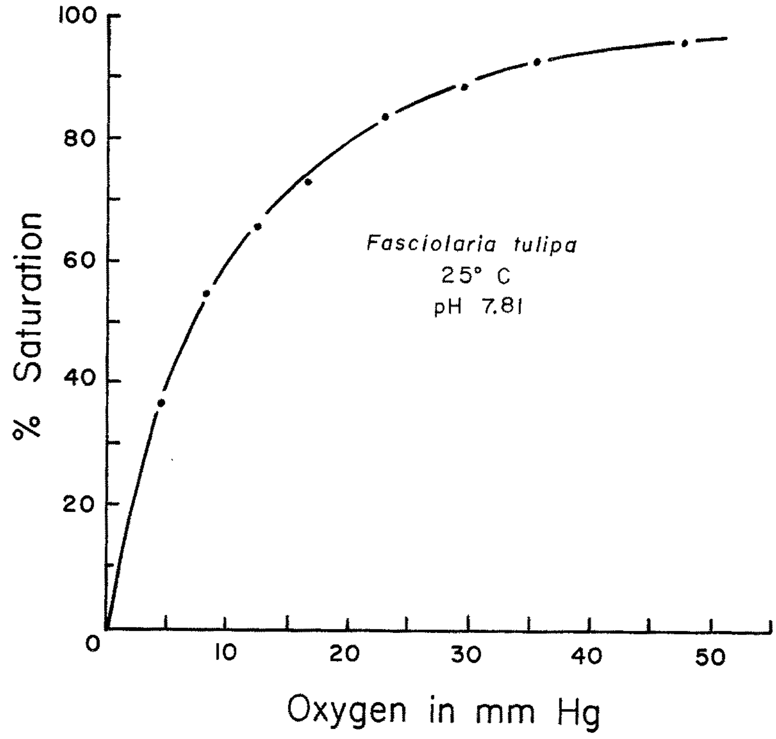

Fig. 1: Oxygen-equilibrium curve of the hemocyanin of the marine snail, *Fasciolaria tulipa*

These and succeeding curves were obtained by the vacuum pump-spectrophotometric method described elsewhere (Redmond 1955). Traces of Tris buffer (1 g Tris: 10 ml sea water) were used to adjust the pH of the blood to desired values. The oxygen affinities of these hemocyanins are greater than those of the cephalopods and chitons. At 25° C and at physiological pH, the half-saturation pressure of *Fasciolaria* and *Fusitriton* hemocyanins is approximately 7 mm Hg oxygen pressure. *Fusitriton* is a cold water species so that at environmental temperatures its oxygen loading tensions are even lower. The low loading pressures of these hemocyanins correspond to what may have been expected of the blood pigments of rather sluggish animals.

Figures 3 and 4 illustrate the effect of changing pH on the half-saturation pressures of the hemocyanins of *Fasciolaria* and *Fusitriton*. In both species a very marked inverse Bohr effect occurs. The dotted vertical lines in Figure 4 indicate a normal range of blood pH in *Fusitriton*. The pH of the blood of ten specimens of *Fusitriton* ranged from 7.73 to 7.9. These blood samples were taken underwater by quickly inserting a hypodermic needle into the extended foot of a snail and withdrawing 1–2 ml of blood from the pedal sinus. Only samples taken quickly and easily were used. The average pH of the ten samples was 7.81. Fewer measurements on *Fasciolaria* indicate a similar blood pH. The hemocyanins from three other species of marine snails, *Livona pica* from Jamaica, W. I., *Melongina corona* and *Busycon contrarium* from Florida, similarly all possess an inverse Bohr effect, so that this phenomenon appears to be a common characteristic of this group of organisms.

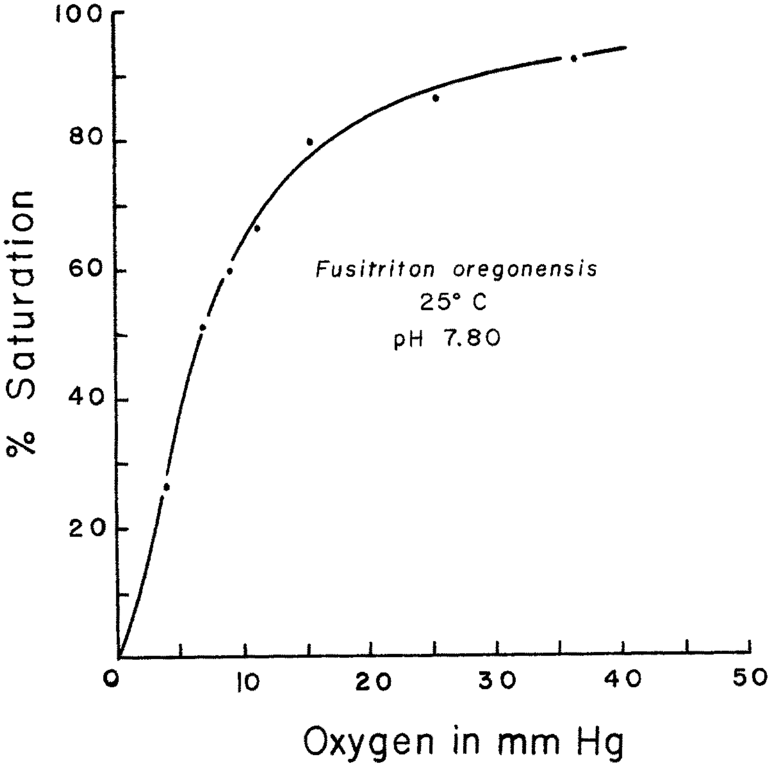

Fig. 2: Oxygen-equilibrium curve of the hemocyanin of the marine snail, *Fusitriton oregonensis*

It has been suggested (Redmond 1962) that many of the preceeding observations may be understood in terms of a hypothetical functional evolution of blood respiratory pigments which consisted of the following general steps.

When these substances first appeared they were probably found intracellularly where they served to speed the diffusion of oxygen. As Scholander (1960) and Hemminsin & Scholander (1960) have demonstrated, oxygen will move along a concen-

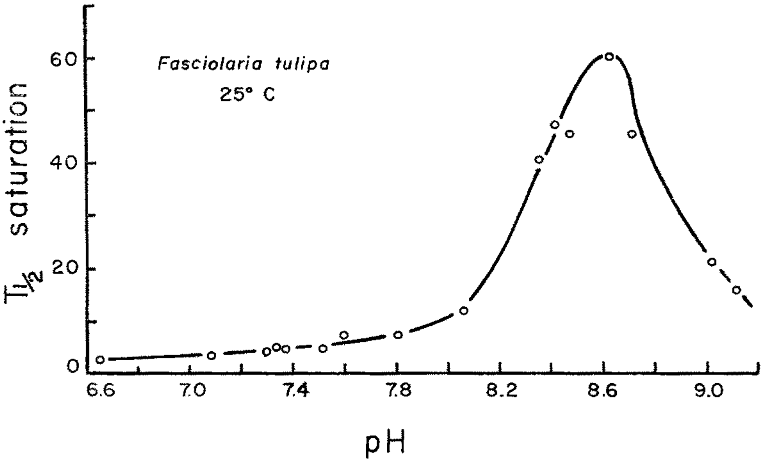

Fig. 3: The effect of pH on the oxygen pressure at which the hemocyanin of *Fasciolaria* becomes half-saturated. The vertical scale is in mm Hg oxygen pressure. Normal blood pH is approximately 7.8

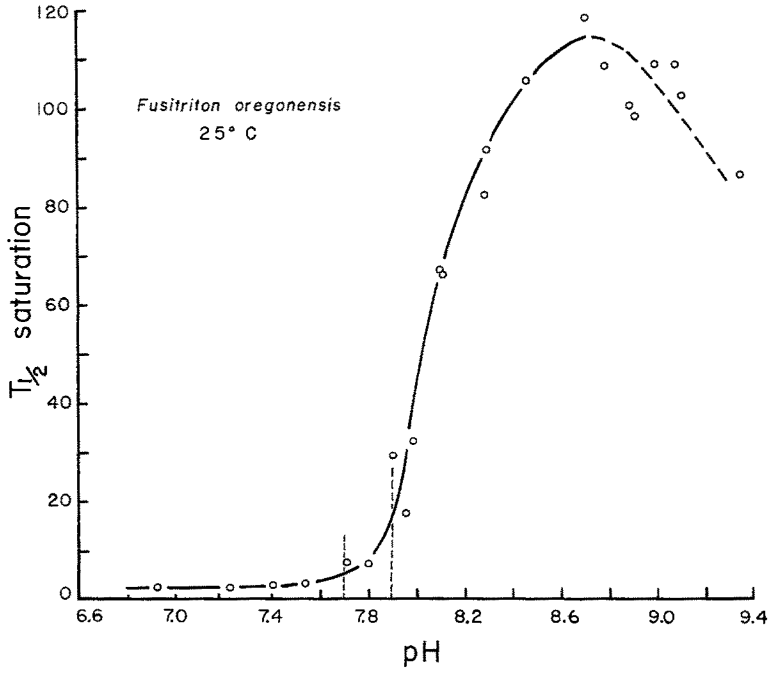

Fig. 4: The effect of pH on the oxygen pressure at which the hemocyanin of *Fusitriton* becomes half-saturated. The vertical scale is in mm Hg oxygen pressure. The two dashed vertical lines enclose a normal range of blood pH. The scatter of points above pH 8.6 suggests that here the hemocyanin is becoming denatured

tration gradient through a solution of hemoglobin much more rapidly than through water alone.

A likely second stage in the functional evolution of these pigments occurred when they moved into extracellular fluids. Here they would help to speed the movement of oxygen from the respiratory surface to the tissues. This would occur whether or not the pigments were circulated, although circulation would greatly increase their efficiency. Another, and in my opinion very important, function of a blood respiratory pigment in such organisms is to maintain a large oxygen diffusion gradient across the respiratory surface. By combining with the oxygen as soon as it enters the blood, the pigment prevents the internal oxygen tension from quickly rising. The large oxygen pressure gradient in turn maintains a high rate of diffusion into the blood so that for a given time interval more oxygen enters the blood than would in the absence of the pigment. While it is impossible to separate this function from that of increasing the oxygen capacity of blood, the low oxygen content reported for *Limulus* and some crustaceans strongly suggests that in many instances the first may be the more important.

Finally, in larger and more active species, greater metabolic demands resulted in the selection of greater concentrations of pigment to further increase the oxygen carrying capacity of the blood and in those special properties, such as the appropriate Bohr effect and most effective loading and unloading tensions, which aid in the pickup and delivery of oxygen. This is the condition exemplified by the blood respiratory pigments of the cephalopods and vertebrates.

If these ideas are partially correct, then the previously described properties of the molluscan hemocyanins may perhaps be rationalized as follows. In the case of the chitons, the respiratory demands are relatively small and there have not been great selective pressures for the hemocyanins to meet critical specifications. The exposed situation of the large gills and the presence of unlimited quantities of well aerated sea water provide a plentiful supply of oxygen. The hemocyanin here would serve principally to maintain a high diffusion gradient across the gills. The loading tension would not be important so long as it was reasonably low but still above the operating pressures of the tissues. The pH change that normally occurs in the blood between tissues and gills must be very small due to the small carbon dioxyde exchange of each circulatory cycle, therefore, the kind and extent of Bohr effect is probably not important. However, since certain of the chitons are subject to periodic exposure during low tides, the possibility exists that the relative insensitivity of their hemocyanins to pH may be of adaptive significance. This point should be tested by measurements of blood pH under field conditions.

In the snails, the hemocyanins investigated had greater oxygen affinities, that is, their loading tensions were lower. This may be an adaptation to the more restricted flow of water across their gills due to the internal location of the gills in the mantle cavity. Because of the lower oxygen pressure maintained in the blood, the greater diffusion gradient would speed the entrance of oxygen into the blood and would partially offset the more restricted flow of water. The absence of a blood respiratory pigment in many of the shelless gastropods, such as the nudibranchs and tectibranchs, may be related to the large body surface available for gas exchange in these forms.

The large reverse Bohr effect that the hemocyanins of many snails exhibit is more difficult to explain. Since it occurs in forms which are found on wave swept rocks as well as in forms which inhabit mud flats, it does not seem to be related to oxygen loading under conditions of high external carbon dioxide. If the blood does become considerably more acid at times, for example during a long period when the snail is withdrawn into its shell, when the gills are again exposed to oxygenated water, oxygen will at first enter the blood more rapidly than usual due to the leftward shift of the oxygen equilibrium curve. However, as carbon dioxide is cleared from the blood the rate of diffusion will return to normal and the slight saving in loading time would not seem great enough to be of physiological significance. A more likely role of the inverse Bohr effect is that under certain circumstances it may aid in the distribution of oxygen internally. Inevitably certain tissues metabolize more rapidly than others, and carbon dioxide concentrations may consequently become somewhat greater in their vicinity. The open circulatory system of the snail is such that the flow of blood past tissues is partially a function of gross body movements and is likely to be somewhat erratic. This would be especially true if the snail were retracted into its shell. In the absence of adequate blood flow, increasing concentrations of carbon dioxide locally would shift the oxygen equilibrium curve of the surrounding hemocyanin to the left and should result in a flow of oxygen into that region from adjacent regions of higher pH. Thus the oxygen diffusion gradients would be augmented which would increase the supply of oxygen to those tissues with greater oxygen requirements. It may be significant that reverse Bohr effects have not be found in species with closed circulatory systems. In closed systems, with the blood flowing continuously past the tissues, such an effect would tend to withhould oxygen from the more rapidly metabolizing tissues.

I would like to emphasize that much of what I have suggested is highly speculative and should be viewed with skepticism. Whether or not the specific ideas presented here have any value remains to be seen. I do believe that present evidence indicates that the detailed manner in which blood respiratory pigments aid in respiration varies among the different species of animals and that only by the study of a variety of organisms may the functioning of these substances become understood.

SUMMARY

1. The detailed manner in which the blood respiratory pigments of many invertebrates function is not well understood.
2. The hemocyanins of the cephalopods studied appear to function in gas transport much like the hemoglobins of the vertebrates although these hemocyanins possess a much larger Bohr effect.
3. The properties of the hemocyanins of the crustacea are fairly uniform but those of the mollusca vary considerably.
4. The hemocyanins of the chitons have relatively high half-saturation oxygen pressures. They may show a normal or reverse Bohr effect, or none at all. Regardless of the direction of the effect, however, these hemocyanins are relatively insensitive to small changes of pH.

5. The hemocyanins of marine snails studied have lower half-saturation pressures and all exhibited a reverse Bohr effect.
6. It is suggested that: *(a)* the properties of the hemocyanins are related to metabolic rate, accessibility and effectiveness of the gills, and the nature of the circulatory system; *(b)* an important function of many invertebrate blood pigments is to maintain a large oxygen diffusion gradient across the respiratory surface; *(c)* the reverse Bohr effect may aid in the internal distribution of oxygen to more rapidly metabolizing snail tissues when circulation is slowed or stopped.
7. A possible functional evolution of blood respiratory pigments is described.

ACKNOWLEDGMENTS

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Discussion following the paper by REDMOND

WIESER: The reversed BOHR-effect might aid the retention of oxygen in animals that by virtue of their calcium carbonate shell and their living in the intertidal are liable to develop low pH body fluid during low tide.

REDMOND: The relative insensitivity of chiton hemocyanins could possibly be of adaptive significance during periods when the chitons are exposed to air. I doubt that it is of importance in conserving the oxygen present in the blood when exposure first occurs since this oxygen should be used up before the blood pH changes appreciably. Possibly the pH insensitivity maintains the oxygen-equilibrium curve in a functional position while the animal is exposed to air. I imagine that the hemocyanin continues to transport some oxygen even though the chiton is out of water.

URICH: Nach MANWELL (1960; *Ann. Rev. Physiol.* **22**, 191–244) soll die Funktion mancher Blutfarbstoffe mit hoher Sauerstoffaffinität darin bestehen, die Gewebe vor hohen O_2-Partialdrucken zu schützen.

REDMOND: Certain species of animals, which normally live under conditions of very low oxygen pressures, for example, the nematode *Ascaris* and the annelids *Tubifex* and *Travisia*, appear to be adversely affected by normal oxygen pressures. Many of these possess a respiratory pigment in one or more of the body fluids. As MANWELL has suggested, one function of their blood respiratory pigments may be to prevent high internal oxygen pressures. These animals usually can exist anaerobically for long periods of time so that delivery of oxygen to the tissues does not seem to be an essential function of the pigments.