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Crustaceans as experimental animals for metabolic and transport physiology

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Abstract

This paper develops briefly four examples illustrating the use of crustaceans as experimental animals in the field of metabolic and transport physiology. In cells of euryhaline crabs, organic substances such as free amino acids contribute much more than inorganic ions to osmotic adaptation, a feature that is now realized to be of general importance in living organisms. Anhydrobiotic and anaerobically dormant embryos of the brine shrimp *Artemia* provide very convenient models to study cellular control of metabolic depression. Studies on amphibious crabs have highlighted the constraints imposed upon gas exchange and acid-base balance by the very contrasted properties of oxygen and carbon dioxide in water and in air. Finally, the blood oxygen transport system of decapod crustaceans has been a model of choice to demonstrate the significance of an increased oxygen affinity for adaptation to environmental hypoxia. These examples illustrate the role played in the past as well as the great future potential of crustaceans as experimental animal models for general studies in Comparative Physiology.

Keywords: Air breathing, cellular volume, compatible osmolytes, crustaceans, hemocyanin, hypoxia, metabolic depression, water breathing.

Les Crustacés en tant que modèles expérimentaux pour des études générales en physiologie du métabolisme et des transports de gaz et d'ions.

Résumé

Le présent article développe brièvement quatre exemples d'études utilisant les Crustacés et se rapportant à des problèmes généraux de physiologie du métabolisme et des transports de gaz et d'ions. C'est sur les cellules de crabes euryhalins qu'a été démontrée l'importance de la contribution des osmolytes organiques tels que les acides aminés à l'adaptation osmotique, une caractéristique maintenant reconnue comme générale dans le monde vivant. Les embryons déshydratés et dormants d'*Artemia* ont fait progresser sensiblement nos connaissances sur les contrôles cellulaires des phénomènes de dépression métabolique. Des études sur les crabes amphibies ont permis de renforcer l'idée que les différences d'état acide-base interne entre les animaux aquatiques et aériens sont liées aux propriétés différentes de l'oxygène et du dioxyde de carbone dans l'eau et dans l'air. Enfin, l'étude des réponses à l'hypoxie du système de transport de l'oxygène par le sang chez les Crustacés Décapodes démontre de façon beaucoup plus évidente que chez les Mammifères l'intérêt adaptatif d'une augmentation de l'affinité du pigment respiratoire pour l'oxygène en environnement hypoxique. Ces exemples illustrent le rôle joué par les Crustacés ainsi que leur potentiel futur en tant que modèles animaux pour des études générales en physiologie comparée.

Mots-clés : Crustacés, dépression métabolique, hémocyanine, hypoxie, osmolytes compatibles, respiration aérienne, respiration aquatique, volume cellulaire.

INTRODUCTION

Crustaceans form a well characterized zoological group with at least one particularly original trait, i.e. the presence of a calcified, and thus inextensible cuticle. As everybody knows, this feature imposes a discontinuous growth with periodical shedding of the exoskeleton and the consequence that many aspects of crustacean physiology vary cyclically with moulting. For this reason and many others, crustaceans are certainly worthy of study for themselves. However, beyond such specific aspects of their biology, crustaceans have also been largely used in many studies aimed at elucidating more general problems. This is certainly because, as already recognized by Claude Bernard (who himself worked on the "glycogenic substance" in the crab's digestive gland), there is always in the animal world the most appropriate species or group to address every general question. Crustaceans are no exception and have indeed often served, and continue to serve, as experimental animals for a variety of general biological studies.

It should, however, be noted that most of these studies have been done on higher crustaceans, mainly Decapods. The reason is that decapod crustaceans display a number of distinctive advantages that make them nicely suitable for physiological research. Many Decapods first have a relatively large size compared to other invertebrates. This makes the amount of available biological material rarely limiting for analyses. A similar advantage is also found in non-decapod crustaceans such as the brine shrimp *Artemia* (see below) that can be obtained by mass culture, or that produce large number of eggs. Secondly, in contrast to the soft body of many invertebrates, the rigid exoskeleton of decapods is very convenient for durably fastening recording equipment, sampling catheters, etc. Thirdly, as a consequence of their open circulatory system, decapod crustaceans have a large relative blood space, allowing sampling of comfortable volumes without undue disturbances to the animals. A drawback, however, is the presence of a very efficient coagulation system in many species, and the lack of any known appropriate anticoagulant substance. Last but not least, decapod crustaceans offer a great variety of lifestyles and habitats, in an otherwise relatively homogeneous group. This is very suitable for comparative studies, particularly in relation to environmental adaptation.

In the following, I would like to take a few examples from the fields of metabolic and transport physiology, in order to illustrate the use of crustaceans in such general and comparative studies.

CELLULAR ISOSMOTIC REGULATION

Many marine invertebrates are euryhaline and behave either as conformers or imperfect osmoregulators with regard to their extracellular fluid. Despite large extracellular osmotic changes, isosmoticity of the intracellular fluid is, however, closely regulated in order to maintain a constant cell volume, this often implying large changes in cellular content of osmotically-active substances. Among these substances, the now well-known major contribution of organic osmoeffectors, principally free amino acids, to this so-called "intracellular isosmotic regulation" was first discovered in crustaceans by the pioneering studies of the Liège School of Comparative Physiology headed by Florkin (Florkin and Schoffeniels, 1969; Schoffeniels, 1976; Gilles, 1988).

In many crabs and shrimps exposed to dilute media, cell concentrations of inorganic ions decrease rather moderately and the bulk of the reduction of intracellular osmolality is accounted for by organic compounds, mainly free amino acids. The reverse changes occur upon increase of ambient salinity. For example, in muscles of the blue crab, *Callinectes sapidus*, transferred from full strength to half strength seawater, the decrease of intracellular concentrations of inorganic ions and free amino acids account for 17% and 83%, respectively, of the total change of cellular osmolality (*fig.* 1) (Gerard and Gilles, 1972).

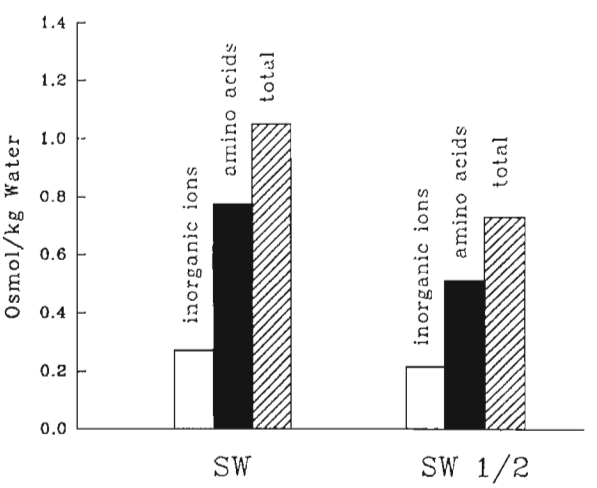

Figure 1. – Intracellular concentrations (in Osmol/kg water) of inorganic ions and free amino acids in muscle fibres of blue crabs *Callinectes sapidus* acclimated to full strength and half strength seawater (Data from Gerard and Gilles, 1972).

What is now realized is that cellular osmotic adjustments by changes of organic osmoeffectors rather than inorganic ions is absolutely general in all cell systems tolerating large osmotic stresses. Organic effectors such as neutral amino acids, polyols (sorbitol, glycerol, inositol, etc.), quaternary amines (betaine for example) have been referred to as "compatible osmolytes" (see Yancey *et al.*, 1982) because, in contrast with inorganic electrolytes, they do not perturb the structure and function of proteins, enzymes and other biological macromolecules. Thus, cellular isosmotic regulation is accomplished such as

to keep electrolyte levels as low as possible while changing the concentration of compatible organic osmoeffectors. This applies even to mammals in which plasma osmolarity is tightly regulated, but where at least one category of cells, those of the renal medulla, are exposed to large osmotic changes according to the concentration of the urine. In the renal medulla of antidiuretic rats for example, extracellular osmolarity may increase up to 2.5 Osmol. Extracellular electrolytes amounts to approx. 1 000 mOsmol, but their concentration in the collecting duct cells remains much lower, about 400 mOsmol, isosmoticity being maintained by accumulation of organic substances such as sorbitol, betaine, inositol, glycerophosphorylcholine and probably also free amino acids (Law, 1991; see also Garcia-Perez and Burg, 1991). Quaternary amines such as betaine and GPC are also known as "counteracting organic osmolytes", (see Yancey *et al.*, 1982) meaning that they provide a protection against harmful effects of urea, which is also accumulated at high concentrations in the renal medulla during antidiuresis.

The mechanisms at work to control the levels of cellular organic osmolytes remain largely unexplored. They may involve modulation of gene expression, regulation of metabolic pathways as well as membrane transport. We can hope that euryhaline crustaceans will continue to serve as favoured models for such studies.

METABOLIC DEPRESSION: THE BRINE SHRIMP EMBRYO AS A MODEL

In the face of environmental stresses of various origins: low oxygen, low temperature, low water availability etc., many animals are able reversibly to depress their metabolic rate and to enter physiological states variously known as facultative anaerobiosis, anhydrobiosis, hibernation, estivation, torpor, diapause etc. Such quiescent states allow organisms to preserve their fuel and energy reserves when exposed to unfavorable conditions for periods of unpredictable durations. Since reversible reduction of physiological activities and hypometabolism are common denominators in all these situations, it is widely held that the molecular mechanisms controlling metabolic depression are similar in all animals and have been conserved across phylogenetic lines (see Storey, 1988).

An extreme example of the ability to undergo reversible metabolic depression is found in encysted embryos of a crustacean, the brine shrimp *Artemia franciscana*. Two situations in fact lead to hypometabolic states in this animal, both being fully reversible: cyst dehydration leading to anhydrobiotic embryos, and oxygen deprivation of normally hydrated cysts, inducing a state called anaerobic dormancy. In both cases, microcalorimetric studies have shown that the metabolic rate falls rapidly to no more than a few per cent of the active rate, with an almost complete cessation of carbohydrate metabolism (Hand and Gnaiger, 1988; Glasheen and Hand, 1989). The trigger to hypometabolism in anhydrobiotic embryos seems to be linked to cell dehydration and this has led to interesting considerations about the physical state of cell water (Clegg, 1981; 1984). Recent work has also shown that dehydrated embryos are rich in "compatible" organic solutes, which may help to keep the integrity of macromolecular structures (Glasheen and Hand, 1989).

The shift to a hypometabolic state during anaerobic dormancy is characterized by an important decrease of intracellular pH (7.9 to 6.3) which appears to be the primary effector mediating depression of energy production (Busa *et al.*, 1982; Busa and Crowe, 1983). Developing *Artemia* embryos use trehalose as the main metabolic fuel. Trehalose breakdown is stopped not only during anaerobiosis but also after induction of cellular acidosis by exposure to high carbon dioxide concentration in presence of oxygen. On the contrary, exposure of anaerobically dormant cysts to low concentrations of ammonium salts, a treatment known to increase cell pH, leads to resumption of both energy production and trehalose utilization (Hand and Gnaiger, 1988). These controls have been shown to occur at three pH-sensitive reactions: trehalase, hexokinase and phosphofructokinase (Carpenter and Hand, 1986). The most important controlling enzyme, trehalase, exists in two catalytically active assembly states differing in kinetic properties. Cell acidification converts the dissociated enzyme into a polymerized form which is inhibited by acidic pH, ATP and the substrate trehalose (Hand and Carpenter, 1986). A decrease of cell pH is also known to accompany metabolic depression in many other systems, particularly hibernating mammals (Malan *et al.*, 1985).

Dormant *Artemia* embryos can remain in a hypometabolic state for months or years (Dutrieu and Chrestia-Blanchine, 1966; Clegg and Jackson, 1989). Since no obvious repayment of an oxygen debt exist during postanoxic recovery, there is a possibility that energy metabolism of anoxic embryos could be brought to a complete standstill (Clegg, 1992, 1993). If this were the case, *Artemia* embryos would not only serve to understand how metabolic depression is controlled but also be the model of choice to test the possibility for a living structure to be maintained in absence of free energy input.

PHYSIOLOGICAL CHANGES ON TRANSITION FROM WATER-TO AIR BREATHING

A further example illustrating use of crustaceans as experimental animals in physiological research stems from the great diversity of habitats occupied by these animals. It concerns the general problem of the transition from life in water to life on land and

in air, two completely different media that have been colonized by crustaceans. This transition affects many functions and particularly respiratory gas exchange as exemplified by two basically different designs of the respiratory system gills in water and lungs in air. Classically, this transition has been known to have occurred on a **phylogenetic** basis such as during vertebrate evolution, and, on a shorter time-scale, on an **ontogenetic** basis, such as during amphibian metamorphosis. Using crabs living in the marine intertidal zone, it has been realized more recently that at least some physiological changes typical of this transition also take place on an **ecological** basis when these animals are alternatively exposed to water and to air during a tidal cycle (see Truchot, 1987).

Let me first recall one of the most fundamental differences between water and air, i.e. that concerning the availability of (or ease of release of) respiratory gases, oxygen and carbon dioxide. The slope of the relationship between concentration C and partial pressure P, $\Delta C/\Delta P$, called the capacitance coefficient, is much higher for oxygen in air compared to water, whereas that for CO_2 is approximately the same in both media and thus is much higher than that of O_2 in water. This is because oxygen is poorly soluble and thus hardly available in water, while CO_2 is easily excreted thanks to its high solubility. This also means that internal CO_2 pressures will be kept much lower in water breathers than in air breathers. Another important aspect is that gill irrigation, sometimes called gill "ventilation" by analogy to lung ventilation, is regulated mainly according to oxygen requirements in water breathers. As a consequence, specific ventilation, i.e. the ventilatory flow rate normalized to oxygen consumption, is at least 10-fold greater in water breathers living in normally oxygenated water than in air breathers respiring at sea level (see Dejours, 1981; 1988). This also helps CO_2 excretion in water, contributing to the maintenance of low internal Pco_2 values during aquatic respiration.

Now, at the shift from water- to air breathing, internal Pco_2 must increase and this is actually seen in both phylogenetic and ontogenetic transitions (*fig.* 2). Arterial blood Pco_2 ranges from 0.5 to 4 Torr in water breathers respiring well aerated water while values from 6 to 45 Torr are found in air breathers (see Truchot, 1987). Such differences apply to both vertebrates and invertebrates. Similarly, the water-breathing tadpole maintains blood Pco_2 at about 3 Torr, this value increasing to 13 Torr in the adult frog after metamorphosis (Erasmus *et al.*, 1970). Such a large difference in blood Pco_2 between water- and air breathers would normally result in a considerable decrease of blood pH. However, acid-base regulatory mechanisms, whose nature is just beginning to be understood, maintain pH almost unchanged during the transition, thanks to an appropriate increase in the blood bicarbonate concentration (*fig.* 2).

These physiological changes are also just those observed during a simulated tidal emersion in the green crab, *Carcinus maenas* (Truchot, 1975). In this situation, blood Pco_2 increases from 1 Torr in water to 4-5 Torr in air (*fig.* 2). After a 4-hour emersion, blood pH had decreased by 0.25 units (not shown) but this acidosis was progressively compensated during the following hours by a corresponding increase of the blood bicarbonate concentration (*fig.* 2). Similar responses have been described in a number of intertidal crustaceans (Burnett, 1988) that are not at all terrestrial animals but that can withstand tidal emersion for many hours. This nicely illustrates the point that not only the structural design of the respiratory organs, but also most functional traits and their regulations as well, are determined according to the physical properties of the ambient media, air and water.

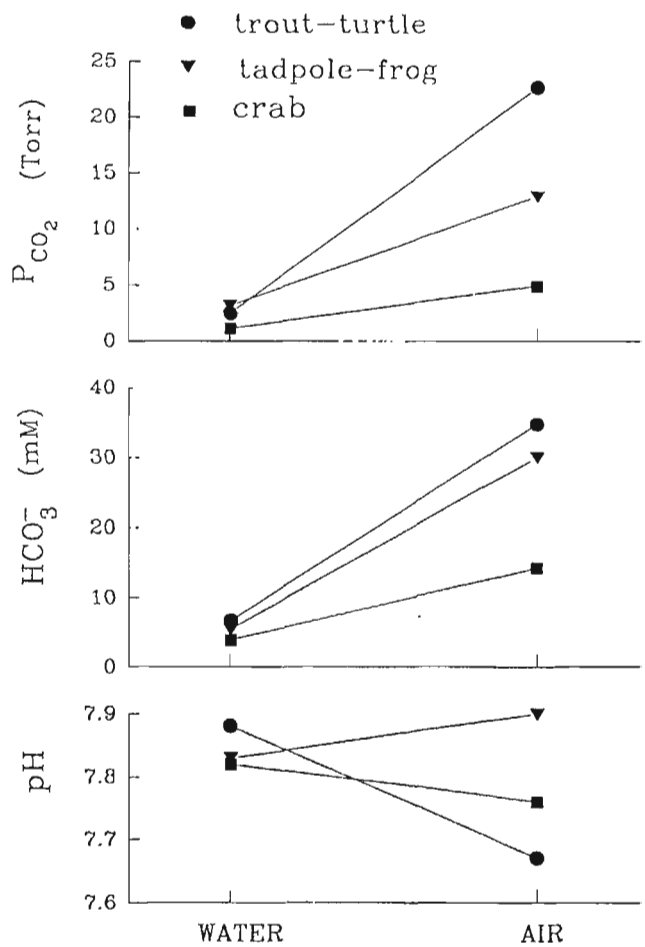

Figure 2. – The changes in blood acid-base parameters (pH, bicarbonate concentration and carbon dioxide partial pressure Pco_2) at the transition from water- to air breathing. Phylogenetic transition during vertebrate evolution is illustrated by values for trout and turtle (from Truchot, 1987); values for frog tadpole and adult show an ontogenetic transition (from Erasmus *et al.*, 1970); emersion at low tide of the shore crab *Carcinus maenas* entails similar acid-base changes during an ecologic transition (Truchot, 1975).

ADAPTATION OF BLOOD OXYGEN TRANSPORT DURING AMBIENT HYPOXIA

In many animals, the most common short-term response to reduced ambient oxygen is a hyperventilation that makes more oxygen available to the gill or lung respiratory exchange surfaces. In addition, it has long been suspected that a change of oxygen affinity of the blood transporting pigment is also instrumental to this adaptation. Such a change, corresponding to a shift of the oxygen dissociation curve along the Po_2 scale, could be brought about either by the Bohr effect via the increase of blood pH that results from the hyperventilatory response, or by allosteric cofactors controlling O_2 binding, for example intraerythrocytic organic phosphates for vertebrate hemoglobins, namely 2,3 diphosphoglycerate (2,3 DPG) in mammals which decreases O_2 affinity. In fact, it has long been controversial whether a left or a right shift of the oxygen dissociation curve is adaptive during hypoxia in Vertebrates, for example upon high altitude exposure in humans. This is mainly because the vertebrate respiratory gas transport system involves a number of

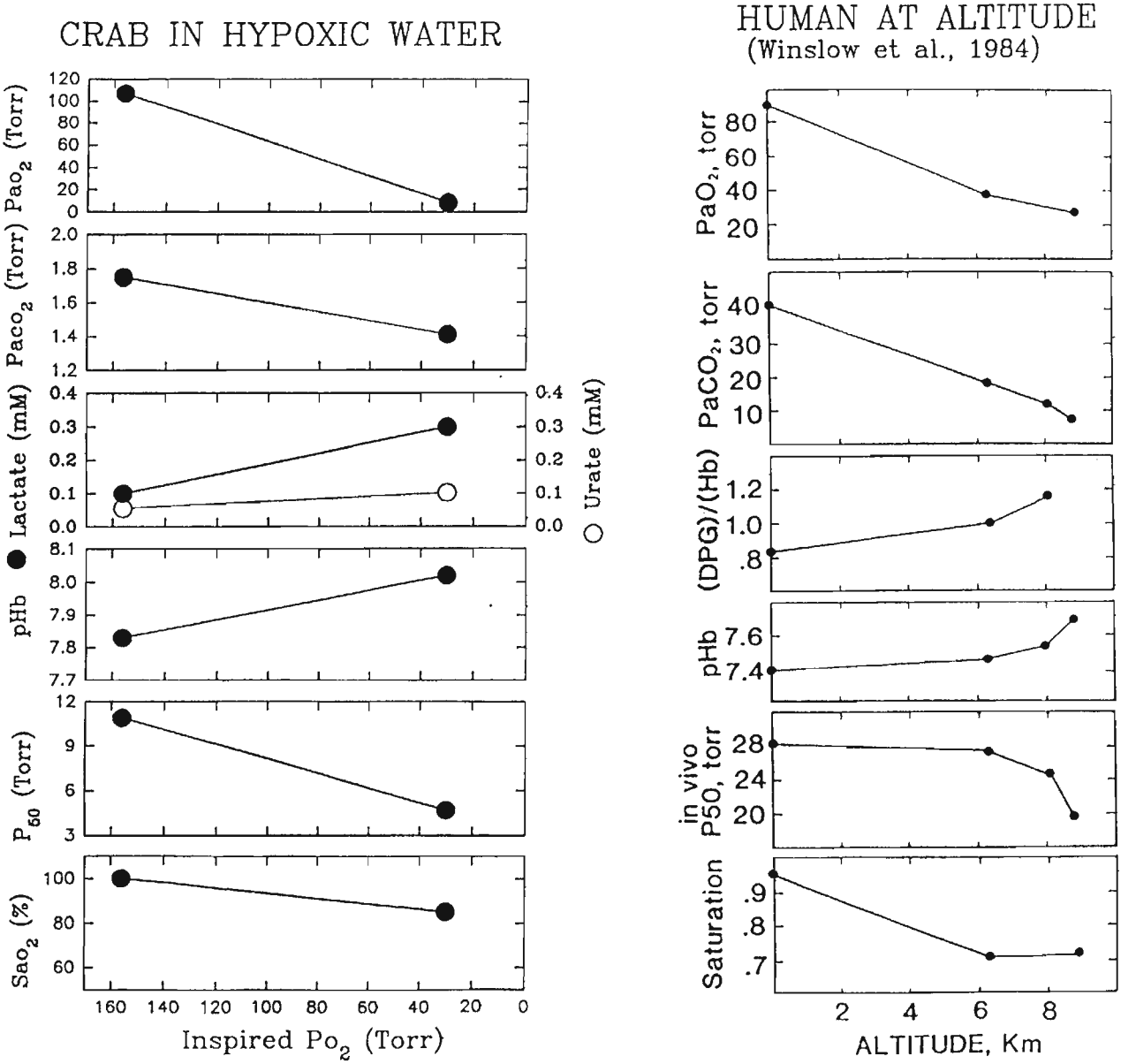

Figure 3. – The adaptation of the blood oxygen transport system to a hypoxic environment compared in the shore crab *Carcinus maenas* (from Lallier and Truchot, 1989) and human at altitude (from Winslow *et al.*, 1984). From top to bottom : oxygen and carbon dioxide partial pressures in arterial blood (Pao_2 and $Paco_2$); concentration of allosteric effectors, lactate and urate in crab, 2,3 diphosphoglycerate (DPG) in human; blood pH; *in vivo* O_2 partial pressure at 50% saturation of the oxygen binding sites (P_{50}); and saturation of arterial blood with oxygen (see text for details).

complexities that makes interpretation of experimental data ambiguous. In contrast, crustaceans provide a much simpler system that allows a more clearcut answer to the same question.

A brief comparison of the oxygen transport systems of crustaceans and mammals is given in *table* 1. The crustacean respiratory pigment hemocyanin is dissolved in the hemolymph rather than contained in blood cells, and this avoids complex effects linked to red cell physiology. Hemocyanin-O_2 binding is nevertheless physiologically controlled by at least two known allosteric cofactors: L-lactate ions (Truchot, 1980), which are the only end product of carbohydrate anaerobic metabolism in crustaceans, and urate ions (Morris *et al.*, 1985). Both cofactors are released to the blood during hypoxia and they increase the O_2 affinity instead of decreasing it as is the case for 2,3 DPG in mammals. Thus, allosteric cofactors and the Bohr effect via the alkalosis resulting from hyperventilation in hypoxia act synergistically to shift the dissociation curve to the left in crustaceans, while their effects are antagonistic in mammals.

Table 1. – A brief comparison of the blood oxygen transport system in Crustaceans and Mammals.

	Crustaceans	Mammals
Respiratory pigment	Hemocyanin Dissolved	Hemoglobin Intracellular
Allosteric cofactors	L-lactate Urate . . .	2,3-DPG
	Increase O_2 affinity	*Decrease* O_2 affinity
Bohr shift	Normal (left shift at increased pH)	Normal

Physiological consequences of these adjustments have been documented in detail in the shore crab *Carcinus maenas* (Lallier and Truchot, 1989; Truchot and Lallier, 1992) and they are summarized in *figure* 3. In hypoxic water, oxygen partial pressure in arterial blood decreases, but high O_2 saturation of the oxygen carrier can be maintained thanks to an increase in hemocyanin-oxygen affinity (i.e. a decrease of the oxygen partial pressure at half saturation P_{50}) brought about by increased blood pH and accumulation of blood lactate and urate (*fig.* 3). This left shift of the oxygen dissociation curve adaptively keeps unchanged the oxygen transport capacity of the hemocyanin during ambient hypoxia.

These physiological responses can be compared to those of human exposed to altitude hypoxia (*fig.* 3). At moderate altitude, the main response is an increase of red cell 2,3 DPG shifting the O_2 dissociation curve to the right (not shown). The adaptive advantage of this adjustment is not clear. But, at extreme altitude (6 to 8 km), strong hyperventilation and ensuing severe blood alkalosis allows the Bohr effect to surpass the DPG effect, resulting in a left shift of the O_2 dissociation curve (i.e. decrease of P_{50}). As in the crab, high O_2 saturation of the arterial blood can be maintained (*fig.* 3), preserving the hemoglobin O_2 transport function (Winslow *et al.*, 1984). This unambiguously demonstrates the adaptive advantage of a left shift in conditions of severe ambient hypoxia.

CONCLUSION

As stated recently by Weibel and Taylor (1992), Comparative Physiology as a research strategy seeks to derive basic physiological principles from studying the diversity of design and function in nature. The four examples summarized above clearly show that crustaceans have a great potential to serve as experimental animals for such a comparative approach. These examples are probably biased by the author's own research work. But others could certainly have been chosen. And, most probably, imaginative carcinologists will continue to find new ideas for which representatives of their favourite animal group, crustaceans, will be appropriate as models for physiological research.

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