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Research Articles

Antarctic fishes survive exposure to carbon monoxide

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Abstract. The extensive in vivo conversion of haemoglobin to the carbon monoxide derivative has no discernible effect on the survival of the red-blooded Antarctic fish *Pagothenia bernacchii*. Analysis of caudally sampled blood of cannulated specimens revealed that reconversion of carbon-monoxy haemoglobin to oxyhaemoglobin was complete within 48 hours. Thus, under stress-free conditions, haemoglobin is not necessary for survival of *P. bernacchii*. Red-blooded Antarctic fishes can carry oxygen necessary for routine delivery dissolved in plasma, in a similar way to the haemoglobinless Channichthyidae, although they lack the morphological and physiological adaptations which allow the latter to prosper without any haemoglobin.
Key words. Antarctic fish; oxygen transport; haemoglobin; carbon monoxide.

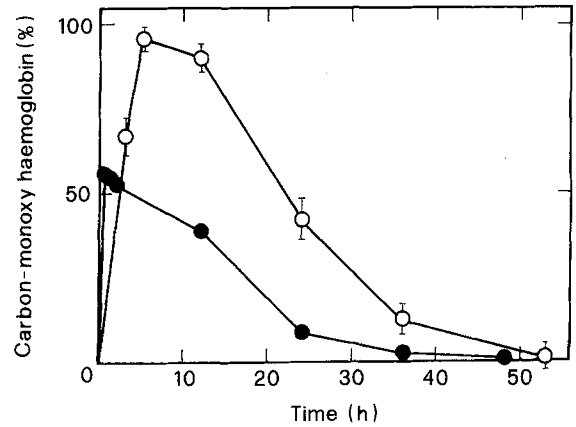
The blood of Antarctic teleost fishes typically contains fewer erythrocytes and less haemoglobin than that of temperate or tropical species[1-3]. This feature reaches its extreme in members of the family Channichthyidae, which are the only adult vertebrates known in which haemoglobin and normal erythrocytes are totally absent[4]. Haemoglobinless and naturally cytopenic red-blooded fish species coexist in the Southern ocean, suggesting that the need for an oxygen-carrying pigment is reduced in both groups. In this paper we report that extensive in vivo conversion of haemoglobin to the carbon monoxide derivative has no discernible effect on the short-term survival of the red-blooded Antarctic species *Pagothenia bernacchii* (family Nototheniidae) under resting conditions.

Originating as a fragment of the ancient supercontinent of Gondwanaland, the Antarctic continent drifted to its present polar position and was isolated climatically and biologically about 50 million years ago by the establishment of a strong circumpolar oceanic circulation[5]. With reduction of heat exchange from northern latitudes, the Antarctic ocean became cold and seasonally ice-covered; at present coastal waters maintain year-round temperatures very near − 1.87 °C, the equilibrium temperature of the seawater-ice mixture. The largely endemic percoid suborder Notothenioidei, comprising the majority of the Antarctic ichthyofauna, appears to have evolved and radiated in situ at sub-zero temperatures from red-blooded ancestors, although there is no fossil record to support this[6].

The haematological peculiarities of Antarctic fishes are considered to be part of a set of characteristics related to a process of cold compensation. The viscosity of corpusculate fluids such as blood is increased substantially by low temperature[7], thus any reduction in cellular content would tend to reduce the energy required for circulation. At the same time, low temperatures reduce the overall metabolic demand for oxygen, while increasing its solubility in plasma, so that more oxygen can be carried in physical solution, and less needs to be bound to haemoglobin.

Nevertheless, only one of the five families of Antarctic notothenioid fishes[8] has completely forsaken haemoglobin as an oxygen carrier. Why have the Channichthyidae alone taken such a radical course, leaving the other families (Nototheniidae, Bathydraconidae, Artedidraconidae, Harpagiferidae) with only partial reductions in haemoglobin? Does haemoglobin remain absolutely vital for adequate oxygen transport in the five red-blooded families, or is it a vestigial relict which may be redundant under stress-free living conditions?

We sought to test the dependence of *P. bernacchii* on haemoglobin by complexing the haemoprotein with carbon monoxide to block oxygen binding, using two alternative experimental protocols (fig.). In the first, whole heparinised blood (4.5 ml, approximately half of the total volume) was removed through a caudal venous cannula from a 250-mm (250 g) specimen of *P. bernacchii*, saturated with carbon monoxide by gentle bubbling, and reinjected into the caudal vein through the cannula. The entire process look less than 3 min. Aliquots of blood were taken periodically and analysed spectrophotometrically for carbon-monoxy haemoglobin[9]. 10 min after reinjecting the blood, the carbon-monoxy derivative accounted for 56% of the total haemoglobin. Carbon-monoxy haemoglobin was thereafter reconverted totally to oxyhaemoglobin within 48 h by slow replacement with oxygen.



Reversible functional incapacitation of the haemoglobin of the Antarctic teleost *Pagothenia bernacchii*, by reversible conversion in vivo to the carbon-monoxy derivative. See text for experimental details of first (●) and second protocol (o). Points are means ± SD (n = 4).

In the second protocol, water of a sealed aquarium was equilibrated with 7% carbon monoxide in air. The aquarium contained cannulated specimens of *P. bernacchii*, as well as control specimens of the haemoglobinless channichthyid *Chionodraco hamatus*. After 5 h, as judged by spectrophotometric analysis[9] of aliquots of blood, periodically taken through the cannula, over 95% of the *P. bernacchii* haemoglobin was converted to the carbon-monoxy derivative, and hence was unable to carry oxygen. Normal aeration and water circulation were then established, and analysis of caudally sampled blood showed that carbon monoxide had been completely removed from the haemoprotein within the next 48 h.

During both regimes, the experimental fish and controls showed no sign of distress; however, they were not challenged metabolically. From this we infer that in *P. bernacchii*, under resting conditions, the incapacitation of haemoglobin may be compensated by other control mechanisms which ensure survival oxygen delivery.

The physiological adaptations which enable the family Channichthyidae to prosper without haemoglobin have been discussed in several papers[10-13]. They include a low metabolic rate; large, well-perfused gills; a large blood volume; a large heart and high stroke volume; large capillary diameter, and cutaneous respiration. The latter is known also to play an important role in gas exchange in several red-blooded Antarctic fishes[14], but otherwise the red-blooded species lack the morphological features which augment the transport of dissolved oxygen in channichthyids.

The survival of red-blooded *P. bernacchii*, in spite of the functional incapacitation of their haemoglobin by carbon monoxide, leaves little doubt that routine oxygen delivery is still possible with virtually no functional haemoglobin in the cold, stable environment of the Antarctic seas. In keeping with these experiments, preliminary observations show that gradual reduction of the haematocrit of cannulated specimens of *P. bernacchii* has no obvious ill effects, even during bouts of enforced exercise (Wells, Macdonald and di Prisco, unpublished results). Like the channichthyids, red-blooded Antarctic fishes can carry the necessary oxygen dissolved in plasma.

It is probably also pertinent that Antarctic teleosts tend to lack the multiplicity of haemoglobins which characterises most other teleosts. All of the Antarctic nototheniids except one have only one single major haemoglobin[15]. When present, a minor component (5% of the total) is functionally indistinguishable from the major component. In contrast, the blood of most other fishes contains multiple haemoglobins[16,17], often showing functional differences which permit oxygen transport even under conditions of extreme acidosis, thereby adapting to environmental changes. In the relatively constant environment of the Antarctic seas, fishes can do very well with only a single haemoglobin, and, under certain conditions, even with none at all.

Natural or experimentally-induced anaemia has also been reported in some non-Antarctic fishes[18-22], and it has been suggested that many fishes rely on haemoglobin for emergencies, and that oxygen carried in the plasma may be sufficient for respiration under minimal working load[23]. In some cases, temperate fishes have been found to be unaffected by carbon monoxide poisoning, especially at low temperatures[12,18,21]; however, the essential physiological role of haemoglobin in oxygen transport in temperate and tropical fish is undisputed. Within the spectrum of biological variability, Antarctic fishes represent one extreme in the reliance of vertebrates on haemoglobin-mediated oxygen transport, with the homeothermic mammals and birds lying at the other extreme. Structural mutations in mammalian haemoglobin are often pathological, and not selectively neutral; however, in notothenioid fishes, survival under routine metabolic conditions is still possible for a long time (≥ 10 h) even with largely incapacitated haemoglobin. This is not wholly surprising, considering the peculiar environmental conditions, and the limited stress experienced by the specimens employed in this investigation.

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