Comparative Biochemistry and Physiology, Part A 168 (2014) 76–81

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

# Blood oxygen- and carbon dioxide-carrying properties in captive penguins: Effects of moulting and inter-specific comparison

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ARTICLE INFO

*Article history:*
Received 23 July 2013
Received in revised form 19 October 2013
Accepted 6 November 2013
Available online 11 November 2013

*Keywords:*
Acid–base balance
*Aptenodytes patagonicus*
*Eudyptes chrysocome*
Moulting
Penguins
$O_2$ storage
Plasma ions
*Pygoscelis papua*

ABSTRACT

Venous blood gas-carrying properties were compared in the three captive species of penguins (king, gentoo and rockhopper) at Océanopolis (France). Captivity permitted to control environmental influences. Given their different ecology and diving behaviour in the wild, it was wondered whether milder conditions and dive privation have repercussions on parameters determining oxygen storage and acid–base status of these birds. In addition, this work provided the opportunity to study the effects of moulting in king penguins. This annual event that imposes deep metabolic adjustments is liable to affect blood gas levels. Because of the regular food supply and probably also of the blood sampling conditions, the blood pH of captive penguins was low. This effect was increased in moulting penguins and supposedly due to both the decreased energetic metabolism and the production of uric acid resulting from new feather synthesis. The decrease in the anion gap also revealed the use of plasmatic albumin for this synthesis. The elevated venous $PO_2$ in all birds is not likely due to stress caused by sampling conditions. The other data, in accordance with those in the literature, show neither major influence of captivity nor fundamental interspecific differences, despite potential diving aptitude.



## 1. Introduction

In sphaeniciformes, haematological values can be strongly influenced by site, season, species, age, sex and physiological and nutritional status (Villouta et al., 1997; Sergent et al., 2004). This underlines the great involvement of blood cells in environmental and physiological adaptations. Most of the previous studies devoted to blood respiratory properties were undertaken in wild birds and concern many geographically and biologically different species; for example: *Aptenodytes forsteri* (Ponganis et al., 2009), *Aptenodytes patagonicus* (Hawkey and Samour, 1988), *Pygoscelis adeliae* (Murrish, 1982), *Pygoscelis papua* and *Pygoscelis antarctica* (Milsom et al., 1973; Murrish, 1982), *Eudyptula minor* (Sergent et al., 2004), *Eudyptes chrysocome* (Karesh et al., 1999), and *Spheniscus humboldti* (Villouta et al., 1997). The overall analysis of these works does not indicate any fundamental differences between the species despite their different diving abilities. This could be explained not only by a variety of factors that can vary simultaneously but also by the dependence of oxygen storage on body mass and the variable distribution of oxygen between respiratory, blood and muscle compartments (Ponganis and Kooyman, 2000). The fact that captive penguins live for a long time in exactly the same conditions allows the researcher to eliminate the variations of surrounding effects. Taking advantage of this, this study compares the three penguin species kept at Océanopolis (the ocean discovery park in Brest, France).

At the time of this experiment (April), about half of the king penguins at Océanopolis were moulting and the others were going later. Moulting is a physiological event liable to affect oxygen storage and consumption as well as the blood acid–base balance because of the deep metabolic adjustments that characterize this period of total fasting (Cherel et al., 1993; Cherel et al., 2005). Indeed, penguins replace their plumage entirely each year and wild birds spend a long time (14–40 days) fasting ashore because the consequent reduction in thermal insulation precludes staying in cold waters to feed (Groscolas and Cherel, 1992). Moulting involves the use of endogenous lipid reserves and body protein to fulfil the nutrient requirement for feather synthesis and the higher thermogenesis associated with the decrease in thermal insulation. Little work has been carried out on energy requirement during moulting and fasting: Le Maho and Despin (1976), Deswasmes et al. (1980), and Fahlman et al. (2004) show that resting energetic metabolism and heart rate decreased throughout the fast. Thus, in addition to an interspecific comparison, this study was also the opportunity to highlight the effects of moulting on the blood gas-carrying properties of king penguins.

The three species of Océanopolis' penguins (king penguin — *Aptenodytes patagonicus* Miller, gentoo penguin — *Pygoscelis papua* Forster and rockhopper penguin — *Eudyptes chrysocome* Forster) were all born in captivity. In their territories of origin (Subantarctic islands and Antarctic peninsula for the first two species, Argentina and

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http://dx.doi.org/10.1016/j.cbpa.2013.11.002

Chile for the third), these marine birds dive to feed and migrate and differ in the depths they frequently reach. Their ability to dive is positively related to body mass (Wilson, 1995). King penguins, being among the largest marine birds, have exceptional diving abilities, reaching a mean maximum depth of 304 m (Kooyman et al., 1992). Gentoo penguins dive to 156 m (Williams et al., 1992) and rockhopper penguins, the smallest of the three, to 66 m (Wilson et al., 1997). In captivity, penguins never dive and the consequent repercussions on blood gas-carrying properties, if there are any, are unknown. Moreover, Océanopolis' penguins are exposed to mild conditions (no polar temperatures, no wind, regular and sufficient food) compared to wild ones. It is hypothesized that these opposing conditions, leading to a less drastic energetic adaptation for captive penguins, may concern the birds' blood properties, particularly when moulting making them temporarily more fragile. The penguins of Océanopolis are primarily destined to a daily public display and consequently they are preserved from all predictable causes of disturbance as much as possible. Thus, this study was carried out during a scheduled clinical examination of the overall population. Our goal was to describe parameters that are relevant in determining oxygen and carbon dioxide storage and their eventual consequences on acid–base status of blood in a comparative study between 1) moulting and non-moulting king penguins and 2) the three bird species kept at Océanopolis.

## 2. Materials and methods

### 2.1. Animals and blood sampling

The study was carried out on all penguins in captivity at Océanopolis: 9 king penguins; (7 ♂ and 2 ♀) weighing on average 12 kg and aged between 6 and 18 years old, 10 gentoo penguins (5 ♂ and 5 ♀) weighing on average 7 kg and aged between 1 and 21 years old and 10 rockhopper penguins (6 ♂ and 4 ♀) weighing on average 3 kg and aged between 1 and 21 years old. They were all born in captivity and were in good physical condition. They were fed daily *ad libitum* with thawed Atlantic herring *Clupea harengus* and Icelandic capelin *Mallotus villotus* except for the day before sampling and for moulting king penguins that refuse food. The slightly low mean body mass of king penguins reflects the moulting and consequent fasting status of some of them.

Venous blood samples of 200 μL were quickly (<1 min) collected in heparinized (lithium heparin 100 IU/mL) syringes from the brachial vein of relatively calm manually-restrained birds. This sampling method was supposedly of minimal disturbance because the birds are used to regular human presence and manipulation. This also avoided the potential effects of sedation and anaesthesia on measured blood parameters. 100 μL of blood was immediately analysed by using a Radiometer ABL77 blood gas analyser (Brønshøj, Denmark) calibrated at 37 °C for a range of respiratory, haematological and ionic data. The time interval between sampling and analysis was always less than 5 min. The gas analyser calculated the measured gas partial pressure ($pO_2$ and $pCO_2$, mm Hg) using the temperature-correction factors given by Burnett and Noonan (1974) assuming a mean penguin temperature of 38.5 °C (Lenfant et al., 1969). The temperature of the birds was not taken to avoid additional disturbance. Moreover, solubility coefficients for $O_2$ and $CO_2$ for penguins in the literature are given at 38.5 °C. The remaining blood was stored on ice (24 h maximum) until required for a red blood cell count and manual checking of haematocrit and haemoglobin concentrations.

### 2.2. Derived acid base characteristics

Using the temperature-corrected values of pH and partial pressure of carbon dioxide, the bicarbonate concentration ($[HCO_3^-]$, $mmol \cdot L^{-1}$) was calculated from the Henderson–Hasselbalch equation assuming a $CO_2$ solubility coefficient ($\alpha CO_2$) and an operational pK′ in penguin plasma at 38.5 °C of 0.0297 $mmol \cdot L^{-1} \cdot mm\ Hg^{-1}$ and 6.086, respectively (Nicol, 1991). The total carbon dioxide content ($CtCO_2$, $mmol \cdot L^{-1}$) was calculated from:

$$CtCO_2 = (\alpha CO_2 \times PCO_2) + [HCO_3^-]$$

(Siggaard-Andersen et al., 1988).

### 2.3. Measured and derived oxygen storage capacity of blood

The Radiometer analyser measured haematocrits (Hct, %) and calculated haemoglobin concentrations ([Hb], $g \cdot L^{-1}$) from Hct. These data were compared to those obtained by manual methods. Hct was measured after centrifugation of 70 μL microcapillaries for 5 min at 12,000 *g*. [Hb] was spectrophotometrically determined by the cyanmethaemoglobin method (kit Sigma 525A). Manually- and automatically-obtained Hct were identical. In contrast, [Hb] was systematically about 15% higher with the manual method. This difference came certainly from the equation used by the Radiometer analyser programme which includes constants for the human blood. Therefore, only manually-obtained [Hb] values were considered. The red blood cell count (RBC, $L^{-1}$) was obtained by means of a Malassez haemocytometer following 1:200 dilution in Marcano solution (5 g sodium sulphate, 1 mL formaldehyde 40%, qs 100 mL distilled water). Mean red blood cell volume (MCV, $\mu m^3 \cdot cell^{-1}$) and mean cell haemoglobin concentration (MCHC, %) were respectively computed as follows:

$$MCV = Hct/RBC \text{ and } MCHC = [Hb]/Hct.$$

### 2.4. Electrolytic assessment

Plasma ion concentrations ($[Na^+]$, $[K^+]$, $[Ca^{2+}]$ and $[Cl^-]$, $mmol \cdot L^{-1}$) were measured by means of the Radiometer analyser's selective sensors. The anion gap (AG, $mmol \cdot L^{-1}$) was calculated from the following equation:

$$AG = [Na^+] + [K^+] - [Cl^-] - [HCO_3^-].$$

### 2.5. Statistical analysis

Statistical analyses were conducted using Sigmastat 4.0 (Systat Software Inc.). All results were expressed as mean ± s.e.m. The effects of sex in gentoo and rockhopper penguins and of moulting in king penguins were tested by a *t*-test. The effects of sex in king penguins could not be performed in king penguins because of the low number of females. The inter-specific differences were tested by a one-way ANOVA analysis. Whenever significant effects were detected, multiple comparisons were conducted using the Tukey test. Differences were considered significant at $p < 0.05$.

## 3. Results

Within gentoo and rockhopper species, no significant differences related to sex in any of the parameters measured. This was probably due to the low number of penguins studied. Therefore, the data are presented as means calculated from all subjects within each species including king penguins, taking the two sexes together. Values for moulting and non-moulting king penguins are shown separately when moulting status was a significant factor.

### 3.1. Acid–base status and ionic data

The mean key blood acid–base parameters are summarized in a Davenport diagram (Fig. 1) where pH, $pCO_2$ and $[HCO_3^-]$ are simultaneously presented according to the Henderson–Hasselbalch equation. Rockhopper penguin blood was significantly more alkaline than one of the two other species. Gentoo and non-moulting king penguins' acid–base balances were very close as shown by the proximity of their points on