Blood Gas Analysis and Acid-Base Status in the Hemolymph of a Spider (Eurypelma californicum) – Influence of Temperature

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Summary. 1. Blood gas content and acid-base status in arterial blood of the North American tarantula, Eurypelma californicum, have been analyzed after acclimation to temperatures between 15 °C and 30 °C. Anaerobic samples were withdrawn from the heart, and the following parameters measured: CaO₂, pHₐ, PaCO₂, CaCO₂. There is no significant influence of acclimation time upon these parameters in tarantula blood.

2. Oxygen concentration of arterial blood, CaO₂, decreases with acclimation temperature from 0.82 mM/l (1.85 vol %) at 15 °C to 0.62 mM/l (1.4 vol %) at 30 °C. Considerable individual variation results from both variation in hemocyanin concentration and variation of in vivo Po₂.

3. The solubility coefficient of CO₂, S, was determined between 15 °C and 30 °C and found not to be influenced by hemocyanin concentration. S is about 6% lower in a solution corresponding to the ionic composition of spider blood if compared to 0.01 n HCl.

4. The influence of temperature on acid-base status of Eurypelma blood is as follows: a) PaCO₂ is generally low and increases with temperature from 7 Torr (15 °C) to 14 Torr (30 °C), b) CaCO₂ (about 13 mM/l) and [HCO₃⁻]a (slightly lower in concentration than CaCO₂) are independent of acclimation temperature, c) pHₐ decreases with increasing temperature, ΔpHₐ/ΔT being −0.0093, about half of the temperature coefficient for the neutral point of water (pN). Consequently, [OH⁻]/[H⁺] increases from 10 (15 °C) to 16 (30 °C), and aₘ from 0.504 to 0.573.

Introduction

Acid-base balance in the hemolymph of invertebrates which contain hemocyanin as respiratory pigment involves the interaction of mainly two buffer systems: the bicarbonate/carbonic acid system and the hemocyanin itself, which appears to represent the major, if not sole hemolymph protein in many species. Thus, hemocyanin is not only responsible for oxygen transport but is also engaged in transport of carbon dioxide as well as in balancing pH within physiological limits.

Carbon dioxide transport and acid-base balance in terrestrial non-insect arthropods have received only little attention. Data on terrestrial crabs (Cameron and Mecklenburg, 1973: Birgus latro, 6-9 Torr; Howell et al., 1973: Gecarcinus lateralis 8-10 Torr, Uca pugilator, 5-7 Torr; McMahon and Burggren, 1979: Coenobita clypeatus, 4-7 Torr) indicate that these animals transport carbon dioxide at CO₂ tensions considerably lower than in terrestrial vertebrates.

Oxygen transport and oxygen binding by the hemocyanin of Eurypelma californicum, a tarantula, have been studied extensively (Angersbach, 1978; Linzen et al., 1977; Loewe, 1978). However, nothing is known about the transport of carbon dioxide in spiders and about the role played by hemocyanin, nor is the interaction between carbon dioxide and oxygen transport understood. An important contribution of hemocyanin in buffering and CO₂ transport may be deduced from the fact that the high concentration of hemocyanin accounts for 20 mM/l of histidine residues (Markl et al., 1976). Due to their pK', the imidazole residues are the major buffering groups in the physiological pH range. Reeves (1972) has shown, that in a mixture of the two weak buffers carbonic acid bicarbonate and imidazole the same changes in pH and PCO₂ with temperature are observed as in
closed system in vitro blood samples (Rosenthal, 1948).

In the present study we have investigated the influence of temperature on respiratory gas transport and acid-base status in the tarantula *Eurypelma californicu*um by measuring O₂ content, C₇CO₂, P₇CO₂, and pH in blood of animals acclimated at 15, 20, 25 and 30 °C.

**Materials and Methods**

**Acclimation and Anaerobic Sampling**

Adult females of *Eurypelma californicu*um were kept in plastic boxes (10 cm × 15 cm) and adapted to temperatures ranging between 15 °C and 30 °C for three to five weeks (one week values having been studied in addition in particular cases); relative humidity was between 30% and 60%. During acclimation, the tarantulas were supplied with water ad lib. and with crickets.

Care was taken to obtain "undisturbed" blood samples: Animals which had been quietly sitting for a prolonged period of time were suddenly pressed down with a piece of foam rubber and stabbed into the heart with a gastight 250 µl Hamilton syringe, the dead space of which had been reduced to 5 µl. Total sampling time was maximally 25 s. The O₂ measurements showed that "resting" blood was obtained (Fig. 2; compare the "in vivo" recordings by Angersbach, 1978). All in vivo parameters (C₇O₂, C₇CO₂, P₇CO₂, and pH) were measured within 2–3 min after sampling.

Hemolymph for in vitro measurements was obtained as described previously (Loewe and Linzen, 1975).

**CO₂ Solubility Coefficient**

The solubility coefficient of CO₂ was determined in a) 0.01 n HCl, b) salt solution prepared according to Rathmayer (1965) but omitting bicarbonate (pH about 3.0), and c) samples of cell free hemolymph treated according to Truchot (1976) and diluted with 0.01 n HCl, if necessary. Aliquots of 200 µl were equilibrated in a thermostated micro titration vessel (Metrohm ES 680-T-1) under continuous magnetic stirring, against water saturated 40% CO₂ (supplied by a Wösthoff gas mixing pump). After 60 min equilibration, the carbon dioxide concentration of 50 µl samples was measured by means of Cameron’s micro method (1971). In samples containing protein, protein concentration was measured before and after equilibration using E₁=600 nm = 11 (Loewe, 1972).

**Blood Gas Analysis**

Oxygen concentration of postpulmonal hemolymph was measured with a Lex-O₂-Con analyzer (Lexington, Mass. USA). Postpulmonal P₇CO₂ (P₇CO₂) was determined with a Radiometer E 602 P₇CO₂ electrode, thermostatted to acclimation temperature. To measure postpulmonal carbon dioxide concentration (C₇CO₂), two methods were used: a) the Micro-Van Slyke technique (Thomas magnematic model manometer) and b) the micro method of Cameron (1971). For our purposes we reduced the volume of the Cameron chamber to 2 ml and inserted the P₇CO₂ electrode from the side. Methylene blue was added to the 0.01 n HCl to be sure, that the pH remained fairly below pH = 4 upon adding the sample.

The Cameron method was compared to the micro Van Slyke technique by means of "Acidbasol" standard solutions (Goedecke, Berlin). Results for "alkalosis" and "normal" solutions were not significantly different by Student’s t-test (Table 1). The difference in case of the "acidosis" standards is presumably due to errors involved in the Van Slyke measurements at low carbon dioxide contents (only small differences in manometric readings), since by the Cameron method appreciable meter deflections are obtained under these conditions. As compared to the data provided with the standard solutions, our results are slightly lower (about 5% with both methods, if the higher acidosis value obtained with the Van Slyke method is excluded), but are within the 2s warning limits. We regard these lower values to be a characteristic of the particular batch since thermostating and shaking of the ampoules as well as sampling have been done with maximum care. Additional proof for the validity of our measurements results from the values of S (CO₂ solubility coefficient) which we determined in acidified water (cf. Table 3) and which are nearly identical to those reported by Bartels and Wrbitzky (1960).

**Determination of pH**

pH was measured with a Metrohm EA 158X electrode connected to a Knick 645 high precision pH meter. This electrode which has a flat pH sensitive membrane, was used for both pH control during equilibration of serum with known CO₂ tension and for measurements of freshly drawn samples. For measurement of the blood samples, the electrode was mounted airtight in a chamber of 30 µl volume, made of acrylic plastic. The whole setup was kept in a controlled temperature cabinet. Blood samples were introduced and visually controlled by aid of a mirror to avoid bubbles. Between measurements the electrode was repeatedly calibrated against NBS precision standard buffers (supplied by Radiometer, Copenhagen).

**Results and Discussion**

**Oxygen Concentration in Postpulmonal (Arterial) Hemolymph**

The oxygen concentration of postpulmonal hemolymph, C₇O₂, was measured in the temperature range between 15 °C and 30 °C. Data obtained after different acclimation times were not significantly different (Table 2). Therefore, at any given temperature, all