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

Renate Loewe* and Helga Brauer de Eggert

Zoologisches Institut der Universität München, Luisenstrasse 14, D-8000 München, Federal Republic of Germany

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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californicum*, have been analyzed after acclimation to temperatures between 15 °C and 30 °C. Anaerobic samples were withdrawn from the heart, and the following parameters measured: $Ca_{O_2}$, $pH_a$, $P_{a CO_2}$, $Ca_{CO_2}$. There is no significant influence of acclimation time upon these parameters in tarantula blood.

2. Oxygen concentration of arterial blood, $Ca_{O_2}$, 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 $P_{a O_2}$.

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) $P_{a CO_2}$ is generally low and increases with temperature from 7 Torr (15 °C) to 14 Torr (30 °C), b) $Ca_{CO_2}$ (about 13 mM/l) and [HCO3]^− (slightly lower in concentration than $Ca_{CO_2}$) are independent of acclimation temperature, c) $pH_a$ decreases with increasing temperature, $d pH_a/dT$ 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 $z_m$ 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 $CO_2$ 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 $CO_2$ 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 $pK_a$, 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 $P_{CO_2}$ 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 californicum* by measuring O₂ content, CₐO₂, PₐO₂, 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 californicum* were kept in plastic boxes (10 cm x 15 cm) and adapted at 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 (CₐO₂, CₐCO₂, PₐCO₂, 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 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 E₁₀₀₀₅₄₀ = 11 (Loewe, 1972).

#### Blood Gas Analysis

Oxygen concentration of postpulmonal hemolymph was measured with a Lex-O₂-Con analyzer (Lexington, Mass. USA). Postpulmonal PₐO₂ (PₐCO₂) was determined with a Radiometer E 605 PₐO₂ electrode, thermostatted to acclimation temperature. To measure postpulmonary carbon dioxide concentration (CₐO₂), 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 PₐCO₂ 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, 1948). The oxygen concentration of postpulmonal hemolymph, CₐO₂, 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