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GAS TRANSPORT IN THE HAEMOLYMPH OF ARACHNIDS

II. CARBON DIOXIDE TRANSPORT AND ACID–BASE BALANCE

R. J. PAUL*, A. PFEFFER-SEIDL, R. EFINGER, H. O. PÖRTNER AND H. STORZ

Zoologisches Institut, Universität München, Luisenstraße 14, D-80333 München, Germany

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Summary

The relationships between P_{CO_2} and pH were determined in cell-free undiluted haemolymph of the arachnids *Eurypelma californicum*, *Pandinus imperator* and *Cupiennius salei*. The pH/bicarbonate diagrams and the CO_2 equilibrium curves were calculated, using the Henderson–Hasselbalch equation, for haemolymph sampled at rest and during recovery from exercise. The calculations of solubility (α_{CO_2}) and dissociation constant (pK″) were based on additional ion concentration measurements. Blood gas analyses corroborate these results: after locomotor activity, there is a metabolic acidosis linked to the accumulation of lactate in the haemolymph. The concentration of bicarbonate in the haemolymph of resting individuals is quite different in the three species and is related to the extent of post-exercise bicarbonate depletion. During early recovery, buffering in the haemolymph strongly depends upon CO_2 release. Potassium and magnesium concentrations in the haemolymph increase after exercise. During cold-acclimation (to 10 °C), there is a metabolic acidosis in the tarantula's haemolymph that is linked to the accumulation of acetate.

Introduction

Many arachnids use a 'sit and wait' style of predation to save energy (Anderson, 1970). A burst of activity (to catch prey or to defend themselves) relies heavily on the anaerobic utilization of muscular phosphagen and carbohydrate stores (Prestwich, 1983*a*,*b*, 1988; Paul, 1991, 1992). Maximum activity is followed by a long-lasting recovery period (Paul *et al.* 1989). At the end of exercise and during early recovery, the final metabolite of glycogenolysis (D-lactate), which was produced in the muscle tissues during exercise (and perhaps additionally during early recovery), enters the haemolymph. The maximum D-lactate concentration is reached approximately 10 min after a 3 min sprint in the tarantula *E. californicum* (Paul and Storz, 1987). Venous haemolymph pH drops sharply after a burst of activity in the tarantula (Angersbach, 1978). During recovery, lactate

*Present address and address for reprint requests: Institut für Zoologie IV, Universität Düsseldorf, Universitätsstraße 1, D-40225 Düsseldorf, Germany.

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accumulation in the haemolymph is similar in *E. californicum* and in the similar-sized emperor scorpion *P. imperator*, but the patterns of CO_2 release (maximum values and time courses) are quite different (Paul and Fincke, 1989). These data indicate close relationships between functional anaerobiosis, acid–base balance, CO_2 transport and CO_2 release. Carbon dioxide transport, acid–base balance and related processes in arachnids have received only a little attention. There are a few reports that deal with the influence of temperature and some other variables on the acid–base status of resting individuals (Loewe and Brauer de Eggert, 1979; Dejours and Ar, 1991), but the influence of activity is hardly studied. This does not do justice to the important role that arachnids play in nature.

In this paper, we describe studies of the relationships between carbon dioxide transport and acid–base balance, together with results on haemolymph buffering capacities, on the influence of oxygen on CO_2 transport and on haemolymph ion concentrations. We also report a metabolic acidosis during cold-acclimation in *E. californicum* linked to the accumulation of acetate in the haemolymph. We hope that this study will contribute to a better understanding of the physiology of this animal group.

Materials and methods

The haemolymph of the North American tarantula *Eurypelma californicum* (Theraphosidae; determined according to Comstock, 1965), the African scorpion *Pandinus imperator* C. L. Koch (Scorpionidae) and the Latin American spider *Cupiennius salei* Keyserling (Ctenidae) was used for these studies. Paul *et al.* (1994) describe the origin and maintenance of animals, sampling procedures and details of the experimental apparatus. Haemolymph was sampled at rest and during early recovery (10 min after 3 min of exhaustive exercise, when D-lactate concentration in the haemolymph is maximal in *E. californicum* and *P. imperator*).

Measurement of P_{CO_2}/pH relationships on undiluted cell-free haemolymph

Gas mixtures containing different CO_2 concentrations were produced using two gas-mixing pumps connected in parallel. The oxygen concentration was kept constant (5 %). The haemolymph pH was measured using a small combination glass pH electrode (SA 4; WPI, USA). To check these data, we also used another (bigger) type of electrode (N6000A; Schott, Germany) to measure the P_{CO_2}/pH relationships of pooled haemolyph of resting *E. californicum*; these results were almost identical to the data given in Table 1. Temperature was maintained at 25±1 °C during all measurements.

Calculation of $[HCO_3^-]$ and C_{CO_2} from P_{CO_2} and pH

$[HCO_3^-]$ and C_{CO_2} were calculated from P_{CO_2}/pH data using the Henderson–Hasselbalch equation (Hasselbalch, 1916). CO_2 solubility (α_{CO_2}) and dissociation constant (apparent pK_1; pK''') (both constants at 25 °C) were calculated according to Heisler (1986). The simplified formula was applied for pK'''. When pH values required averaging, they were first transformed to concentrations. The mean $[H^+]$

values were than re-transformed to pH values. The calculated constants (α_{CO_2}, pK‴) were: 0.03959 mmol l^{-1} $mmHg^{-1}$ and 6.1517 (for *E. californicum*); 0.03959 mmol l^{-1} $mmHg^{-1}$ and 6.1432 (for *P. imperator*); and 0.03942 mmol l^{-1} $mmHg^{-1}$ and 6.1400 (for *C. salei*). An α_{CO_2} value of 0.0413 mmol l^{-1} $mmHg^{-1}$ was used in a previous study of *E. californicum* haemolymph (Loewe and Brauer de Eggert, 1979), and pK‴ was estimated from the nomogram of Severinghaus *et al.* (1956) for human serum. At 25 °C and pH 7.5 (arterial haemolymph), the nomogram value is slightly above 6.15.

Buffer values

Non-bicarbonate buffer values (β) of whole blood were calculated using the Henderson–Hasselbalch equation (following a linear regression analysis of the logP_{CO_2}/pH data): $\beta=\Delta[HCO_3^-]/\Delta pH$.

Measurement of in vivo *haemolymph pH and* C_{CO_2}

Arterial haemolymph was sampled from *E. californicum* and *P. imperator* at rest and 10 min after 3 min of exhaustive locomotor activity. The animals had been adapted for at least 1 week to 25 °C. The needle of a gas-tight syringe (the dead space filled with paraffin oil) was used to prick the cuticle. Haemolymph was rapidly withdrawn from the pericardium (within approximately 30 s) and analysed. pH was measured with a capillary pH electrode (G299; Radiometer, Copenhagen, Denmark) regulated at 25±0.1 °C and calibrated with precision phosphate buffers (Radiometer, Copenhagen). Total CO_2 was analysed in 50 μl blood samples using the gas chromatography method of Lenfant and Aucutt (1966) modified after Boutilier *et al.* (1985).

Measurement of inorganic ions

Sodium and chloride

Cell-free haemolymph samples were diluted with double-distilled water and denaturated by heat (10 min, 85 °C). After centrifugation (5 min, 12 000 ***g***), the supernatants were analysed by flame photometry (sodium) or electrometric titration (chloride). Standard solutions (containing Na^+ and Cl^-) were subjected to the same protocol. These analyses were performed by Dr J. P. Hildebrandt, FU Berlin, Germany.

Potassium, calcium and magnesium

Cell-free haemolymph samples were diluted with strontium solution (7.6 g of $SrCl_2$ in 1 l of double-distilled water), the volume depending on the cation to be analysed. Determinations were carried out with a Perkin Elmer 400 atomic absorption spectrophotometer equipped with a flame. Titrisol (Merck, Germany) was used as standard (dissolved in double-distilled water, diluted with strontium solution).

Measurement of metabolite concentrations

Metabolite concentrations in cell-free haemolymph samples were determined after protein denaturation by heat (15 min at 95 °C). D-Lactate was analysed enzymatically according to Noll (1970). Acetate was determined using a Boehringer kit. All

biochemical reagents were obtained from Sigma (Germany) or Boehringer (Germany), and were of the highest available purity. All reactions were checked with appropriate standards and blanks.

Determinations of metabolite concentrations in cell-free and protein-free haemolymph samples were also carried out using high-pressure liquid chromatography (Abimed, Germany) and a LiChroCART 125-4 Superspher 100 RP-18 column (Merck, Germany), with an eluent consisting of 0.35 mol l^{-1} NaCl (pH 2.7) at a flow rate of 1 ml min^{-1} and a temperature of 21 °C.

Differences were tested for significance at the 5 % level using a two-tailed Student's *t*-test for unpaired samples. Any difference mentioned in the text met this statistical criterion.

Results

Rest and recovery

The relationships between P_{CO_2} and pH of cell-free undiluted haemolymph of resting *E. californicum*, *P. imperator* and *C. salei* are shown in Table 1. Haemolymph pH of resting *E. californicum* was significantly lower than in *P. imperator* at equal P_{CO_2} values. According to the Henderson–Hasselbalch equation, the concentration of bicarbonate ($[HCO_3^-]$) and of total CO_2 (C_{CO_2}) must be much higher in resting *P. imperator*. In comparison with *E. californicum*, *C. salei* also showed significantly higher pH values at rest, indicating greater amounts of $[HCO_3^-]$ and a greater C_{CO_2}.

During recovery, there was a metabolic acidosis (Table 1). At the same P_{CO_2} values, haemolymph pH dropped significantly in all three species by approximately 0.36 units. An HPLC analysis of organic acid concentrations in the haemolymph at rest and during recovery showed that it is almost exclusively lactate (and to a much lower extent

Table 1. *Relationships between* P_{CO_2} *and pH (mean values ± S.D.; 25 °C) in the undiluted haemolymph of* Eurypelma californicum, Pandinus imperator *and* Cupiennius salei

	P_{CO_2} (mmHg)	Resting pH	Recovery pH
E. californicum	6.9	7.77±0.05 (13)	7.36±0.14 (8)
	17.3	7.44±0.05 (13)	7.09±0.11 (8)
	34.5	7.17±0.05 (13)	6.86±0.09 (8)
P. imperator	17.3	7.68±0.11 (12)	7.28±0.18 (6)
	34.5	7.39±0.10 (12)	7.03±0.15 (6)
	103.5	6.94±0.10 (12)	6.61±0.12 (6)
C. salei	6.9	7.85±0.08 (4)	7.42±0.08 (4)
	17.3	7.51±0.08 (4)	7.16±0.06 (4)
	34.5	7.25±0.09 (4)	6.94±0.04 (4)

The pH differences at rest and during recovery in all three species, as well as those between the different species at rest, were all statistically significant ($P<0.05$, unpaired *t*-test).

Haemolymph was sampled at rest and after 10 min of recovery from a 3 min period of exhaustive activity.

Values in parentheses indicate the number of tested individuals.

pyruvate) that increases during recovery (Werner, 1991). In *E. californicum*, the determination of haemolymph D-lactate concentration by enzymatic assays (Werner, 1991) revealed values of 0.05 mmol l^{-1} at rest and 10.6 mmol l^{-1} during recovery (10 min after a 3 min phase of exhaustive exercise). In *P. imperator*, the corresponding values were 0.17 mmol l^{-1} and 12 mmol l^{-1}. No such measurements have been made in *C. salei*.

Pairs of data from Table 1 were transformed and plotted in a pH/bicarbonate diagram (Fig. 1). Because P_{CO_2} was fixed during these measurements, standard deviation lines are aligned to the isobars. In the range between pH 7 and pH 8, mean [HCO_3^-] was 13 mmol l^{-1} in the haemolymph of resting *E. californicum*, 24 mmol l^{-1} in resting *P. imperator* and 16 mmol l^{-1} in resting *C. salei* (Fig. 1). The slopes of the non-bicarbonate buffer lines ($\beta=\Delta[HCO_3^-]/\Delta pH$) yield buffer values (in mmol l^{-1} pH $unit^{-1}$) of 4.53 in the haemolymph of *E. californicum*, 3.51 in *P. imperator* and 5.97 in *C. salei*.

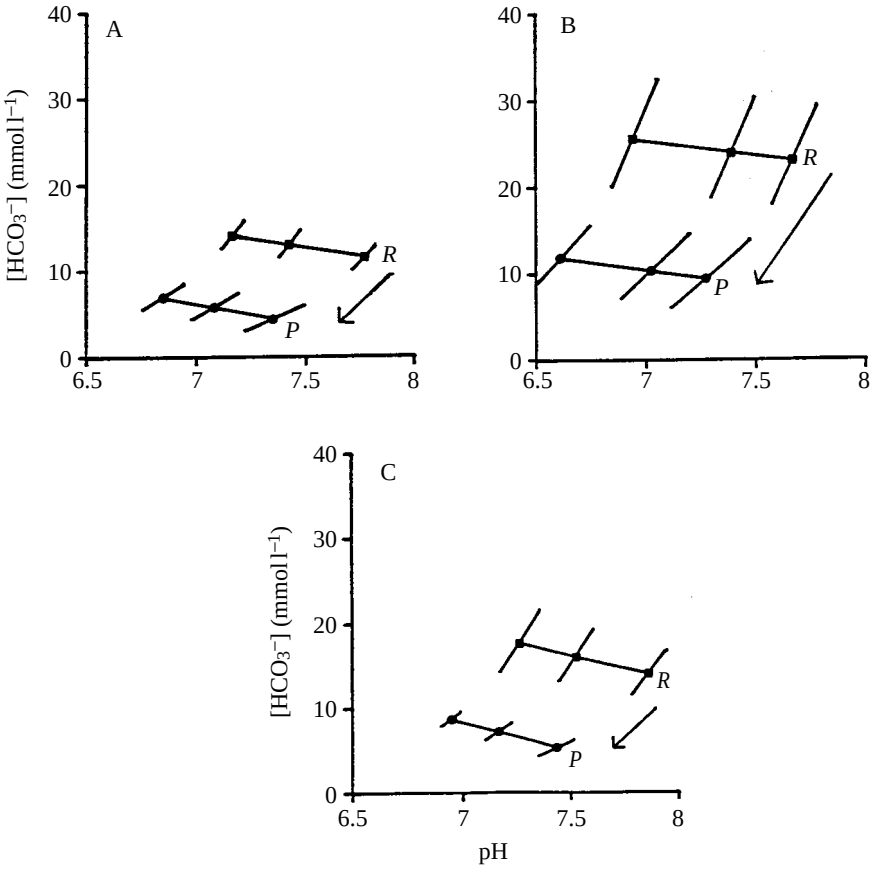

Fig. 1. pH/[HCO_3^-] diagrams (mean values ± S.D.) of cell-free undiluted haemolymph of *Eurypelma californicum* (A), *Pandinus imperator* (B) and *Cupiennius salei* (C) at rest (*R*) and after 10 min of recovery from 3 min of exhaustive exercise (*P*) calculated from the data shown in Table 1. The downward shift of the curves reflects the strong metabolic acidosis during recovery in all three species.

There were no, or only weak, correlations between individual haemolymph protein concentrations (measured by the absorbance of the haemolymph at 280 nm) and respective buffer values. In *E. californicum*, a linear least-squares correlation yielded: β=0.16+0.09[protein]; r=0.67 (N=13 individuals; [protein] was between 16 and 69 mg ml^{-1}). In *P. imperator*, there was no such correlation: β=2.82+0.014[protein]; r=0.08 (N=12 individuals; [protein] was between 31 and 75 mg ml^{-1}).

The bicarbonate concentration (at pH 7.5) was significantly higher at rest than during recovery in all three species: 12.7 *versus* 4 mmol l^{-1} in *E. californicum*, 23.8 *versus* 8.8 mmol l^{-1} in *P. imperator* and 16 *versus* 4.9 mmol l^{-1} in *C. salei* (Fig. 1). Comparing the mean values in the three different species, there is a highly linear relationship between the bicarbonate concentration at rest and the decrease during recovery: $\Delta[HCO_3^-]_{rest–recovery}$=1.88+0.56$[HCO_3^-]_{rest}$ (r=0.99).

Haemolymph pH in resting *E. californicum* depended on O_2 concentration in the gas mixtures. As the partial pressure of oxygen increased, pH decreased a little, indicating lower $[HCO_3^-]$ and C_{CO_2} (Haldane effect). The equilibration experiments were carried out (on the haemolymph of four individuals) first using a gas mixture without oxygen and then using a gas mixture with an identical carbon dioxide concentration, but with 50 % oxygen (P_{O_2} approximately 350 mmHg). In this way, even small pH differences could be measured and verified. Linear regression analyses yielded: $[HCO_3^-]_{deoxygenated}$=78.09−8.50pH (r=0.94) and $[HCO_3^-]_{oxygenated}$=85.45−9.59pH (r=0.94). The mean difference in $[HCO_3^-]$ between deoxygenated and oxygenated haemolymph depends on pH (values in mmol l^{-1}): $\Delta[HCO_3^-]$=1.37 (pH 8), 1.10 (pH 7.75), 0.83 (pH 7.5), 0.56 (pH 7.25) and 0.28 (pH 7). For measurements of the relationships between P_{CO_2} and haemolymph pH at rest and during recovery (Table 1), the oxygen concentration in the gas mixtures was kept constant at 5 %; P_{O_2} was approximately 35 mmHg. (This P_{O_2} is near to the P_{50} values of the haemocyanins of the three species.)

The carbon dioxide equilibrium curves were calculated from pairs of data in Table 1 and are plotted in Fig. 2. At equal P_{CO_2} values (e.g. 17 mmHg), total CO_2 is highest in *P. imperator* (24 mmol l^{-1}), lower in *C. salei* (approximately 17 mmol l^{-1}) and lowest in *E. californicum* (approximately 14 mmol l^{-1}). At the same P_{CO_2} (17 mmHg), C_{CO_2} dropped to approximately 10 mmol l^{-1} in *P. imperator*, 8 mmol l^{-1} in *C. salei* and 7 mmol l^{-1} in *E. californicum* during recovery.

Gas analyses were carried out on *E. californicum* and *P. imperator* haemolymph samples during rest and recovery (Table 2). It is difficult to measure accurate blood gas values using samples from these species, and there is a risk of systematic deviations from the correct values (see Discussion), but the measurements were valuable to check the P_{CO_2}/pH measurements and the calculations based upon them. These data (Table 1; Figs 1, 2) were confirmed in principle: haemolymph $[HCO_3^-]$ and C_{CO_2} were approximately twice as high in resting *P. imperator* as in *E. californicum*. The drop in $[HCO_3^-]$ and C_{CO_2} during recovery was much smaller in *E. californicum* than in *P. imperator* (3 *versus* 9 mmol l^{-1}). In addition, pH was lower, and P_{CO_2} higher, in resting *P. imperator*. During recovery, haemolymph pH dropped to a lower value and P_{CO_2} was again much higher in *P. imperator*.

Cold-acclimation

When *E. californicum* was kept at lower temperatures (10 °C) for more than a few hours, the measurements (at 25 °C) of the relationships between imposed P_{CO_2} values and haemolymph pH revealed a metabolic acidosis (Fig. 3A). After 7 days of cold-acclimation, pH dropped by approximately 0.3 units (pH/bicarbonate diagrams were not calculated; see Discussion).

An analysis by HPLC of organic acid concentrations in the haemolymph of individuals at room temperature and after different cold-acclimation periods (10 °C) showed an

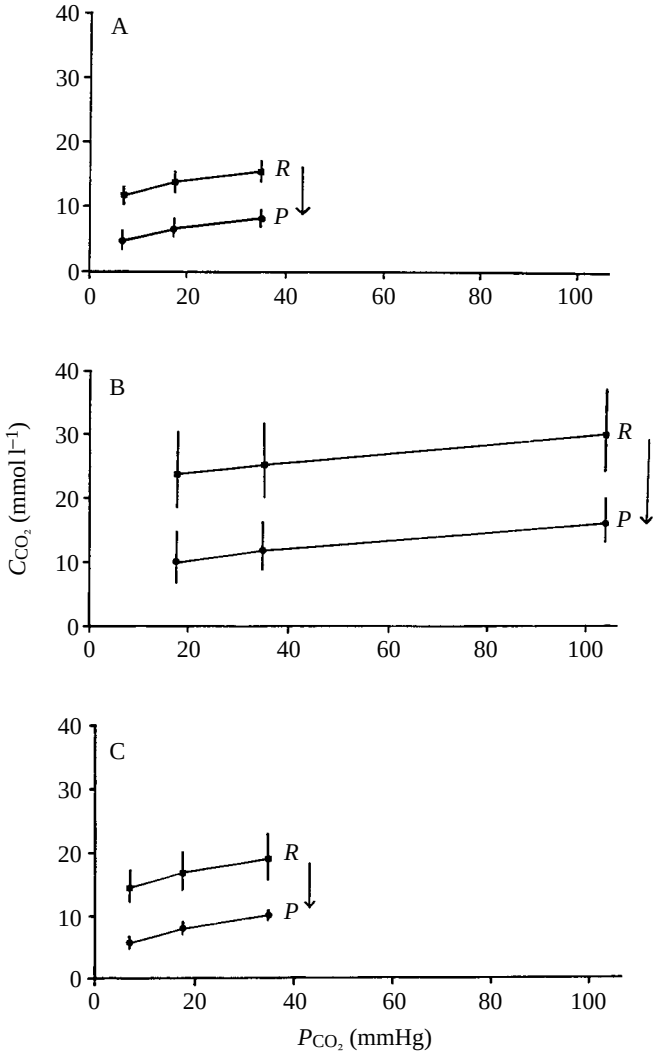

Fig. 2. CO_2 equilibration curves (mean values ± S.D.) of undiluted haemolymph of *Eurypelma californicum* (A), *Pandinus imperator* (B) and *Cupiennius salei* (C) at rest (*R*) and after 10 min of recovery (*P*) calculated from the data shown in Table 1.

Table 2. *Values of arterial pH and* C_{CO_2} *in* Eurypelma californicum *and* Pandinus imperator *haemolymph* in vivo *(mean values ± S.D.; 25 °C)*

	E. californicum	*P. imperator*
Rest		
pH	7.64±0.03 (5)	7.49±0.07 (5)†
C_{CO_2} (mmol l^{-1})	9.24±0.95 (5)	18.19±2.68 (5)†
P_{CO_2} (mmHg)	7.46±1.09 (5)	19.67±2.94 (5)
$[HCO_3^-]$ (mmol l^{-1})	8.95±0.91 (5)	17.41±2.61 (5)
Recovery		
pH	7.24±0.06 (4)*	7.12±0.04 (4)*†
C_{CO_2} (mmol l^{-1})	6.28±1.20 (4)*	9.09±0.78 (4)*†
P_{CO_2} (mmHg)	12.05±2.82 (4)	21.73±2.07 (4)
$[HCO_3^-]$ (mmol l^{-1})	5.80±1.11 (4)	8.23±0.73 (4)

*Values are significantly different ($P<0.05$, unpaired *t*-test) from corresponding rest values.

†Values are significantly different ($P<0.05$, unpaired *t*-test) from corresponding *E. californicum* values.

P_{CO_2} and $[HCO_3^-]$ values were calculated using the Henderson–Hasselbalch equation.

Haemolymph was sampled at rest and after 10 min of recovery from a 3 min period of exhaustive activity.

Values in parentheses indicate the number of tested individuals.

increase in acetate concentration alone (Pfeffer-Seidl, 1991). Using enzymatic assays, the acetate concentrations were determined in the haemolymph of individuals acclimated to 10 ˚C for different periods (Fig. 3B). There was a marked increase from approximately 1.3 mmol l^{-1} at room temperature to approximately 12.3 mmol l^{-1} after 7 days of cold-acclimation.

Haemolymph ion concentrations

To compute α_{CO_2} and pK‴, the concentrations of inorganic cations (sodium, potassium, calcium, magnesium) and anions (chloride) were determined in the haemolymph of resting *E. californicum* and *P. imperator* (Table 3).

We studied activity-related changes of the cation concentrations by analysing samples from *E. californicum* at rest and during the post-exercise recovery phase. (To measure changes more accurately, we took two samples from each individual, one at rest and the other after different periods of recovery. Control experiments were carried out in a similar way, but without provoking activity.) There was a significant increase in haemolymph potassium concentration from 2.5 mmol l^{-1} at rest to 5.4 mmol l^{-1} directly after a 3 min phase of exhaustive exercise (Fig. 4A). During the following recovery phase, the potassium concentration decreased and reached an approximately constant level within the ‘control range’ after about 20 min. The haemolymph magnesium concentration also showed a significant increase during recovery, reaching a maximum (approximately 0.7 mmol l^{-1}) after 30 min (Fig. 4B). The magnesium concentration at rest (0.4 mmol l^{-1}) varied according to the month of sampling (experiments were carried out between February and April), so the data were normalized, with the resting values set to 100 %.

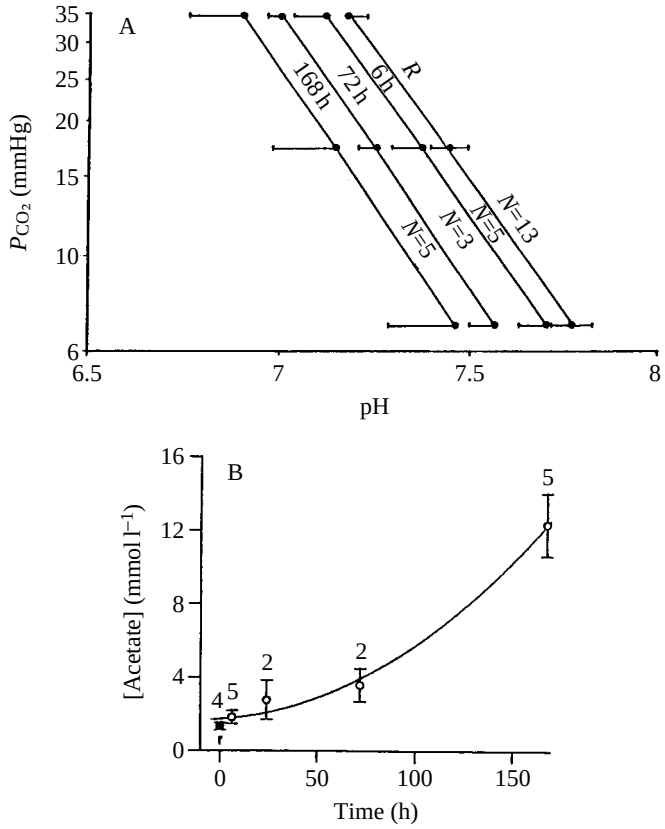

Fig. 3. (A) logP_{CO_2}/pH curves (mean values ± S.D.) of undiluted haemolymph of resting *Eurypelma californicum*, either control (*R*; 25 °C) or cold-acclimated (10 °C) for 6, 72 and 168 h. The left shift of the curves demonstrates a strong metabolic acidosis during cold-acclimation. The number of individuals tested is marked beside the lines. (B) The acidosis was due to the accumulation of acetate in the haemolymph (see text). A quadratic polynomial curve was fitted to the enzymatically determined data (mean values ± S.D.). The number of individuals tested is marked beside the points.

Table 3. *Haemolymph ion concentrations in resting* Eurypelma californicum *and* Pandinus imperator

	E. californicum	*P. imperator*
Sodium	188.5±4.4 (4)	230.5±3.4 (4)*
Potassium	2.5±0.4 (25)	2.9±0.2 (4)
Calcium	4.3±0.2 (7)	4.1±0.7 (12)
Magnesium	0.4±0.1 (28)	1.1±0.1 (12)
Chloride	215.6±6.6 (4)	219.2±3.3 (4)

*Significantly different from the respective *E. californicum* value ($P<0.05$, unpaired *t*-test).
Values are mean ± S.D. and are expressed in mmol l^{-1}.
Values in parentheses indicate the number of tested individuals.

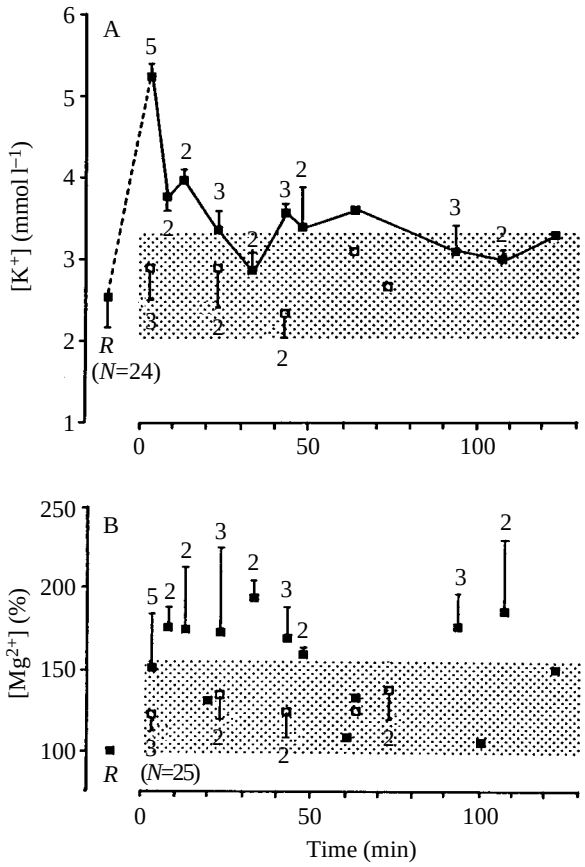

Fig. 4. Potassium (A) and magnesium (B) concentrations (mean values ± S.D.) in the haemolymph of *Eurypelma californicum* increase markedly after exhaustive exercise (3 min). During the following recovery phase, the concentrations returned to the shaded range set by the control values (open squares). Magnesium values are normalized, with the resting values (*R*) set to 100 % (see text for details). The time axis starts with the onset of activity. The increase during recovery (rest *versus* first recovery value) was statistically significant ($P<0.05$, unpaired samples). Numbers of individuals tested are marked beside the points.

There were no significant changes in sodium or calcium concentrations during recovery or in different seasons.

Discussion

Calculations

The relationships between P_{CO_2} and haemolymph pH of *E. californicum*, *P. imperator* and *C. salei* were measured in order to investigate exercise-induced changes in haemolymph acid–base status. The results were transformed using the Henderson–Hasselbalch equation and depicted in pH/[HCO_3^-] and P_{CO_2}/C_{CO_2} diagrams.

The constants α_{CO_2} and pK‴ were calculated using data on haemolymph ion concentrations of resting *E. californicum*, *P. imperator* and *C. salei*. These constants were also used for calculations on haemolymph sampled during recovery, because this caused only a minor error. For the cold-acclimation experiments, we refrained from making further calculations, because larger changes in haemolymph variables relevant for the calculation of the constants could not be excluded. The precision of the pH measurements is essential for any further calculations (Burton, 1987). So we confirmed our measurements using a different type of pH electrode.

Haemolymph ion concentrations

To calculate α_{CO_2} and pK‴, we determined haemolymph ion concentrations in resting *E. californicum* and *P. imperator* (Table 3). The data for *E. californicum* agree well with previous reports (Schartau and Leidescher, 1983) with the exception of magnesium, for which our values are lower by a factor of ten. Data are not available for *P. imperator* haemolymph. Haemolymph values reported for other scorpions are quite variable (Bowerman, 1976; Müller, 1987), so we refrain from discussing them. For *C. salei*, we mostly used data from a previous study by Loewe *et al.* (1970).

In *E. californicum*, we found a marked increase in haemolymph potassium and magnesium concentrations after locomotor activity and, possibly, a dependence of magnesium concentration on season. Exercise-dependent changes in blood potassium concentration have been found in other animals (Turner *et al.* 1983*a*,*b*; Jensen, 1987), including man (Tibes *et al.* 1974). These increases are assumed to reflect K^+ efflux from the muscle cells during exercise. There are additional speculations that a rise in intracellular H^+ concentration may cause the Na^+/K^+ pumps to be inhibited, thus contributing to a net K^+ efflux from, and Na^+ influx into, the muscle cells (Stegemann, 1984). It is well known that many spiders exhaust very rapidly during exhaustive locomotor activity (Bristowe and Millot, 1933). A loss of muscle cell excitability due to K^+ efflux may be at least one of the reasons for this. The mechanisms responsible for the changes in haemolymph magnesium concentration remain unclear.

Gas analysis of haemolymph samples

At equal P_{CO_2} values, haemolymph pH was much higher in resting *P. imperator* than in *E. californicum*, with the pH in *C. salei* being in between. In arterial haemolymph samples from *E. californicum* (Loewe and Brauer de Eggert, 1979), P_{CO_2} was found to be approximately 10.7 mmHg, pH was approximately 7.57 and C_{CO_2} approximately 12 mmol l^{-1} (25 °C). These values for P_{CO_2} and pH correspond approximately with the result of a linear regression analysis of the data from resting *E. californicum* shown in our study [Table 1: P_{CO_2}=antilog(9.8−1.153pH)]. In his *in vivo* study of *E. californicum*, Angersbach (1978) measured a resting pHa of 7.49 and a pHv of 7.45 (22–24 °C). Using these data, arterial and venous P_{CO_2} in resting *E. californicum* can be calculated using the linear equation mentioned above: Pa_{CO_2}=14.59 mmHg and Pv_{CO_2}=16.22 mmHg. These values are higher than previously reported data, because the pH values measured by Angersbach were lower than corresponding values determined by Loewe and Brauer de Eggert (1979). Excluding significant differences between the respective pH measurement

techniques, we think, based on own experiences (see Paul *et al.* 1994 and Table 2), that pH was higher in the haemolymph samples because of an escape of CO_2, especially considering the low non-bicarbonate buffer values of the blood.

It is difficult in these species to measure correct blood gas values under well-defined conditions using haemolymph samples, for several reasons. (i) The circulation time between the book lungs and the pericardium/heart (the only place where sufficient amounts of arterial haemolymph can be sampled) is very short. An invasive sampling procedure (which takes more than a few seconds) may influence both heart activity and book lung function (by changing spiracle entrance area; see Fincke and Paul, 1989), and thus arterial blood gas values. (ii) Many arachnids seem to use anaerobic metabolism during locomotor activity, and protons swiftly appear in the haemolymph during recovery. Angersbach (1978) reported in *E. californicum* a drop of pHv by up to 0.5 units, sometimes within a few seconds after activity. It is therefore difficult to measure resting values, because haemolymph sampling may cause bouts of activity. (iii) The haemolymph O_2-carrying capacity is low, increasing the probability that atmospheric gases could modify the haemolymph gas values. (iv) For arachnids, no known drugs prevent clotting under physiological conditions. This makes quick and anaerobic haemolymph sampling difficult. Considering these measurement problems, we think that *in situ* measurements of haemolymph variables (by using electrodes) are a better way to obtain correct blood gas values in many arachnids. Gas equilibration experiments may also be carried out to describe haemolymph gas transport fully. But of course an analysis of haemolymph samples is a valuable way to check calculated results.

Rest and recovery

The differences in haemolymph pH at equal P_{CO_2} values (Table 1) show that resting $[HCO_3^-]$ and C_{CO_2} are different in the three species: high pH correlates with high $[HCO_3^-]$. The mechanisms that regulate the ionic composition of the haemolymph of the three species obviously have different set points. Chloride concentrations were comparable in *E. californicum* and *P. imperator*, whereas sodium concentrations were much higher in *P. imperator* (Table 3). Taking bicarbonate anions into account, the sum of the cations ($196\,mmol\,l^{-1}$) is less than the sum of the anions ($229\,mmol\,l^{-1}$) in *E. californicum*. In *P. imperator*, these quantities ($239\,mmol\,l^{-1}$ cations, $243\,mmol\,l^{-1}$ anions) are more or less equal. A cation deficit in *E. californicum* has already been reported (Schartau and Leidescher, 1983) and merits further studies.

In *E. californicum* haemolymph, pH at a set P_{CO_2} value was found to depend on the oxygen concentration in the gas mixtures (Haldane effect). This effect is more distinct at lower P_{CO_2} values. In *E. californicum*, a maximum difference in CO_2 concentration of $0.9\,mmol\,l^{-1}$ was measured in a previous study between deoxygenated and oxygenated haemolymph at P_{CO_2} values between 7 and 10 mmHg (R. Loewe, unpublished C_{CO_2} analyses). Above 20 mmHg there is no further dependence of C_{CO_2} on oxygen. Until we have additional information about arterial and venous haemolymph gas variables (e.g. relationships between P_{O_2} and P_{CO_2}), it will remain unclear whether this small Haldane effect has a physiological role.

During locomotor activity, the energy metabolism of *E. californicum* and *P. imperator*

(and of many other arachnid species) depends strongly on the anaerobic utilization of muscular phosphagen and carbohydrate stores (with D-lactate as the final product in the tissues). After 10 min of recovery from 3 min of exercise, the haemolymph pH (at equal P_{CO_2}) drops by 0.36 pH units in all three species (Table 1) which, owing to the logarithmic pH scale, corresponds to different changes in $[HCO_3^-]$: 8.7 mmol l^{-1} in *E. californicum*, 15 mmol l^{-1} in *P. imperator* and 11.1 mmol l^{-1} in *C. salei* (Fig. 1; pH 7.5). The gas analyses of haemolymph samples verified these results (Table 2): resting C_{CO_2} was about twice as high in *P. imperator* (see above); the drops in $[HCO_3^-]$ and C_{CO_2} after activity were also much higher in *P. imperator*. The drop in haemolymph bicarbonate concentration (which should correspond to the number of protons released from the tissues) was different in *E. californicum* and in *P. imperator*. The increase in haemolymph D-lactate concentration, however, was more similar in both species: 10.6 *versus* 12 mmol l^{-1}. During glycogenolysis, 1 mole of protons is generated per mole of end product lactate (Pörtner *et al.* 1984). Determinations of organic acids in the haemolymph by HPLC showed that, during recovery, it was almost exclusively D-lactate (and to a lesser extent, pyruvate) concentration that increased. The differences between the drop in $[HCO_3^-]$ and the increase in [D-lactate] could be due to additional regulatory mechanisms acting on intra- and extracellular $[H^+]$, because non-bicarbonate haemolymph buffers have no importance in this situation (the bicarbonate drops were considered at a constant pH of 7.5).

Cold-acclimation

A strong metabolic acidosis was found in *E. californicum* haemolymph after locomotor activity, but also after acclimation to moderately cold temperatures (10 °C; Fig. 3A). This species is found in arid zones in the Unites States close to the Mexican border, where winter temperatures may fall below this value. The analysis of organic acid concentrations in the haemolymph of cold-acclimated individuals by HPLC and enzymatic assays showed that the acidosis was due to an accumulation of acetate (Fig. 3B), which suggests that specific anaerobic processes participate in energy metabolism during cold-acclimation (de Zwaan, 1983; Hochachka and Somero, 1984; Urich, 1990), although the reasons for a lack of oxygen in (some) tissues are unknown. It is especially interesting that cold-acclimated carp and goldfish do not accumulate much lactate under anoxic conditions (Blazka, 1958). Lactate produced by some tissues is partially oxidized to acetyl CoA and CO_2 in other tissues (Shoubridge and Hochachka, 1979), and a further reaction from acetyl CoA to acetate may occur (Hochachka, 1980). Further studies on this subject are necessary.

Gas exchange and gas transport

How are gas exchange at the book lungs and acid–base balance related? Angersbach (1978) showed that pHa and pHv change in different ways after exercise in *E. californicum*. During early recovery, pHa dropped by at most 0.26 units and pHv decreased by at most 0.5 units.

Our haemolymph gas analyses revealed a larger decrease of approximately 0.4 pH units in both *E. californicum* and *P. imperator* (Table 2). But, because haemolymph was

sampled from the pericardium (pHa), venous admixtures caused by puncturing the pericardial wall cannot be excluded. Resting pHa was lower in *P. imperator* than in *E. californicum* (Table 2), even if there were deviations from the correct values, as discussed above. Dejours and Ar (1991) reported a very low pH (7.15) and a very high P_{CO_2} (29 mmHg) in the haemolymph of the scorpion *Leiurus quinquestriatus* at rest. On the basis of our analyses in *P. imperator*, we cannot confirm these data for scorpions in general.

Angersbach (1978) also reported that the extent of pH reduction during recovery from exercise was dependent on the intensity of the exercise. The difference of 0.24 units between the drop in pHa and that in pHv can be explained by respiratory compensation due to an increased expiration of CO_2: i.e. carbon dioxide is released to stabilize haemolymph pH. In studies on the expiration of CO_2 during recovery, we found marked differences between *E. californicum* and *P. imperator* (Paul and Fincke, 1989). Maximum CO_2 release was approximately twice as high in *P. imperator* and recovery was much slower in *E. californicum*, but the excess CO_2 expired above resting level (Me_{CO_2}) was almost identical in both species.

The decrease in bicarbonate concentration in the haemolymph (at pH 7.5) was 8.7 mmol l^{-1} in *E. californicum* and 15 mmol l^{-1} in *P. imperator* (for details, see above). Haemolymph volume in these animals is about 20 % of body mass (Stewart and Martin, 1970). In 15 g individuals, the reduction in the amount of haemolymph CO_2 after 10 min of recovery is therefore 26 μmol in *E. californicum* and 45 μmol in *P. imperator*. During recovery from a 3 min run, Me_{CO_2} of 15 g individuals was 62 μmol in *E. californicum* and 65 μmol in *P. imperator*. If the changes in pHa are disregarded for this estimation (which is justified), *E. californicum* expires about 40 % and *P. imperator* about 70 % of Me_{CO_2} within the first 10 min of recovery. This is reflected by the animals' typical CO_2 release patterns (Paul and Fincke, 1989): *P. imperator* shows a rapid increase to a maximal value that is twice as high as that in *E. californicum*, with a fast decrease afterwards, whereas *E. californicum* releases CO_2 more steadily.

These considerations are supported by the haemolymph gas analyses (Table 2). *P. imperator* has a higher resting C_{CO_2} and a much larger decrease in [HCO_3^-] and C_{CO_2} during recovery than does *E. californicum*. Maximal CO_2 release during recovery is twice as high in *P. imperator*, which correlates with an arterial P_{CO_2} that is also approximately twice as high in this species.

Both *E. californicum* and *P. imperator* release CO_2 during early recovery to stabilize haemolymph pH, but this mechanism to regulate haemolymph acid–base balance is much faster in *P. imperator* than in *E. californicum*, a difference that may exist between scorpions and spiders in general (see Paul and Fincke, 1989). The pattern of CO_2 expiration during recovery is obviously related to the morphometry of the respiratory organs. *E. californicum* and *P. imperator* have similar body masses, but the respiratory surface area is higher in *P. imperator* because it has more book lungs (Paul and Fincke, 1989).

Among different arachnid species, the importance of CO_2 release for the stabilization of haemolymph pH during recovery from exercise seems to vary. *P. imperator*

haemolymph has the highest C_{CO_2} and shows the greatest decrease during recovery, *E. californicum* haemolymph has the lowest resting C_{CO_2} and the smallest decrease during recovery, and *C. salei* has intermediate values of both resting level and decrease. There is a strong correlation between resting C_{CO_2} and the extent of CO_2 depletion during recovery.

Apart from the respiratory compensation, non-bicarbonate buffers should also play a role in the regulation of acid–base status. Non-bicarbonate buffering is weak in the tested species, however, being lower in *P. imperator* than in *E. californicum* (β 3.51 *versus* 4.53). It is puzzling that there is little or no correlation between non-bicarbonate buffer capacity and haemolymph protein concentration. In *E. californicum* only, haemocyanin (which represents about 80 % of haemolymph proteins) and the haemolymph lipoprotein seem to contribute significantly to non-bicarbonate buffering. This indicates that there are different numbers of buffering amino acid residues in the haemolymph proteins of *E. californicum* and *P. imperator*. The lipoprotein has been reported to serve as a carrier for a carbonic anhydrase (Stratakis and Linzen, 1984), which is thought to function in CO_2 release and acid–base regulation. In the land crab *Gecarcinus lateralis* (and in the blue crab *Callinectes sapidus*), however, carbonic anhydrase activity seems to be involved more in blood ion regulation than in CO_2 release (Henry and Cameron, 1983). More studies on the role of carbonic anhydrase in arachnid haemolymph are necessary.

The haemolymph of arachnids carries large amounts of carbon dioxide, thus permitting the stabilization of haemolymph pH by respiratory mechanisms. Haemolymph pH drops during recovery because lactate is formed during muscular activity and the associated protons are released into the haemolymph. The importance of haemolymph CO_2 for acid–base regulation may be made clearer by comparing the amounts of dissolved oxygen and carbon dioxide in the haemolymph. At a P_{O_2} at which haemocyanin is almost completely saturated (approximately 100 mmHg), the concentration of oxygen is (depending on haemolymph protein concentration) on average 0.69 mmol l^{-1} in *E. californicum* (0.52 mmol l^{-1} bound to haemocyanin) and 0.78 mmol l^{-1} in *P. imperator* (0.62 mmol l^{-1} bound to haemocyanin). At a physiological P_{CO_2} of 17 mmHg, C_{CO_2} is 13.89 mmol l^{-1} in *E. californicum* and as high as 23.94 mmol l^{-1} in *P. imperator*; values that are more than 20 or 30 times higher than the respective oxygen concentrations. The very high CO_2 concentration (compared with the O_2 concentration) and the marked decrease observed during recovery from exercise reflect the important role that carbon dioxide transport in the haemolymph plays in acid–base regulation of body fluids in arachnids. A look at the other big group of terrestrial arthropods, the insects, does not seem to be very informative in this context, because the physiological and metabolic differences are too numerous (e.g. haemolymph *versus* tracheal oxygen transport, mainly diffusive *versus* ventilatory gas exchange, continuous *versus* discontinuous respiration, well-developed *versus* poorly developed circulatory systems and high *versus* low anaerobic capacities; e.g. Paul, 1991, 1992; Kerkut and Gilbert, 1985). Land crabs, however, frequently have large differences between their haemolymph oxygen and carbon dioxide concentrations, similar to that seen in *E. californicum* (Burggren and McMahon, 1988). It is also interesting to consider terrestrial vertebrates. In birds and

mammals, the O_2 capacity is between 4.5 and 9 mmol l^{-1}; the concentration of CO_2 is even more variable, with values usually being well above 15 mmol l^{-1} at physiological P_{CO_2} values (Dejours, 1981). In humans, the O_2 capacity of blood is approximately 9.3 mmol l^{-1} and the CO_2 concentration of arterial or venous blood is between 20 and 23 mmol l^{-1} (Silbernagel and Despopoulos, 1983).

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