J Comp Physiol B (1989) 159:419–432

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Book lung function in arachnids

II. Carbon dioxide release and its relations to respiratory surface, water loss and heart frequency

Rüdiger Paul and Till Fincke*

Zoologisches Institut, Universität München, Luisenstrasse 14, D-8000 München 2, Federal Republic of Germany

Accepted May 23, 1989

Summary. 1. Carbon dioxide release, a major variable of gas exchange, was measured during rest, maximum activity and recovery in the tarantula, *Eurypelma californicum,* the ctenid spider, *Cupiennius salei,* and in the scorpions, *Pandinus imperator* and *Leiurus quinquestriatus.* In *Eurypelma* and *Pandinus,* water loss was measured simultaneously with CO_2. In *Eurypelma,* heart frequency, and in both *Eurypelma* and *Pandinus,* the respiratory surfaces of the book lungs were also determined. In most experiments, gas exchange of the whole animal was measured, and the animals were not restrained.

2. At rest, $\dot{V}_{CO_2}$ was similar in *Eurypelma* and *Pandinus.* During activity, $\dot{V}_{CO_2}$ often came to a halt in the spiders but not in the scorpions. During recovery, the pattern of CO_2 release was rather different in spiders and scorpions. In a series of experiments, CO_2 release after different activity periods was measured. In *Eurypelma,* maximum $\dot{V}_{CO_2}$ was much lower than in *Pandinus,* and recovery time was much longer in the tarantula. The surplus release of CO_2 was similar in both species, and it was linearly related to the duration of activity.

3. There were no significant differences in water loss during rest and recovery between *Eurypelma* and *Pandinus,* although these species live in quite different habitats (dry and humid environments). Special adaptations respecting a reduction of water loss seem to be absent in *Eurypelma.*

4. The additional measurement of heart frequency in *Eurypelma* revealed that slow fluctuations in CO_2 release ($1–3 \cdot min^{-1}$) at rest and in a later phase of recovery are probably caused by slow fluctuations in heart frequency. The time courses of $\dot{V}_{CO_2}$ and heart frequency during recovery differed, with heart frequency declining much faster than CO_2 release.

5. The respiratory surface in *Pandinus* is about 1.7 times larger than that in *Eurypelma,* which is in agreement with a much higher maximal CO_2 release in *Pandinus* during recovery.

6. CO_2 release pattern differences in the spiders and scorpions during recovery are probably caused by a different proportion of CO_2 release for buffering metabolic acidosis. Because of the high postactive CO_2 release due to the buffering of D-lactate, respiration and acid-base control are strongly related in the arachnids investigated. It is proposed that the control of the spiracle entrance area from the ambient to the book lungs is an essential element in controlling the acid-base state of body fluids.

Key words: Arachnids – Book lungs – Carbon dioxide release – Water release – Heart frequency

Introduction

Respiration and circulation play an essential part in clearing metabolically produced CO_2 and eventually the further CO_2 released from the buffering of metabolic acids. There has been a lot of work carried out, especially on vertebrates but also on invertebrates like crustacea, to understand the role of CO_2, e.g., in buffering processes or in the control of respiration.

For arachnids, only a few papers deal with the involvement of CO_2, especially in acid-base balance (e.g., Loewe and Brauer de Eggert 1979). The control of the openings of the book lungs to the ambient environment, the spiracle entrance areas, was related to the prevention of a high water loss (cf. Angersbach 1978). A continuous registration

* Present address: Zoologisches Institut, Universität Erlangen-Nürnberg, Staudtstrasse 5, D-8520 Erlangen, Federal Republic of Germany

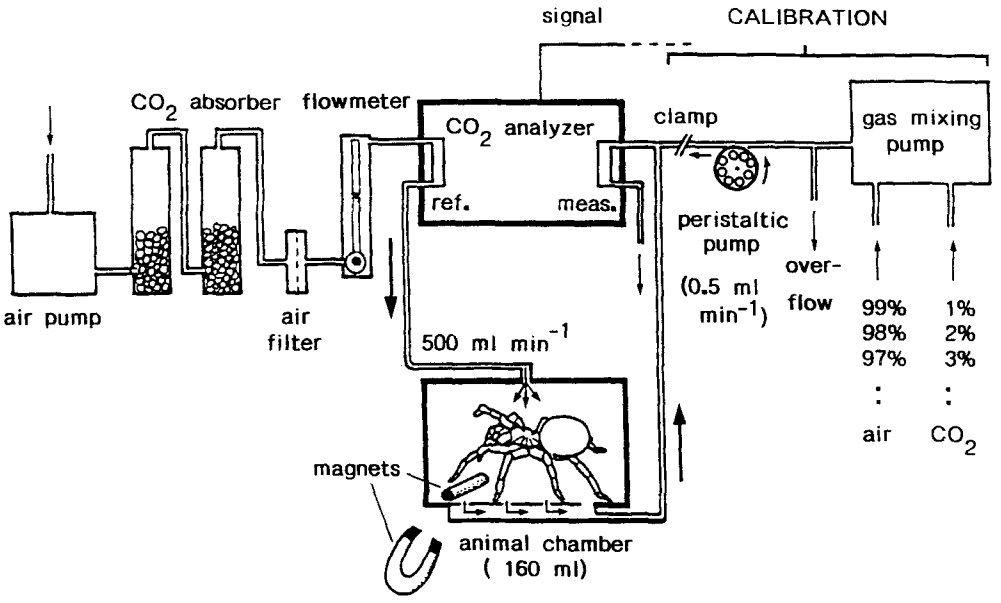


Fig. 1. Set-up for measurements of CO_2 release (see text for details)

of variables of respiration and circulation in arachnids to study cardiopulmonary coupling is not known to us.

Therefore, measurements with a high time resolution of CO_2 release, water release and heart frequency of unrestrained animals during rest, activity and recovery seemed to be promising for new insights in arachnid physiology. The main aspects to be explored are the comparison of CO_2 release in spiders and scorpions and the evaluation of maximum gas exchange rates. The high sensitivity of the CO_2 analyzer used here allowed measurements of $\dot{V}_{CO_2}$ without using a respiratory mask. Simultaneously with CO_2, either water release of the whole animal or heart frequency was measured. Together with data on respiratory surfaces and CO_2 transport in the haemolymph, the role of CO_2 in buffering is discussed.

Material and methods

Animals. Origin and keeping of *Eurypelma californicum* and *Pandinus imperator* were discussed in Paul et al. (1989b). The Latin and South American ctenid spider, *Cupiennius salei* (mass: 2.5 to 4.8 g), was bred according to Melchers (1963), and the scorpions, *Leiurus quinquestriatus* (Buthidae) (mass: 1.4 to 4.3 g), were caught in the Sahara. They were kept in preserving jars, filled either with moistened peat dust or with sand and were fed small crickets and flies. Water was given ad libitum. The temperature in the animal room was 25 °C.

General conditions. See Paul et al. (1989b).

Measurement of the release of carbon dioxide (Fig. 1). The animals were in a small plastic chamber (ca. 160 ml) which was streamed through from top to bottom by air free of CO_2 and H_2O. A magnetic bar in the chamber could be moved from the outside to provoke activity. The flow rate was 500 ml (ATPD)·min^{-1} (the optimum to receive a high time resolution and a high sensitivity of the CO_2 analyzer), controlled by a flow meter with an adjustable valve (Rotameter Serie L, Rota, Wehr, FRG). The calibration of this flow meter was periodically tested. The CO_2 and water from the room air were absorbed by two serially connected containers, filled with KOH pellets and lye. After that, the air passed a dust filter. CO_2- and H_2O-free air streamed through the reference channel of the CO_2 analyzer (URAS 2T, Hartmann & Braun, Frankfurt, FRG), while the air from the chamber streamed through the measuring channel of the device. All tubing material was made of PVC (inside diameter 4 mm; lengths as short as possible). The CO_2 analyzer was calibrated with mixtures of CO_2- and H_2O-free air and CO_2 (99% air/1% CO_2; 98% air/2% CO_2, etc.) prepared by a gas mixing pump (Wösthoff 201/a-F, Bochum, FRG). A peristaltic pump (Minipuls II, Gilson, France) was used to add 0.5 ml (ATPD)·min^{-1} of these mixtures to the main stream of 500 ml (ATPD)·min^{-1}. The flow rate of the pump was controlled prior to each calibration. In this way CO_2 concentrations of 10, 20, 30 ppm, etc. were produced to calibrate the analyzer. From the CO_2 concentration and the total flow rate, the CO_2 flux ($\dot{V}_{CO_2}$) was calculated. During calibration the clamp was opened and the animals removed. The voltage signal of the analyzer was amplified and recorded with an ADC-computer unit (12-Bit A/D converter, Apple II). The CO_2 analyzer was checked to give a voltage linear to the CO_2 concentration or flux [from 0 to >150 µl CO_2 (ATPD)·min^{-1}]. The maximum delay between the animal's CO_2 release and the corresponding signal of the analyzer could be estimated from the flow rates and from the volumes of the chamber, the tubes and the measuring channel of the analyzer to be smaller than 30 s. Because CO_2 was released ventrally from the animals' book lungs and could stream directly out of the chamber, the time resolution was even better than this estimation of maximum delay. Applying steps of 0 to 10, to 20 or to 30 µl CO_2 (ATPD)·min^{-1} (and backwards) at the bottom of the chamber revealed a $\tau_{63\%}$ of 6.5 (6.5–7) s and a $\tau_{90\%}$ of 10–12 (10.5–13) s.

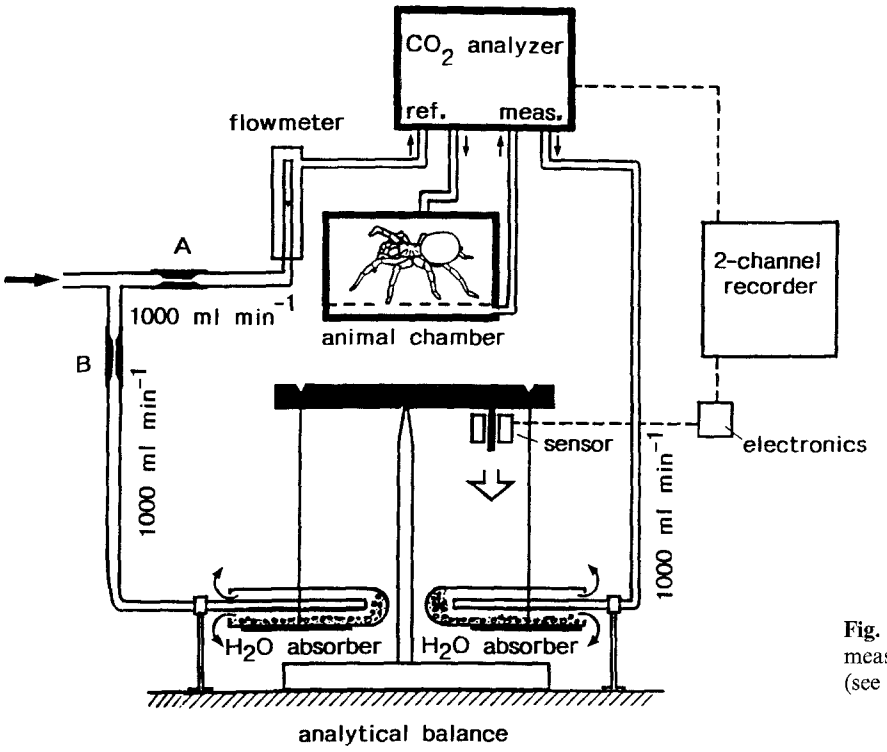


Fig. 2. Set-up for simultaneous measurements of CO_2 and water release (see text for details)

Simultaneous measurement of carbon dioxide and water release (Fig. 2). The experimental set-up (Fig. 1) was extended by a water vapour measuring device. The CO_2- and H_2O-free air was divided into two streams [1000 ml (ATPD)·min^{-1} each]. Flow rates were stabilized by narrow cannulas (cannulas with an inside diameter of 0.4 mm and a length of 30 mm). Air flow B served as a reference for the water vapour analyzer. Flow A streamed through a flow meter (Rotameter), the reference channel of the CO_2 analyzer, the animal chamber, the measuring channel of the CO_2 analyzer to the measuring part of the water vapour analyzer. An analytical balance (DP 3, Sartorius, Göttingen, FRG) with two plastic containers on the scales, which were filled with selective water-absorbing material (granulated P_2O_5), was used as analyzer. The streams into the containers were orientated horizontally, to prevent a turn of the scales due to the air stream. The small tubes entering the containers were fixed to the laboratory table and touched neither the absorbing material nor the containers. The high outflow from the containers of 1000 ml (ATPD)·min^{-1} prevented an invasion of *ambient* water vapour. A turn of the scales due to water absorption was recorded with an inductive displacement transducer (IWE 09/8 and OVE 35-1, TWK Elektronik, Düsseldorf, FRG). Linearity between mass and the signal of the displacement transducer was checked (from 0 to 15 mg). A built-in device for an addition of 'riding weights' to the left arm (reference) was used to calibrate and also to reset the balance, if the deflection became too high. Two signals, one from the CO_2 analyzer and one from the water vapour analyzer, were fed to a two channel chart recorder. The water analyzer at work needed attention (P_2O_5 handling, permanent control of the small tubes inside the plastic containers to prevent any contact). An injection of 1, 2 and 5 µl of water (distilled) with a micrometer syringe into the animal chamber led to an increase in mass (downward movement of the right scale) of 1, 2, and 5 mg. The CO_2 analyzer did not show any response during water calibration, and the water vapour analyzer did not show any response during CO_2 calibration (see above), which means both devices worked selectively.

One problem, however, was that the dynamic properties of the analyzer were not completely clear. A rapid injection of moistened room air (CO_2 concentration $\approx$300 ppm, 100% relative humidity) into the animal chamber caused a response with a $\tau_{90\%}$ of ca. 6 s (a little higher than the $\tau_{90\%}$ of the CO_2 analyser), but the measurements of the animals' water loss did not show any more rapid changes (see Paul et al. 1989b; Fig. 4). Perhaps there was some damping by condensation in the chamber or tubes. But it might also be true that the water release of whole animals does not show any rapid changes. Nevertheless, resting values, release patterns in general and the order of magnitude of the maxima are certainly reliable (see 'Discussion'). In some experiments, the animals release fluid from the mouth or from the poison gland during activity. In these cases, the maximum $\dot{M}_{H_2O}$ was higher, the time course was clearly not typical, and therefore these experiments were not taken into account.

Simultaneous measurement of carbon dioxide release and heart frequency (Fig. 3). Measurement of CO_2 release and calibration were done as above (Fig. 1). To measure heart frequency simultaneously a special chamber was built (i) to allow an easy exchange of animals (because the animals lay on their back, the recording device for heart activity could not be fixed to the opisthosoma) and (ii) to adjust the mounting to the specific anatomy of each spider. The carapace of the tarantula was glued to a square pipe (Fig. 3, bottom) with a mixture of wax and colophonium. This pipe fitted exactly around a square bar on a stand which could be screwed to the bottom of the chamber in variable positions. The legs remain free, and the prosoma can be move up and down to some degree. A magnetic bar was used to provoke activity (leg and prosoma movements, bites into the bar). The opisthosoma of the animals rested on a water-filled container covered with a Teflon membrane and

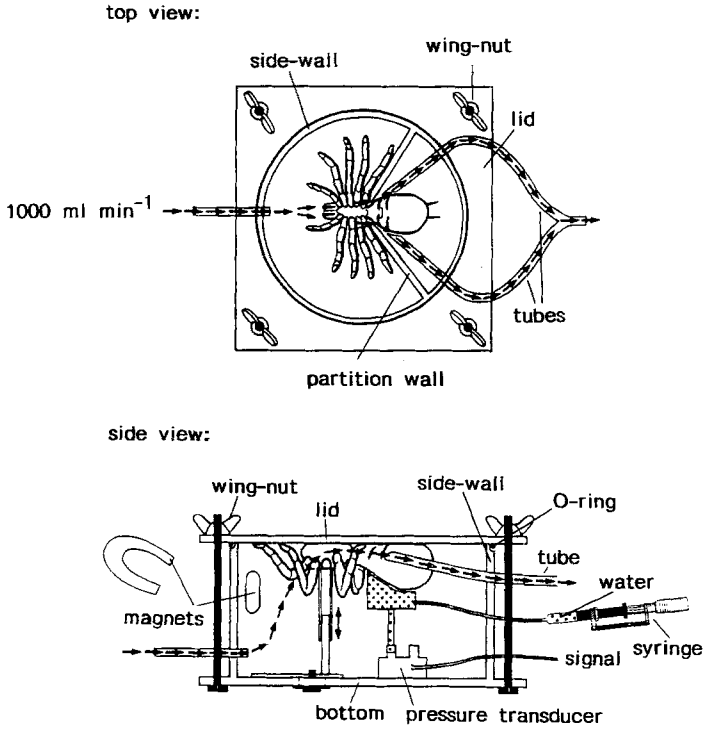


Fig. 3. Set-up for simultaneous measurements of CO_2 release and heart frequency (see text for details; the partition wall is not shown in side view)

connected to a differential pressure transducer (LX 06005D, SenSym, Sunnyvale, CA, USA) and to water-filled syringe. With the syringe, the amount of water in the container could be changed to fit the membrane to the animal's shaved opisthosoma. The beating of the heart led to changes of the curvature of the dorsal cuticle which caused pressure changes inside the container recorded by the pressure transducer. The plastic chamber was closed with a lid held tight by screws. CO_2- and H_2O-free air entered the front part of the chamber separated from the back part by a partition wall to improve the speed of response by limiting dead space (Fig. 3, top). The air streamed around the pedicel and passed the spiracles of the book lungs. Two tubes were used to exhaust the air with the expired CO_2 to obtain a fast response. In contrast to Fig. 1, the air was sucked by a pump placed behind the measuring channel of the CO_2 analyzer [flow rate: 1000 ml (ATPD)·min^{-1}]. The partition wall also fixed the opisthosoma laterally and prevented a disturbance of the heart recording by the posterior legs. The signal of the pressure transducer (voltage reference: LH 0070-0H, National Semiconductor, USA) was amplified (instrumentation amplifier: INA 101, Burr-Brown, Tucson, AZ, USA) and filtered (high pass, $f_0 = 0.1$ Hz; low pass, $f_0 =$ 1 Hz). This signal was permanently controlled to be simply periodic (sinusoidal) by using an oscilloscope. Subsequently, the signal was again amplified and fed into a frequency-to-voltage converter (AD 451 J, Analog Devices, Norwood, MA, USA). This device provided a voltage linear to frequency and therefore also to heart frequency. The frequency-dependent voltage signal enables a parallel plot of CO_2 release and heart frequency on a two-channel recorder, without further computation of heart frequency from the period length of single beats. Linearity was tested with periodics from smaller than 0.1 Hz to more than 2 Hz. A sudden change of frequency from e.g. 30 to 70 periods·min^{-1} – as tested by using a waveform generator – was followed by the frequency-voltage converter within 2.6 s. The $\tau_{90\%}$ of the CO_2 analyzer was ca. 5 s at a flow rate of 1000 ml (ATPD)·min^{-1}. Two signals, one from the frequency-voltage converter and one from the CO_2 analyzer were recorded either by a two-channel recorder or by an A/D converter-computer unit.

Measurement of the respiratory surface. The surface of the book lungs' lamellae was determined in *Eurypelma* and *Pandinus*. Individual book lungs were dissected, and after removal of the posterior atrial wall, a photograph (slide) was taken of the entrance area to the lamellae (by using a Zeiss binocular microscope, FRG). On the projected slide, the number of lamellae could easily be counted. Furthermore, several stacks of lamellae (up to 8) were dissected from one book lung and put on a sheet of graph paper. Then again a slide was taken. The outlines of the lamellae and a distinct number of millimeter squares were drawn from the projection onto cardboard, cut out and weighed. The surface of the lamellae was calculated by comparing the weights. The averaged surface of all stacks of lamellae was multiplied by the total number of lamellae and then by two (top and bottom surface), to get the respiratory surface of one book lung. Different book lungs of one animal were treated in this way. The mean respiratory surface of these lungs multiplied by their number (4 in *Eurypelma*, 8 in *Pandinus*) was considered to be the mean respiratory surface of an animal.

Results

Measurement of CO_2 release

CO_2 release, as one essential variable of respiration, differed significantly in the spiders and scorpions investigated, especially during recovery. Examples of typical release patterns are shown in Fig. 4. After an activity period of 60 s, maximum $\dot{V}_{CO_2}$ was about 45 µl CO_2 (ATPD)·min^{-1} in one

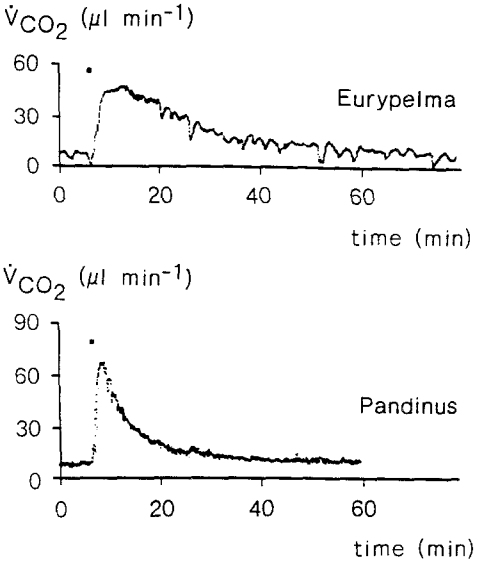
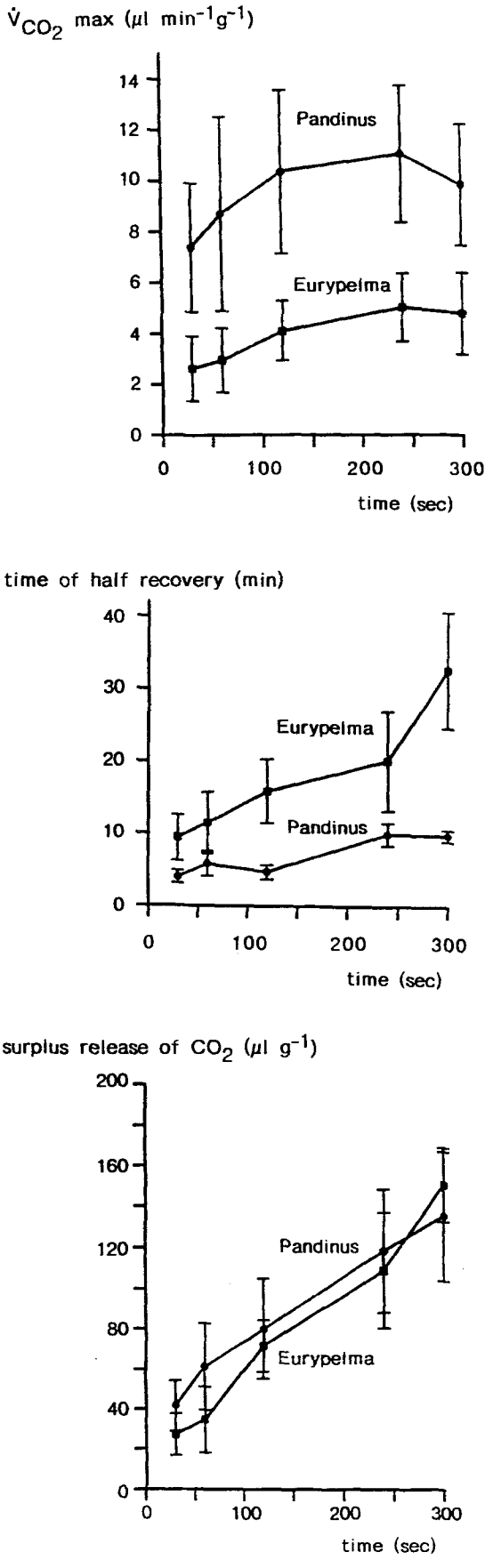


Fig. 4. Two examples of CO_2 release (µl ATPD) during rest, 60 s of locomotory activity (*black bar*) and recovery in *Eurypelma* (*top*) and *Pandinus* (*bottom*). Note the stop of CO_2 release during activity and the slow fluctuations, especially in a later phase of recovery in *Eurypelma* and the higher peak value and the shorter recovery time in *Pandinus*

Eurypelma californicum, whereas the maximum was about 70 µl CO_2 (ATPD)·min^{-1} in one *Pandinus imperator*. The recovery period (the time until resting level was reached again) was long in *Eurypelma* (ca. 60 min) but definitely shorter in *Pandinus* (ca. 40 min). While in *Eurypelma* $\dot{V}_{CO_2}$ gradually decreased after it had reached its maximum, in *Pandinus* the decrease was more rapid in the beginning and then slowed down, which resulted in a more distinct $\dot{V}_{CO_2}$ peak. During the first phase of activity, $\dot{V}_{CO_2}$ usually came to a halt in *Eurypelma* but not in *Pandinus*. In *Eurypelma,* quite regularly slow fluctuations of $\dot{V}_{CO_2}$ were observed during rest or in a later phase of recovery (with frequencies of ca. 1–3·min^{-1}). In the phase of maximum $\dot{V}_{CO_2}$ (the first 10 min of recovery), these fluctuations did not occur. In *Pandinus* these fluctuations were usually absent.

In a quite extensive series of experiments ($n=70$), CO_2 release during rest, different periods of maximum activity and recovery was measured in *E. californicum* ($n=7$ animals with body masses between 5.7 and 22 g; mean mass = 15 g) and *P. imperator* ($n=6$ animals with body masses between 7.5 and 22 g; mean mass = 15.1 g). Activity periods lasted 30 s, 1, 2, 4 and 5 min. For each period ca. 6 tests in *Eurypelma* and 5 tests in *Pandinus* were performed. Although it looks as if the parameters of CO_2 release, e.g. $\dot{V}_{CO_2max}$, do not depend on species and body mass only but show very strong individual variations, $\dot{V}_{CO_2}$ values were calculated as $\dot{V}_{CO_2}$ per g body mass (mass-specific $\dot{V}_{CO_2}$) to allow comparison with data in other species. Furthermore, it is obvious that identical stimulation periods do not necessarily evoke an identical response of the animals which causes an increase in standard deviation.

During rest $\dot{V}_{CO_2}$ was found to be very similar in both species: 0.75 ± 0.3 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} in *Eurypelma* ($n=37$ experiments) and 0.73 ± 0.35 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} in *Pandinus* ($n=32$ experiments). These 'resting values' are certainly not basal values, because no precautions were taken to minimize e.g. acoustical or optical stimulation. During locomotory activity, *Eurypelma* often stopped to release CO_2 for a certain period of time. The phase of interrupted or at least reduced CO_2 release did not depend on stimulation time and normally lasted between 26 and 42 s. Only in a few cases did the stop or reduction exceed 1 min. In *Pandinus* no stop or reduction was observed. In both *Eurypelma* and *Pandinus,* the maximum $\dot{V}_{CO_2}$ was always reached within 1–2 min after the end of activity, independent of stimulation time; in *Eurypelma* this was usually later than in *Pandinus*. The CO_2 release during recovery was very strongly different in both species. In either case, maximum CO_2 release slightly increased when stimulation was prolonged up to 4 min (Fig. 5, top). The mass-specific $\dot{V}_{CO_2max}$, calculated from the experiments with a stimulation period of 4 min, was 5.02 ± 1.34 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} in *Eurypelma* ($n=7$ experiments) and more than twice that much in *Pandinus,* 11.05 ± 2.69 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} ($n=5$ experiments). The highest non-mass-specific values in single experiments have been 94 µl CO_2 (STPD)·min^{-1} in *Eurypelma* and 180 µl CO_2 (STPD)·min^{-1} in *Pandinus*. Recovery was much faster in *Pandinus* (Fig. 5, center). As total recovery time was difficult to determine, we used the parameter 'half recovery time' starting with the end of activity and lasting until the mean $\dot{V}_{CO_2}$ between maximum and resting level was reached. This value generally was ca. 2.5 times higher in *Eurypelma*. In both species, half recovery time increased with longer stimulation periods and was approximately twice as high after 4 min activity than after 30 s. Total recovery times were maximally 98 min in *Eurypelma* and 50 min in *Pandinus* in single experiments. As a third parameter the surplus of CO_2 release during recovery ('additional CO_2 release'), which is the total amount of CO_2 (per g body mass) above resting level released during recovery, was calculated (Fig. 5, bottom). In



both species this parameter was similar and increased linearly with stimulation time, somewhat faster in *Eurypelma* than in *Pandinus*. A linear regression analysis revealed a slope of 0.44 in *Eurypelma* and 0.34 in *Pandinus*. Absolute, non-mass-specific amounts were maximally 2926 µl CO_2 (STPD) in *Eurypelma* and 2467 µl CO_2 (STPD) in *Pandinus* in single experiments.

In summary, maximum CO_2 release is twice as high in *Pandinus*, and half recovery time is approximately twice as long in *Euypelma*. Therefore, a similar surplus of CO_2 release may be expected and indeed occurs.

Additional experiments were done in the much smaller arachnids *Cupiennius salei* and *Leiurus quinquestriatus*. The spider and the scorpion again have similar body masses. Although these tests were not so numerous, they fit well into the general scheme of CO_2 release in spiders and scorpions.

In *Cupiennius*, the pattern of CO_2 release during recovery matched that of *Eurypelma* (as described above) closely, as the pattern of *Leiurus* matched that of *Pandinus*. During activity, the CO_2 release was reduced in *Cupiennius* but not in *Leiurus*, and again maximum $\dot{V}_{CO_2}$ was reached within 1–2 min after the end of activity, usually faster in *Leiurus* than in *Cupiennius*. Most of the experiments were performed with stimulation periods of 1 and 2 min. The evaluated data are shown in comparison with the data from *Eurypelma* and *Pandinus* [Table 1; mean body masses: 15 g (*Eurypelma*), 15.1 g (*Pandinus*), 3.16 g (*Cupiennius*), 2.75 g (*Leiurus*)]. Mass-specific $\dot{V}_{CO_2max}$ was approximately twice as high in *Leiurus* compared with *Cupiennius* and was much higher in the smaller species than in the bigger ones (difference about threefold in the two spiders and about twofold in the two scorpions). The highest non-mass-specific values in single experiments were 43 µl CO_2 (STPD)·min^{-1} in *Cupiennius* and 83 µl CO_2 (STPD)·min^{-1} in *Leiurus*. Half-recovery time in *Leiurus* was approximately only one-third of that in *Cupiennius*, but the values of the two spider or the two scorpion species, respectively, are similar. Total recovery times were maximally 54 min in *Cupiennius* and 25 min in *Leiurus*. In the two spider species, the mass-specific surplus of CO_2 release

Fig. 5. *Top:* Maximum CO_2 release (µl STPD; mean values ± SD) plotted against duration of activity in *Eurypelma* and *Pandinus*. *Center:* Time of half recovery (mean values ± SD) plotted against duration of activity in *Eurypelma* and *Pandinus*. *Bottom:* The surplus release of CO_2 during recovery (µl STPD; mean values ± SD) plotted against duration of activity in *Eurypelma* and *Pandinus*

Table 1. Comparison of maximum CO_2 release, 'half recovery time' and additional CO_2 release in *Eurypelma, Cupiennius, Pandinus* and *Leiurus* after stimulation periods of 60 and 120 s

	Eurypelma	*Cupiennius*	*Pandinus*	*Leiurus*
$\dot{V}_{CO_2max}$ (µl CO_2 STPD·min^{-1}·g^{-1})				
60 s	3.0± 1.3 (9)	8.6± 1.8 (4)	8.7± 3.8 (7)	19.3± 7.5 (4)
120 s	4.1± 1.2 (7)	11.7± 4.1 (5)	10.4± 3.2 (4)	18.0± 2.8 (3)
Time of half recovery (min)				
60 s	11.5± 4.3 (8)	12.0± 0.8 (4)	5.8± 1.7 (8)	4.0± 1.2 (4)
120 s	15.9± 4.4 (5)	12.1± 4.2 (5)	4.7± 1.0 (6)	3.8± 0.9 (5)
Additional CO_2 release (µl CO_2 STPD·g^{-1})				
60 s	34.7±16.4 (9)	67.1±10.7 (4)	61.1±21.7 (7)	59.8±40.2 (4)
120 s	71.5±13 (4)	148.2±51.2 (5)	79.9±24.9 (4)	64.6±35.2 (3)

( ) = number of experiments

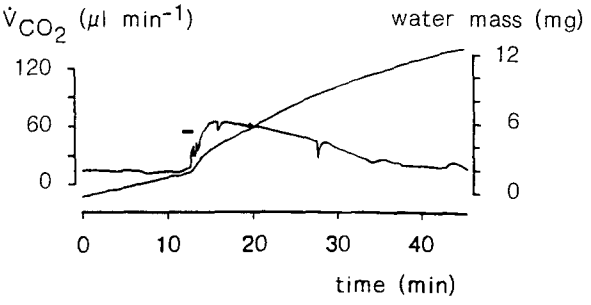


Fig. 6. Example of a simultaneous measurement of CO_2 (µl ATPD) and H_2O release in *Eurypelma* during rest, 1 min of locomotory activity (*black bar*) and recovery. ($\dot{M}_{H_2O}$ values were obtained by differentiating this signal vs time)

(additional CO_2 release) increased more strongly with stimulation time and was still ca. two times higher in *Cupiennius*. Non-mass-specific amounts were maximally 570 µl CO_2 (STPD) in *Cupiennius* and 416 µl CO_2 (STPD) in *Leiurus*. In summary, $\dot{V}_{CO_2max}$ is higher in scorpions, and recovery takes longer in spiders. The consequences will be discussed.

Simultaneous measurement of CO_2 and H_2O release

Reduction of water loss is an essential factor in keeping the body water content constant, especially in organisms (like *Eurypelma*) living in arid zones. CO_2 and H_2O release were measured simultaneously in *Eurypelma* (see for example, Fig. 6) and *Pandinus*. In all cases CO_2 release during recovery (after 60 s of maximum activity; black bar) followed the typical pattern, with a maximum of ca. 60 µl CO_2 (ATPD)·min^{-1} in this example. $\dot{M}_{H_2O}$ was calculated by differentiating the water vapour analyzer signal (Fig. 6) vs time in sections by applying tangents. During rest and in the late phase of recovery, water loss is similar. In the beginning of recovery, water release increases considerably.

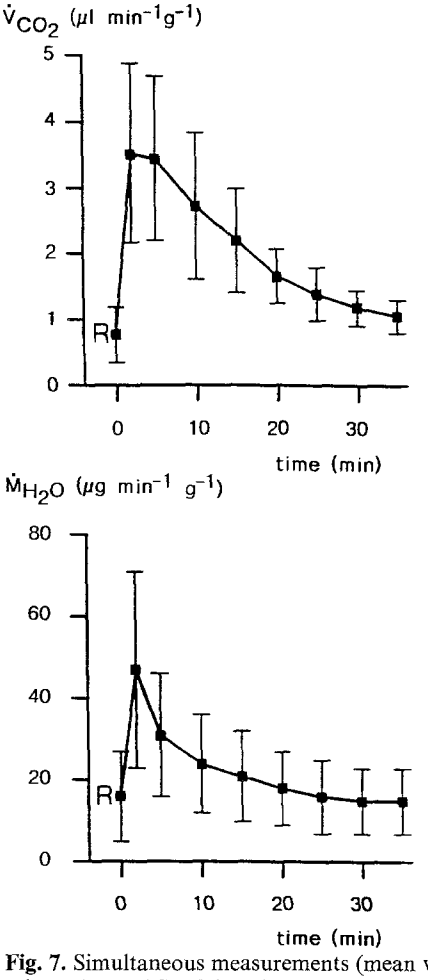


Fig. 7. Simultaneous measurements (mean values ± SD) of CO_2 release (*top;* µl STPD) and water release (*bottom*) in *Eurypelma* during rest (*R*) and recovery from 1 min of locomotion

Several experiments of this type in *Eurypelma* led to the result in Fig. 7: $n=17$ experiments in 6 animals (body masses between 6.7 and 12.3 g;

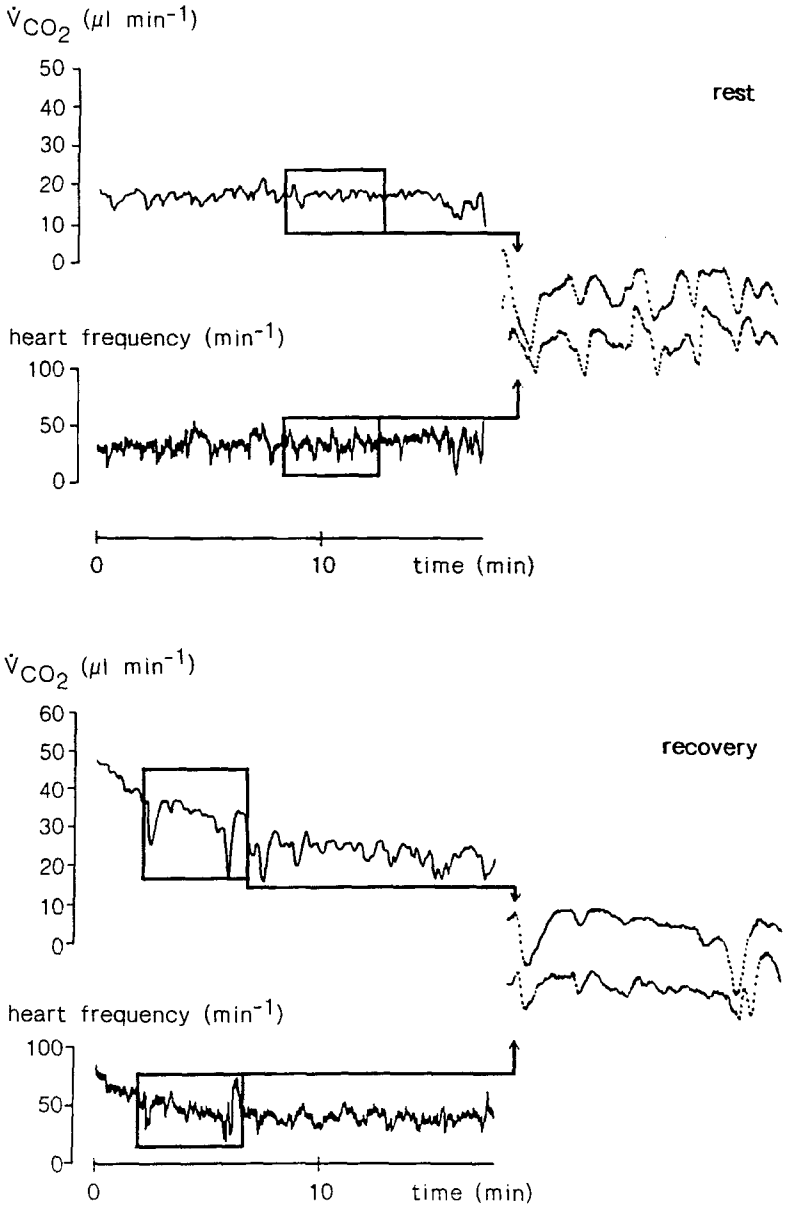


Fig. 8. Two examples of simultaneous measurements of CO_2 release (µl ATPD) and heart frequency in *Eurypelma* during rest (*top*) and recovery (*bottom*). Parts of the signals are magnified to demonstrate the similarity of heart frequency and CO_2 release (at *right*)

mean mass = 9.5 g). CO_2 release at rest was 0.77 ± 0.42 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} and water loss at rest was 16 ± 11 (SD) µg H_2O·min^{-1}·g^{-1}. After 1 min of locomotory activity, maximum CO_2 release was 3.52 ± 1.35 (SD) µl CO_2 (STPD)·min^{-1}·g^{-1}, and maximum water loss was 47 ± 24 (SD) µg H_2O·min^{-1}·g^{-1} (Fig. 7). As mentioned above in 'Material and methods', the water release registrations do not show any more rapid changes (Fig. 6), and the dynamic behaviour of the set-up is not completely clear. Thus, recovery time and additional water release were not determined, but it might be that the resting level was reached earlier in H_2O release (Fig. 7). In *Pandinus* 4 experiments in 4 animals (body masses between 9.2 and 17.7 g; mean mass = 13.6 g) were done. Values at rest were $\dot{V}_{CO_2} = 0.63 \pm 0.26$ (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} and $\dot{M}_{H_2O} = 19 \pm 11$ (SD) µg H_2O·min^{-1}·g^{-1}. Maximum values were $\dot{V}_{CO_2} = 13.7 \pm 6.1$ (SD) µl CO_2 (STPD)·min^{-1}·g^{-1} and $\dot{M}_{H_2O} = 35 \pm 20$ (SD) µg H_2O·min^{-1}·g^{-1}. No significant differences in $\dot{M}_{H_2O}$ could be found for *Eurypelma* and *Pandinus* during rest and recovery.

Simultaneous measurement of CO_2 release and heart frequency

CO_2 release (as an essential variable of respiration) and heart frequency (as an essential variable of perfusion) were recorded in parallel to obtain information about cardiopulmonary coupling. One major observation was that the fluctuations in CO_2

release mentioned above are reflected and probably caused by fluctuations in heart frequency (Fig. 8). The fluctuations of heart frequency and CO_2 release had a frequency of ca. 1–3·min^{-1}, they were usually absent during the first phase of recovery, when both variables were maximal, and the changes in heart frequency always occurred ahead of the changes in CO_2 release (0–10 s). Heart frequency variations probably cause slow variations in book lung perfusion and thus changes in CO_2 release (see 'Discussion').

In a series of experiments, the two variables were measured simultaneously during rest and recovery (after 60 s of activity) to estimate the general pattern ($n=14$ experiments in 6 animals with body masses between 9.2 and 14 g; mean mass = 9.7 g). In the following, $\dot{V}_{CO_2}$ data are not given as mass-specific but rather as absolute values to make comparison with the heart frequency values more feasible (to avoid the dimension: heart frequency ·g^{-1}). Mean resting $\dot{V}_{CO_2}$ was 12.7±3.7 (SD) µl CO_2 (STPD)·min^{-1}, and mean resting heart frequency was 21.2±7.2 (SD) beats·min^{-1} (Fig. 9, top and center; first three data points). After 60 s of locomotion, $\dot{V}_{CO_2max}$ was 40.55±12.21 (SD) µl CO_2 (STPD)·min^{-1}, and maximum heart frequency was 79.5±16.8 (SD) beats·min^{-1}. Maximum $\dot{V}_{CO_2}$ was reached ca. 2 min and maximum heart frequency ca. 1 min past the end of activity. Half-recovery time (as defined above) was ca. 8 min for CO_2 release and ca. 5 min for heart frequency. The time courses of CO_2 release and heart frequency during recovery were not parallel. This can be demonstrated by plotting $\dot{V}_{CO_2}$ against heart frequency (Fig. 9, bottom). A hysteresis occurs running clockwise as heart frequency declines much faster than CO_2 release.

Respiratory surface

The respiratory surface in *Pandinus* is much larger than that in *Eurypelma* (Fig. 10). The variables of the allometric equation: $S_r = a^* M^b$ (S_r = respiratory surface in cm^2, M = body mass in g) were calculated using a least-squares linear regression analysis after transformation to common logarithms ($\log S_r = \log a + b^* \log M$). In *Eurypelma* (body mass between 7.5 to 18.6 g), the resulting factors are $a=8.59$ and $b=0.64$ (with a 95% confidence interval for b of 0.33) and in *Pandinus* (body mass between 6.5 and 19.5 g) $a=14.29$ and $b=0.64$ (95% confidence interval for b of 0.33). Thus, the respiratory surface of *Pandinus* is 1.66 times larger than that of *Eurypelma*, independent of body mass.

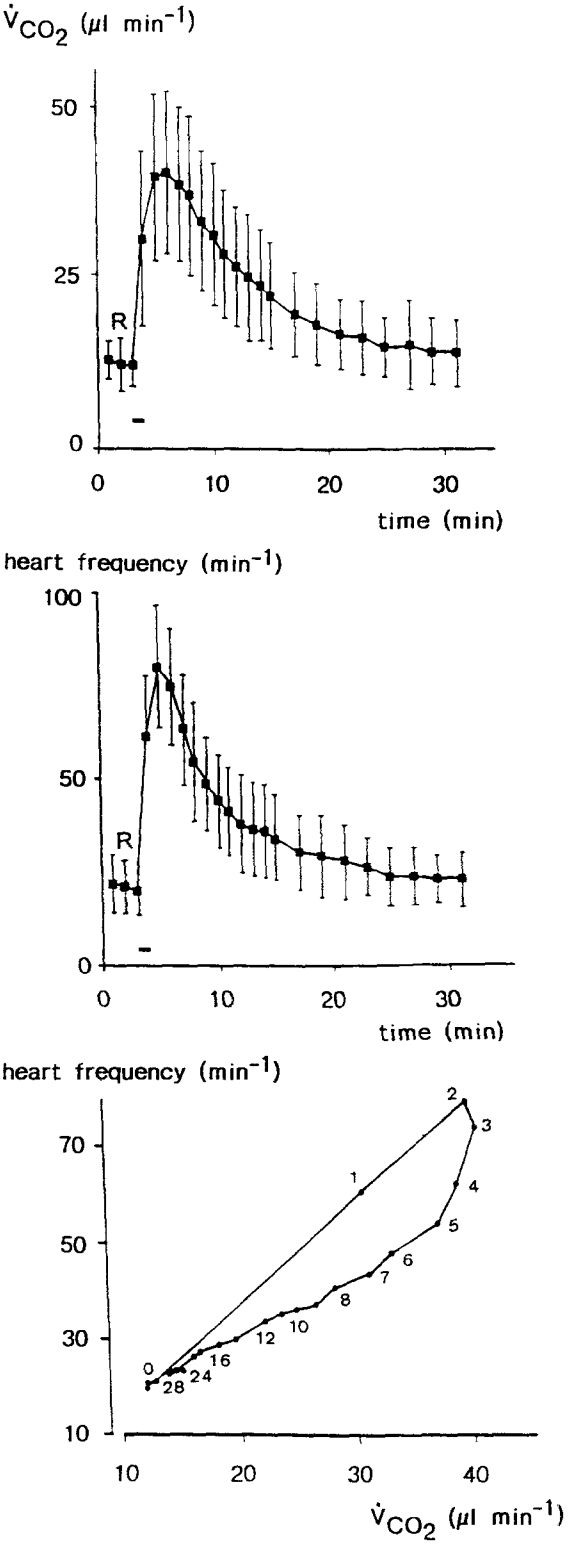


Fig. 9. Simultaneous measurements (mean values ± SD) of CO_2 release (*top;* µl STPD) and heart frequency (*center*) in *Eurypelma* during rest (*R*) and recovery from 1 min of maximum activity (*black bar*). The plot of heart frequency vs CO_2 release (*bottom*) shows a distinct hysteresis. *Numbers* designate subsequent intervals of time from the state of rest (*0*), through the maximum of heart frequency (*2*) and through the whole recovery period (*3–28*); time between numbers is 1 min

This corresponds to a higher CO_2 release in *Pandinus* (Fig. 5), although a still higher value (ratio of 2 in $\dot{V}_{CO_2max}$) would be expected (see 'Discussion').

Discussion

Carbon dioxide release

The pattern of CO_2 release is quite different in spiders and scorpions. Fluctuations of $\dot{V}_{CO_2}$ occurred in *Eurypelma* but were usually absent in *Pandinus* (Fig. 4). During maximum activity, $\dot{V}_{CO_2}$ usually came to a halt in the spiders but not in the scorpions (Fig. 4). Maximum values were much higher and recovery periods much shorter in the scorpions (Fig. 5, Table 1). Only mean values at rest and the surplus release of CO_2 during recovery were comparable in *Eurypelma* and *Pandinus* (Fig. 5).

CO_2 release at rest (22° C) was 675 µl CO_2 (STPD)·h^{-1} in a 15-g *Eurypelma*. This value is ca. 3.8 times higher than the basal value, which was measured by manometric respirometry and found to be 180 µl CO_2 (STPD)·h^{-1} in a 15-g animal, interpolated for $T=22°$ C (see Paul et al. 1989b). Special precautions had been taken to minimize optical and acoustical stimulations, and only the lowest gas exchange rate from a 24-h period was taken into account. Obviously, a certain alertness in the arrangement shown in Fig. 1 could not be avoided (see Paul et al. 1989b).

The stop of $\dot{V}_{CO_2}$ (up to 1 min) in *Eurypelma* at the beginning of maximum activity correlates with a stop of $\dot{V}_{O_2}$ (Paul et al. 1987, Paul et al. 1989b) and with a closure of the spiracles (Fincke and Paul 1989). Additionally, a stop or reduction of prosomal perfusion was measured during locomotion, because of high prosomal haemolymph pressures due to hydraulic leg movement (Paul et al. 1989a). Scorpions, in contrast to spiders, most probably do not have a hydraulic locomotory system (Manton 1958).

Maximum CO_2 release during recovery is much higher in the scorpions than in the spiders investigated (Fig. 5, Table 1). After 4 min of activity, the highest mean $\dot{V}_{CO_2max}$ values were reached: 75.3 µl CO_2 (STPD)·min^{-1} in *Eurypelma* and 165.8 µl CO_2 (STPD)·min^{-1} in *Pandinus* (at $T=22°$ C; mean body mass = 15 g). Respiratory surfaces are also much smaller in the tarantula (Fig. 10): 48.7 cm^2 in *Eurypelma* vs 80 cm^2 in *Pandinus* (in 15-g animals). The mean respiratory surface per lung is ca. 12 cm^2 in the four-lunged *Eurypelma* and ca. 10 cm^2 in the eight-lunged *Pandinus* (in 15-g animals). For $\dot{V}_{CO_2max}$ values, the ratio is ca.

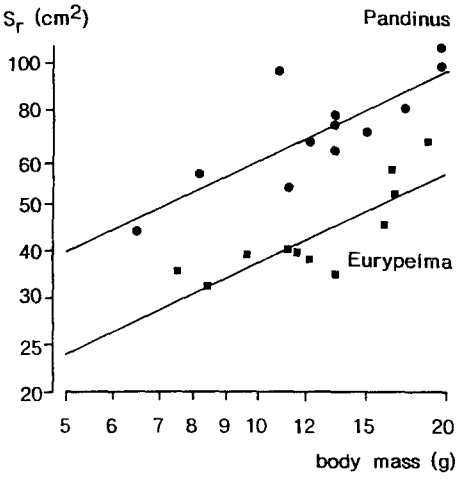


Fig. 10. Regression lines between respiratory surface S_r (cm^2) and body mass (g) after transformation to common logarithmy in *Eurypelma* (*squares*) and *Pandinus* (*circles*)

1:2.2; for respiratory surfaces, it is ca. 1:1.7 (*Eurypelma* to *Pandinus*). Maximum CO_2 flux per cm^2 is 1.55 in *Eurypelma* and 2.1 µl CO_2 (STPD)·min^{-1}·cm^{-2} in *Pandinus* (15-g animals).

From these data, two conclusions may be drawn:

1. The increase in respiratory surface from *Eurypelma* to *Pandinus* is mainly due to an increase in book lung units and hardly due to an increase in respiratory surface per lung (addition of functional subunits; cf. Günther 1975; Anderson and Prestwich 1982). Book lungs, at least in these two species, essentially are diffusion lungs, as 'ventilatory' volumes are much lower than gas exchange volumes in *Eurypelma* (Paul et al. 1987), and similar results have been found in *Pandinus* (unpublished data). An increase of the respiratory surface by increasing the anterior-to-posterior length of the lamellae would probably impede diffusion in the air space by too long diffusion distances. On the other hand, an increase of the lateral-to-medial width of the lamellae would not improve gas exchange much, because the haemocyanin in the haemolymph, which streams in parallel to this width, already is almost completely saturated during recovery (Angersbach 1978). A further increase of either length or width of lamellae would not increase the effective respiratory surface. The only real alternative would be an increase in the number of sacculi, but this would probably require changes in anatomy which would cause even more problems, e.g., a conflict with the position of other organs. The construction and the dimensions of book lungs are the result of an optimizing process for metabolic and various physical requirements (see below).

2. Since the ratio for $\dot{V}_{CO_2max}$ values is higher than the ratio for respiratory surfaces for *Eurypelma* to *Pandinus* or, in other words, because the maximum CO_2 flux per cm^2 of respiratory surface is higher in *Pandinus*, the P_{CO_2} gradient across the lung wall should be higher in the scorpion. The chitinous lung walls are more or less equally thin, in *Eurypelma* about 0.2 µm (electron microscopically determined by Peter Seifert, unpublished data), in *Pandinus* about 0.3 µm (Rödl 1987). The assumption of a higher gradient is supported by measurements of the CO_2 transport properties of the haemolymph (Paul, unpublished data). Haemolymph samples were taken from the pericardium, during rest or 10 min after a 3-min phase of maximum activity. The pH of the undiluted haemolymph was measured after equilibration with a certain P_{CO_2}. The CO_2 dissociation curves (C_{CO_2} vs P_{CO_2}) were calculated from these data by applying the Henderson-Hasselbalch equation (solubility coefficients and pK values were calculated after Heisler 1986). In the haemolymph of resting animals, C_{CO_2} was much lower in *Eurypelma* than in *Pandinus* at corresponding P_{CO_2} values. For a P_{CO_2} of 15 torr for example, C_{CO_2} was 13.6 µmol·ml^{-1} in *Eurypelma* and 23 µmol·ml^{-1} in *Pandinus* at 25° C. Post-active samples revealed a strong metabolic acidosis. The C_{CO_2} lowered considerably in both *Eurypelma* and *Pandinus* (almost parallel downward shifts of the CO_2 dissociation curves). These drops, which were due to an accumulation of anaerobic metabolites, were ca. 7 µmol·ml^{-1} in *Eurypelma* and ca. 13.4 µmol·ml^{-1} in *Pandinus*. The reduction in C_{CO_2} is not very dependent on specific arterial or venous P_{CO_2} values, because in the physiological range the slopes of the CO_2 dissociation curves are low. Assuming a blood volume of about 20% of body mass (3 ml in 15-g animals; see Stewart and Martin 1970), the total loss of CO_2 from the haemolymph can be calculated to be ca. 470 µl CO_2 (STPD) in *Eurypelma* and ca. 900 µl CO_2 (STPD) in *Pandinus*. A comparison with the data on total additional CO_2 release (Fig. 5, bottom) reveals that a considerable share of CO_2 release during recovery must be due to the buffering of a metabolic acidosis: 34% in *Eurypelma* [additional CO_2 release: 1378 µl CO_2 (STPD)] and 62% in *Pandinus* [additional CO_2 release: 1460 µl CO_2 (STPD)]. Total CO_2 release values are interpolated on the basis of 3 min of locomotion. The high share of CO_2 release in *Pandinus* due to buffering seems to be correlated with a low concentration of non-bicarbonate buffers in its haemolymph (Paul, unpublished data). The accumulation of anaerobic metabolites (D-lactate) in the haemolymph 10 min after a 3-min phase of locomotion is roughly the same (regarding the order of magnitude): maximally 13 µmol·ml^{-1} in *Eurypelma* and 15 µmol·ml^{-1} in *Pandinus* (Paul and Storz 1987). Note the similarity of D-lactate accumulation and C_{CO_2} drop in *Pandinus*. At rest the D-lactate concentration is below 1 µmol·ml^{-1} in both species.

As buffering of metabolic acidosis by CO_2 release during recovery is more distinct in *Pandinus*, it might be the main reason for the different release pattern of CO_2 compared with *Eurypelma*. Since in both species accumulation of D-lactate in the haemolymph is maximum 10 min after the end of 3 min of locomotion, and since the metabolic acidosis occurring is buffered by CO_2 release – more strongly in the scorpion than in the tarantula – CO_2 release is much higher in *Pandinus*, especially during the first 10 min of recovery, causing a higher $\dot{V}_{CO_2max}$ value and a shorter half recovery time (cf. Fig. 4). The high CO_2 release in the first phase of recovery in *Pandinus* is strongly facilitated by the larger respiratory surface.

The loss of CO_2 from the haemolymph due to buffering has to be balanced. Oxygen consumption, which is strictly coupled with CO_2 production, shows a faster time course than CO_2 release (Paul et al. 1989b). The refilling of CO_2 (bicarbonate) stores therefore probably occurs only with a small delay to the CO_2 release from buffering. The second drop in haemolymph pH during recovery, as reported by Angersbach (1978), may reflect this metabolic production of CO_2.

The acidosis occurring suggests that the muscles of the locomotory system strongly depend on anaerobic metabolism in both species, which probably is caused by a lack of capillaries in muscle tissues (Bihlmayer et al. 1989): oxygen supply has to occur via diffusion from the haemolymph in the haemocoele with long diffusion distances (see Angersbach 1975). In some spiders (*Cupiennius, Pholcus*) it is known that mitochondria are very rare in most of the leg muscles (Linzen and Gallowitz 1975; Foelix 1979: Fig. 23). In *Eurypelma* there is also some evidence for a low mitochondria content in the prosoma and leg muscles (Paul, unpublished data).

For the two smaller species, *Cupiennius* and *Leiurus*, the results are similar (Table 1). During activity, $\dot{V}_{CO_2}$ came to a halt in *Cupiennius;* during recovery, the scorpion had a much higher CO_2 release, and the recovery period was much shorter. The situation most probably is similar to that in *Eurypelma* and *Pandinus*, yet not so many data were collected. Mass-specific CO_2 release is higher

in the smaller species. In *Cupiennius,* the mass-specific $\dot{V}_{CO_2}$ is exceptionally high. Since this species has only two book lungs and a smaller respiratory surface, there must be some peculiar features, e.g. high P_{CO_2} differences across the lung wall. The total additional CO_2 release increased more pronouncedly with stimulation time in the spiders, and especially strongly in *Cupiennius,* which may be caused by a higher participation of anaerobic processes during locomotion.

H_2O release

In contrast to CO_2 release H_2O release in *Eurypelma* (Fig. 7) and *Pandinus* is quite similar: 16 vs 19 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ in resting animals (which corresponds to 0.096% or 0.114% body mass $\cdot h^{-1}$) and 47 vs 35 µg $H_2O \cdot min^{-1} \cdot h^{-1}$ maximally (22° C). Judging these results, an environmental adaptation regarding the variable water release is not deducible (*Eurypelma* living in the arid zones of North America, *Pandinus* living in hot humid areas in West Africa).

The data in *Eurypelma* are supported (regarding the order of magnitude) by measurements with a radiolabelling technique (Löw 1983): ca. 8 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ in resting animals, with an increase of 48.6% after activity. A similar water loss has been demonstrated for *Aphonopelma* sp: 0.073% body mass $\cdot h^{-1}$ at 30° C (Seymour and Vinegar 1973) and *Eurypelma* sp. (Cloudsley-Thompson 1967): 0.147% body mass $\cdot h^{-1}$. Herreid (1969) reported in *Eurypelma californicum* a water loss about five times higher than ours but under different environmental conditions (higher relative humidity): 0.45% body mass $\cdot h^{-1}$ at 25° C. For scorpions (all living in dry habitats) several data are given by Hadley (1970), which are within the range of our results: 0.028% body mass $\cdot h^{-1}$ in *Hadrurus arizonensis,* 0.044% body mass $\cdot h^{-1}$ in *Diplocentrus* sp. and 0.091% body mass $\cdot h^{-1}$ in *Centruroides sculpturatus* (all data at 30° C). Pulz (1986) measured water loss in tarantulas with a humidity sensor. He differentiated between transpiration (primary gaseous water loss) and secretion (primary liquid water loss). Transpiration in *Phormictopus* sp. (body mass between 3.7 and 17.4 g) was 12 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ and 8.3 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ in *Brachypelma smithi* (body mass between 5.2 and 27.4 g) (all data at 30° C). He also reported that activity leads to an increase of non-mass-specific water loss ('transpiration peaks') up to 500 µg $\cdot min^{-1}$ in individual tarantulas. By sealing the spiracles he concluded that apart from a minor respiratory water loss the transpiration peaks 'are likely to be elicited by activity-induced turbulence within the humid boundary layer near the cuticular surface'.

So far only data about water loss of whole animals have been discussed. In a set of experiments, water loss from the book lungs was measured by using a respiratory mask and a humidity sensor (see Paul et al. 1989b). Water loss was much lower: ca. 3 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ in resting animals and ca. 7 µg $H_2O \cdot min^{-1} \cdot g^{-1}$ maximally. Respiratory water loss obviously is only ca. 20% of total water loss in resting animals and 15% during recovery. Löw (1983) estimated respiratory water loss to be 12% of the total loss in *Eurypelma.*

The spiracles of the book lungs are nearly closed at rest. This was considered a mechanism to prevent water loss (cf. Angersbach 1978). Considering the relatively small proportion of respiratory water loss one has to look for additional advantages. One major point might be the control of the acid-base state of the haemolymph. In *Eurypelma* and *Pandinus,* the pH of body fluids depends very much on CO_2; at a low P_{CO_2} (a few torr), the haemolymph becomes rather alkaline (pH > 8; Paul, unpublished data). Increasing the P_{CO_2} in front of the spiracles causes their opening after a certain latency (Hazelhoff 1926; Fincke and Paul 1989). The latter may be explained as part of the acid-base control system. An increase of P_{CO_2} in the body fluids due to buffering in a closed system (prosoma) or to an increase of metabolic CO_2 production should cause a widening of the spiracle entrance area, with a subsequent increase of CO_2 release (see Fincke and Paul 1989). Therefore, the spiracle entrance area is probably regulated to stabilize the haemolymph pH by controlled CO_2 release.

Heart frequency and CO_2 release

The parallel measurement of heart frequency and $\dot{V}_{CO_2}$ in *Eurypelma* revealed that fluctuations in CO_2 release mostly are preceded, reflected and probably caused by changes in heart frequency (Fig. 8). Fluctuations in heart frequency probably cause changes in lung perfusion. Reduced lung perfusion might cause a faster equilibration of partial pressures between the haemolymph and lamellar air space during lung passage of the haemolymph, thus reducing the effective partial pressure gradient across the lung wall with the consequence of a reduced gas exchange.

Similar fluctuations have been reported for $\dot{V}_{O_2}$

in *Brachypelma smithi* (Anderson and Prestwich 1985) with a frequency of 0.2–2·min^{-1}. We found fluctuations also in $\dot{V}_{O_2}$ and even in respiratory water loss (Paul et al. 1989b). Investigating book lung 'ventilation' with a microbarometric method (Paul et al. 1987), we found not only heart-synchronous pulsations of the atrial wall but also non-heart-synchronous movements, which occured at frequencies of ca. 5–10·min^{-1} and slower and are probably related to the measured fluctuations in gas exchange.

The general time courses of heart frequency and CO_2 release are not parallel (Fig. 9). Heart frequency declines faster after having reached its maximum. To keep up expiration on a high level, the CO_2 release per heart beat (CO_2 pulse) has to increase towards the maximum $\dot{V}_{CO_2}$. The CO_2 pulse increases from 0.5 µl CO_2 (STPD) per beat immediately after activity to 0.71 µl CO_2 (STPD) per beat about 6–9 min after the end of locomotion. The accumulation of D-lactate in the haemolymph shows a similar time course, with a maximum 10 min after the end of activity (Paul and Storz 1987). Buffering and metabolic production of CO_2 cause an increase of CO_2 release per heart beat.

Respiratory surface

The respiratory surface in *Pandinus* is independent of body mass and 1.7 times larger than in *Eurypelma* (Fig. 10). The slope *b* in the allometric equation $S_r = a^* M^b$, is identical in both species: 0.64. Anderson and Prestwich (1982) reported a slope of 0.83 in a regression of different spider species with individuals between 40 and 3000 mg and somewhat higher slopes in intra-specific regressions. A comparison with data in similar-sized mammals reveals some distinct differences in lung anatomy. A 15-g *Eurypelma* has a respiratory surface of 50 cm^2 and a total lung volume of 0.03 cm^3 (Paul et al. 1987). Small mammals like shrews and mice (15 g) have a much larger respiratory surface of ca. 600 cm^2 (Tenney and Remmers 1963) and a lung volume of 0.9 cm^3 (Schmidt-Nielsen 1979, p 191). Surface per volume is ca. 1670·cm^{-1} in *Eurypelma* and only 670·cm^{-1} in a similar-sized mammal. Thus, an increase of respiratory surface by folding is much more pronounced in the book lungs of *Eurypelma*. In *Eurypelma* the CO_2 flux per cm^2 of respiratory surface is 1.55 µl CO_2 (STPD)·min^{-1}·cm^{-2} maximally; in a similar-sized mammal this value is ca. 5.7 µl CO_2 (STPD)·min^{-1}·cm^{-2}, which partly is due to the very different materials the lungs are built from (chitin vs epithelium). There is a distinct difference between tarantulas and mammals in the size of their body surfaces compared with their lung surfaces. In *Eurypelma*, both values are quite similar: the body surface is 78 cm^2 (Stewart and Martin 1970), the lung surface 50 cm^2; in the mammal, the lung surface is ten times the body surface: 600 cm^2 vs 60 cm^2 (Benedict 1934). The increase of the inner (respiratory) surface demonstrates the growing importance of aerobic metabolism in higher organisms.

References

Anderson JF, Prestwich KN (1982) Respiratory gas exchange in spiders. Physiol Zool 55:72–90

Anderson JF, Prestwich KN (1985) The physiology of exercise at and above maximal aerobic capacity in a theraphosid (tarantula) spider, *Brachypelma smithi* (F.O. Pickard-Cambridge). J Comp Physiol B 155:529–539

Angersbach D (1975) Oxygen pressures in haemolymph and various tissues of the tarantula *Eurypelma helluo*. J Comp Physiol 98:133

Angersbach D (1978) Oxygen transport in the blood of the tarantula *Eurypelma californicum*: P_{O_2} and pH during rest, activity and recovery. J Comp Physiol 123:113–125

Benedict FG (1934) Die Oberflächenbestimmung verschiedener Tiergattungen. Ergeb Physiol 36:300–346

Bihlmayer S, Zahler S, Paul R (in press) Zur Struktur und Funktion des Kreislaufsystems der Vogelspinne *Eurypelma californicum*. Verhandl Dtsch Zool Ges

Cloudsley-Thompson JL (1957) Studies in diurnal rhythms. V. Nocturnal ecology and water relations in the British cribellate spiders of the genus Ciniflo. J Linn Soc Zool 43:134–152

Fincke T, Paul R (1989) Book lung function in arachnids III: the function and control of the spiracles. J Comp Physiol B 159:433

Foelix R (1979) Biologie der Spinnen. Thieme, Stuttgart

Günther B (1975) Dimensional analysis and theory of biological similarity. Physiol Rev 55:659–699

Hadley NF (1970) Water relations of the desert scorpions, *Hadrurus arizonensis*. J Exp Biol 53:547–558

Hazelhoff EH (1926) Die Regulierung der Atmung bei Insekten und Spinnen. Z Vergl Physiol 5:179–190

Heisler N (1986) Acid-base regulation in animals. Elsevier, Amsterdam Oxford New York

Herreid CF (1969) Water loss of crabs in different habitats. Comp Biochem Physiol 28:829–839

Linzen B, Gallowitz P (1975) Enzyme activity patterns in muscles of the lycosid spider, Cupiennius salei. J Comp Physiol 96:101–109

Löw A (1983) Untersuchungen zur cuticulären und pulmonären Transpiration der Vogelspinne *Eurypelma californicum*. Diploma thesis, University of Munich, Faculty of Biology

Loewe R, Brauer de Eggert H (1979) Blood gas analysis and acidbase status in the hemolymph of a spider (*Eurypelma californicum*) – influence of temperature. J Comp Physiol 134:331–338

Manton SM (1958) Hydrostatic pressure and leg extension in arthropods, with special reference to arachnids. Ann Mag Nat Hist Zool Botany Geol Ser 13:161

Melchers M (1963) Zur Biologie und zum Verhalten von *Cupiennius salei* (Keyserling), einer amerikanischen Ctenide. Zool Jb Abt System Ökol Geogr 91:1–90

Paul R, Storz H (1987) Zur Physiologie der Hämolymphe von Spinnentieren. Verhandl Dtsch Zool Ges, p 221

Paul R, Fincke T, Linzen B (1987) Respiration in the tarantula *Eurypelma californicum:* evidence for diffusion lungs. J Comp Physiol B 157:209–217

Paul R, Tiling K, Focke P, Linzen B (1989a) Heart and circulatory functions in a spider (*Eurypelma californicum*): the effects of hydraulic force generation. J Comp Physiol B 158:673–687

Paul R, Fincke T, Linzen B (1989b) Book lung function in arachnids I: oxygen uptake and respiratory quotient during rest, activity and recovery – relations to gas transport in the haemolymph. J Comp Physiol B 159:409–418

Pulz R (1986) Patterns of evaporative water loss in tarantulas (Araneae, Theraphosidae): transpiration and secretion. Proc 9th Int Congr Arachnol, Panama 1983

Rödl S (1987) Raster- und transmissionselektronenmikroskopische Untersuchungen an der Buchlunge des Kaiserskorpions *Pandinus imperator*. Diploma thesis, University of Munich, Faculty of Biology

Schmidt-Nielsen K (1979) Animal physiology: adaptation and environment. Cambridge University Press, Cambridge

Seymour RS, Vinegar A (1973) Thermal relations, water loss and oxygen consumption of a North American tarantula. Comp Biochem Physiol 44A:83–96

Stewart DM, Martin AW (1970) Blood and fluid balance of the common tarantula, *Dugesiella hentzi*. Z Vergl Physiol 70:223–246

Tenney SM, Remmers JE (1963) Comparative quantitative morphology of the mammalian lung: diffusing aerea. Nature 197:54–56