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Direct and indirect calorimetric investigations on some snakes☆

B. Schaarschmidt [a,*], F.-R. Matuschka [b], I. Lamprecht [a]

[a] *Institut für Biophysik, Freie Universität Berlin, Thielallee 63, D-14195 Berlin, Germany*
[b] *Institut für Pathologie, Freie Universität Berlin, D-14050 Berlin, Germany*

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Abstract

The energy metabolism of three species of snake *(Coluber gemonensis, Coronella austriaca* and *Elaphe longissima)* was investigated by polarography and direct calorimetry at 25°C. The snakes produced a mean specific heat of 0.61 mW g^{-1}, a maximum of 0.94 mW g^{-1} during locomotory activities and a minimum of 0.45 mW g^{-1} during periods of rest. From simultaneous measurements of heat dissipation and oxygen consumption, calorimetric to respirometric ratios of 457, 637 and 309 kJ (mol O_2)$^{-1}$ for the three conditions were calculated.

Keywords: Calorimetry; Direct calorimetry; Indirect calorimetry; Metabolic rate; Snake

1. Introduction

Anaerobic metabolism plays an essential role in reptiles during maximal activity. In indirect calorimetry, heat dissipation can be calculated from the determination of only oxygen consumption and carbon dioxide production [1]. However, when metabolism changes to short- or long-term anaerobic glycolysis this method fails because of the absence of gas exchange during these processes. Therefore direct estimations of lactate concentration in the animal's blood or tissue [2,3] are

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* Corresponding author.

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necessary to measure energy production. On the other hand, direct calorimetry monitors both, the aerobic as well as the anaerobic metabolism of the animal.

The first calorimetric experiments on reptiles, among them a 2.5 m long *Python molurus*, were carried out as early as 1899 by Krehl and Soetbeer [4]. The first quantitative data were reported by Hill in 1911/1912 [5] for a grass snake. Although McDonald [6] has stated that calorimeters are prospective tools for metabolic investigations, and although Bennett [3] pointed out that, for the exact determination of anaerobiosis, simultaneous measurements of heat production and oxygen consumption were necessary, no such experiments on snakes have so far been reported in the literature.

2. Materials and methods

2.1. Experimental animals

The following animals were used for the investigations: one Smooth snake *Coronella austriaca*, one Balkan Whip snake *Coluber gemonensis*, and seven Aesculapian snakes *Elaphe longissima*. The living weight of the snakes was measured before and after each experiment; it varied between 7.4 and 18.2 g for the different animals but did not change individually by more than 4.8% during the different investigations. This reasonable stability revealed that the animals were not severely stressed by the experimental procedures.

The snakes were kept in separate plastic cages (20 cm × 20 cm × 10 cm) containing a bedding of paper towels and several flower pot fragments for hiding places. They had access to food and water ad libitum. The animals were kept under conditions of natural daylight at a room temperature of $(23 \pm 2)^{\circ}C$.

2.2. Calorimetry

Two nearly identical batch calorimeters of the heat-conduction type (Setaram, Lyon, France) were used for the heat dissipation measurements. A detailed description of the experimental handling of reptiles was given previously [7]. The sensitivity of the instruments amounted to 55 mV W^{-1} with a lower limit of detection in the μW range. The temperature was set at 25°C with a precision of 1 mK. The hermetically closed calorimetric vessels had a volume of 100 ml. Within the vessels, the snakes remained in complete darkness at steadily decreasing oxygen concentrations.

The calorimetric signals were fed to two multichannel recorders (type BD5, Kipp & Zonen, Delft, Netherlands). The calorimetric device had a time constant of 8 min, so that the recorded graphs (power–time curves, i.e. heat production rate P versus time t) had to be corrected mathematically to get the "true" heat signal [7]. The power–time curves were then processed from maximum and minimum heat dissipation rates and mechanically integrated by a polar planimeter to give the mean heat output Q. Dividing by time yields the mean heat production rate of the animal.

2.3. Polarography

Two different polarographic systems were used to register continuously the oxygen consumption of the snakes.

1. A Beckman-Monitor-System (type 123301 O_2/T, Beckman, Munich, Germany) was adapted for use with the calorimetric vessel. It measured the partial pressure of oxygen simultaneously with the heat dissipation. The time constant was 20 s.

2. An oxymeter with transoxode (Dräger, Lübeck, Germany) fitted to a glass vessel of 780 ml volume registered continuously the decrease of the oxygen partial pressure. This system was not synchronized to the calorimetry and was only used for long term measurements of aerobic metabolism. Because of the larger size of this container, the animals could remain in it for more than one day without causing the oxygen tension to drop below 80% of its initial value. This respiration chamber could be placed inside a temperature-controlled incubator.

3. Results

3.1. Heat production rate

The calorimetric curves correspond to the rates of maximum, mean and minimum heat production for the animals under the experimental conditions. Phases of constant heat dissipation (minimum) predominate (Fig. 1), representing the resting metabolism of the inactive animal, whereas locomotory periods of voluntary spontaneous movement are infrequent but give transient peaks of heat production.

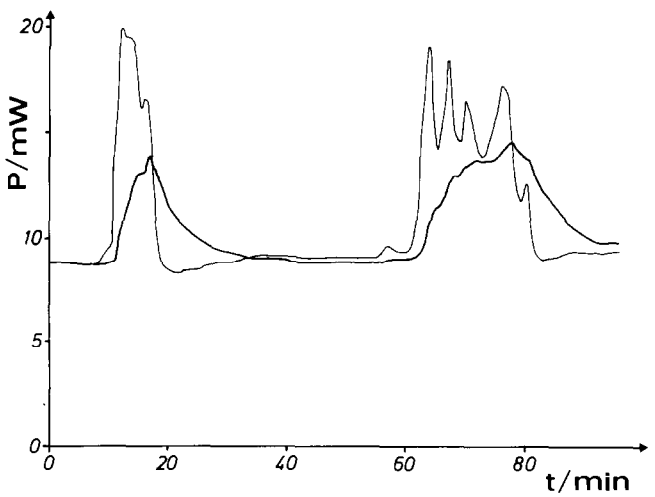

Fig. 1. Calorimetric response P (heavy line) of *E. longissima* No. 3 at 25°C. The fine line represents the "true" power–time curve, i.e. after mathematical treatment of the calorimetric response.

Table 1
Weight (m) and maximum (p_{max}), mean (p_{mean}) and minimum (p_{min}) specific heat production rates at 25°C of all snakes

Species	n	m/g	p_{max}/(mW g^{-1})	p_{mean}/(mW g^{-1})	p_{min}/(mW g^{-1})
Coronella austriaca	19	7.41	0.83	0.51	0.40
Coluber gemonensis	18	9.20	0.98	0.59	0.41
Elaphe longissima					
No. 1	4	14.22	0.74	0.55	0.50
No. 2	6	15.08	0.93	0.58	0.48
No. 3	4	18.22	1.01	0.68	0.57
No. 4	15	7.52	1.66	1.11	0.88
No. 5	11	10.78	0.77	0.6	0.54
No. 6	4	14.80	0.62	0.37	0.24
No. 7	5	17.77	0.88	0.53	0.36
Mean of all *Elaphe*	49	14.06 ± 3.79	0.94 ± 0.32	0.63 ± 0.23	0.51 ± 0.20
Mean of all snakes	86	13.34 ± 3.39	0.94 ± 0.30	0.61 ± 0.18	0.45 ± 0.18

n is the number of observations.

The $P-t$ curves of snakes are less structured than those of other reptiles [8], and are often without any structure at all because of reduced locomotion or its total absence. In the short section of the calorimetric graph shown as Fig. 1, the peaks represent periods during which the animal crept around after a long period of resting, depicted as smooth straight signals parallel to the zero line.

Throughout the experiment the maximum and minimum rates and the mean value (integrated over several hours) were taken from the mathematically desmeared curves and divided by the living mass of the animals. Table 1 shows the corresponding mass-specific heat production rates for all snakes investigated. It is evident that the smallest animal (*E. longissima* No. 4) exhibited by far the highest rate of heat production.

The maximum contribution of momentary activities to the heat production rate (heat bursts) is calculated from the difference $p_{max} - p_{min}$, and the averaged total contribution is quantified by $p_{mean} - p_{min}$. The maximum difference between locomotory and resting heat production determined in this way is in the mean larger than the resting heat production by a factor of 1.36 ± 0.38 for all *E. longissima* animals and 1.09 ± 0.36 for all snakes investigated. Exceptionally high single values of 4 are observed for *C. gemonensis*, with a mean of 2.39 ± 1.05. The contribution of locomotory activity to the total heat output is of course much smaller: (20 ± 4)% for all *E. longissima* and (19 ± 3)% for *C. austriaca*, and (26 ± 6)% for the more vivid *C. gemonensis*. These figures are given for 25°C and increase for higher temperatures.

3.2. Oxygen consumption

The data from the polarographic oxygen determinations are compiled as Table 2. As expected from the rate of heat production, *C. gemonensis* exhibited the highest

Table 2
Specific oxygen consumption rates in the larger glass container

Species	n	$\dot{v}_{max}$/(ml O_2 h^{-1} g^{-1})	$\dot{v}_{mean}$/(ml O_2 h^{-1} g^{-1})	$\dot{v}_{min}$/(ml O_2 h^{-1} g^{-1})
Coronella austriaca	10	0.252 ± 0.157	0.095 ± 0.072	0.088 ± 0.067
Coluber gemonensis	9	0.365 ± 0.234	0.120 ± 0.056	0.073 ± 0.040
Elaphe longissima	12	0.235 ± 0.110	0.151 ± 0.088	0.092 ± 0.025
Mean of all snakes	31	0.278 ± 0.174	0.124 ± 0.075	0.085 ± 0.043

n is the number of observations.

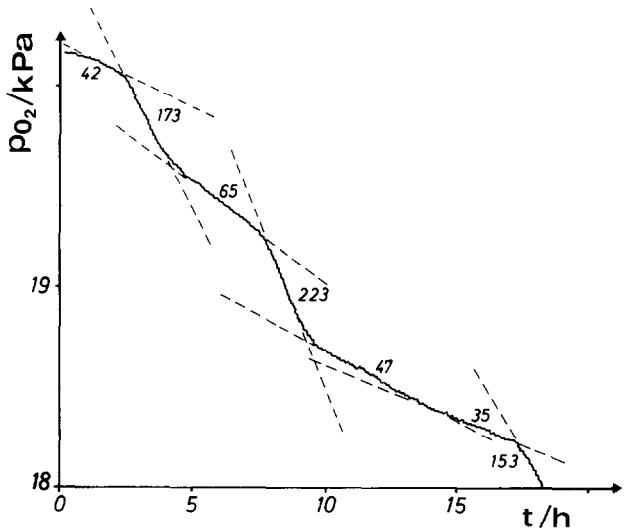

Fig. 2. Change of oxygen tension p_{O_2} with time in a 780 ml container due to respiration of *C. austriaca* at 25°C. The values on the slope indicate the specific rate of oxygen consumption in μl O_2 h^{-1} g^{-1}.

maximum rate, whereas the mean value corresponds to that for the other snakes. The same holds for the minimum figures. The oxygen consumptions of *C. austriaca* and *C. gemonensis* show Q_{10} values between 1.6 and 2.5 (mean 2.0) for the range between 20 and 30°C. Fig. 2 gives the changes in the rate of oxygen consumption during an experiment in the glass vessel with *C. austriaca*. In contrast to the transients in the $P-t$ curves, clearly different respiratory levels are attained and remain constant for longer times, and do not show such strong fluctuations as do the calorimetric signals.

Simultaneous measurements of heat production and oxygen consumption of *C. austriaca* are presented in Fig. 3. In this and all other graphs, no correspondence can be recognized between the rather smooth course of oxygen concentration and the peaks in heat production.

Calorimetric–respirometric ratios were obtained by dividing the heat output (over intervals of 5–20 min) by the amount of oxygen consumed during the same

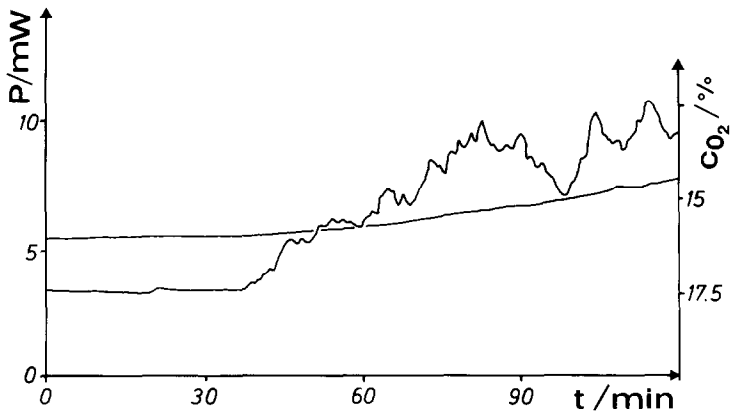

Fig. 3. Simultaneous measurements of heat production rate P (structured slope) and oxygen concentration c_{O_2} (straight line) for *C. austriaca* in a calorimetric vessel.

Table 3
Calorimetric–respirometric ratios CR averaged over 5–20 min (n = number of observations)

Species	n	CR_{max}/[kJ (mol O_2)$^{-1}$]	CR_{mean}/[kJ (mol O_2)$^{-1}$]	CR_{min}/[kJ (mol O_2)$^{-1}$]
Coronella austriaca	7	856 ± 459	529 ± 181	284 ± 49
Coluber gemonensis	7	585 ± 276	450 ± 148	289 ± 49
Elaphe longissima	18	571 ± 175	432 ± 76	327 ± 54
Mean of all snakes	32	638 ± 307	457 ± 130	309 ± 58

The thermochemically derived oxycaloric equivalent for aerobically catabolized glucose is 470 kJ (mol O_2)$^{-1}$. The predicted oxycaloric equivalent for a mixed nutrition for reptiles [6] is 441–450 kJ (mol O_2)$^{-1}$.

period (Table 3). During heat bursts this ratio increased, and during phases of rest it dropped sharply. The total heat output and the total oxygen consumption over the whole time of investigation showed a mean value of 457 kJ (mol O_2)$^{-1}$, in good agreement with the figure of 441–450 kJ (mol O_2)$^{-1}$ given by McDonald [6] for reptiles.

Because of the limited oxygen supply in the calorimetric vessels, no 24-h experiments were performed in the calorimeter to compare the heat production during day and night. Thus, only polarographic investigations were run longer. In some cases, but with all three species, the rate of oxygen consumption remained absolutely constant for more than one day. This happened mainly at elevated temperatures (>27.5°C). When different rates of metabolism occurred, the lowest was always observed in the middle of the night, or in a few cases also after the morning burst of activity. Compared with the highest level of activity during the day, the nocturnal rates amounted to (30 ± 11)% for *C. austriaca*, (27 ± 5)% for *C. gemonensis* and to (47 ± 21)% for *E. longissima*, the value for all snakes being (36 ± 16)%. This figure corresponds well to that found for lizards [7].

The changes in the respiration rate during the period of sloughing for *E. longissima* No. 1 in the glass vessel was observed by chance. The rate abruptly increased by a factor of 3.6 for a period of 25 min and then decreased smoothly to 90% of the initial value for 7 h. This decrease seems to be due to a time of rest after the exertion, which compensates approximately for the additional energy expenditure of sloughing. Then follows a second active period of 4 h with a 1.8 times higher respiration rate, which might be connected with the last phases of shedding the skin. Sloughing was never observed during a calorimetric run.

With an oxycaloric equivalent of 457 kJ (mol O_2)$^{-1}$ in the present experiments, corresponding to 20.4 J (ml O_2)$^{-1}$, the maximum respiration rate of 0.94 ml O_2 h^{-1} g^{-1} during sloughing transforms to 19.17 J h^{-1} g^{-1} or 5.25 mW g^{-1}, which is far above the usual mean heat production rate of 0.55 mW g^{-1} for "No. 1" or even the maximum value of 0.94 mW g^{-1} for all snakes. Thus, even without additional fermentative metabolism, enough energy for sloughing is available by respiration.

4. Discussion

The first calorimetric data from Hill [5] refer to a grass snake of 84 ml volume at 23.7°C. He found that cold-blooded animals produce heat at a constant rate during rest. Taking the specific weight of snakes to be 1.0 g ml^{-1}, Hill's data of 0.55 cal h^{-1} ml^{-1} transform to 0.64 mW g^{-1}. Most of the snakes investigated for their metabolism by other authors were much heavier than the animals in the present experiments. For a mutual comparison, those results have to be corrected by an equation which gives the metabolic activity as an allometric function of the body weight. Bennett and coworkers [2,3] found for resting snakes the following relationship between O_2 consumption dV/dt given in ml O_2 h^{-1} and weight m given in g at 20°C (35 species)

$$dV/dt = 0.12m^{0.77}$$

and at 30°C (13 species)

$$dV/dt = 0.28m^{0.76}$$

With the assumed value of 20.4 J (ml O_2)$^{-1}$ for the present experiments, these oxygen values correspond to a heat production rate of 0.67 mW g^{-1} or 1.57 mW g^{-1} respectively. This means that the rates of oxygen consumption given in ml O_2 h^{-1} have to be multiplied by a factor of 5.68 to give the rates in mW. Both transformed functions are shown as dotted lines in Fig. 4. The direct calorimetric values observed in our experiments at 25°C are given in the same figure as single points.

With a mean weight of 14.1 g for all animals of *E. longissima* in the present study, our figures transform to 0.065 ml O_2 h^{-1} g^{-1} or 0.36 mW g^{-1} at 20°C and 0.15 ml O_2 h^{-1} g^{-1} or 0.83 mW g^{-1} at 30°C. The observed mean of (0.61 ± 0.18) mW g^{-1} at 25°C averaged over all snakes (Table 1) coincides well with the estimations given above. Except for *E. longissima* No. 4 (p_{mean} is higher), the p_{min} and p_{mean} values fall

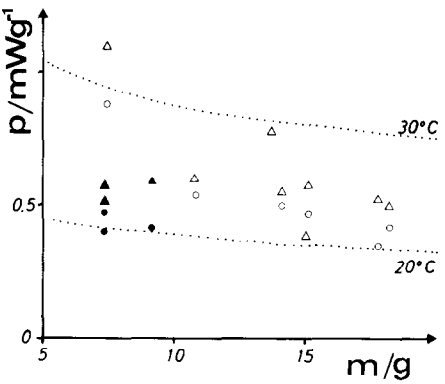

Fig. 4. Specific mean heat production rate (△,▲) and minimum heat production rate (○,●) of all snakes at 25°C as a function of their weight m. The dotted lines correspond to the allometric equations of Bennett and Dawson [2]. Open symbols are for *E. longissima*, filled ones for *C. gemonensis* and *C. coluber*.

into the interval between the two allometric curves. Allometric calculations of our data at 25°C yield an exponent of 0.74 for p_{mean} and 0.70 for p_{min}. These values are somewhat lower than those from Bennett and thus nearer to a surface dependence of the oxygen consumption (exponent of 0.67).

Correspondingly, the maximum oxygen consumption and the maximum rate of heat production of the animals in the test can be compared with data cited by Bennett [3] using the allometric relationship for aerobic power output in active reptiles. His data transform (for a mean weight of 14.1 g) to 0.41 ml O_2 h^{-1} g^{-1} or 2.29 mW g^{-1} at 20°C and 0.97 ml O_2 h^{-1} g^{-1} or 5.41 mW g^{-1} at 30°C. The values found in our investigations are much smaller (Tables 1 and 2). This indicates that under our experimental conditions the maximum aerobic power output was never obtained.

On the other hand, allometric relationships should be used with care because there are large inter- and intraspecific differences with reptiles. Thus, the data reported in this paper reveal that not only the species and the body weight determine the rate of metabolism but also the individual "temper". So, *E. longissima* No. 6 seems to have a low metabolic rate whereas No. 1, in spite of having nearly the same weight (Table 1), exhibits generally higher heat production rates. Nevertheless, without exception, animals with the smallest weight show the highest specific rates of metabolism.

The different calorimetric–respirometric ratios (Table 3) reflect the extent to which the tissue in the snakes uses anaerobic oxidation during short bursts of activity and to which oxygen is replenished during recovery periods. Higher ratios point to an anaerobic contribution to total heat dissipation, wheareas during the resting phase replenishment of the oxygen stores increases the measured rate of oxygen uptake relative to the rate of cellular respiration.

Chodrow and Taylor [9] determined the net cost of locomotion for a 24 g garter snake (*Thamnophis sirtalis*) as being 0.52 ml O_2 g^{-1} km^{-1}, which amounts to only

31% of that calculated for a lizard of equal weight. Bennett [3] supposed that this could indicate an advantage of limbless locomotion over quadrupedal movement in an energetic sense. We cannot confirm this observation because the ratio of p_{mean} to p_{min} indicates that, within the range of error, the cost of movement is equal for lizards (2.0 ± 0.4, after [7]) and snakes (2.1 ± 0.5, this paper).

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