

MICROCALORIMETRIC INVESTIGATIONS OF THE ENERGY METABOLISM OF SOME REPTILES

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ABSTRACT

Energy metabolism of 16 lacertide lizards and 12 snakes was investigated by means of two Calvet microcalorimeters. Heat production is compared with oxygen consumption, rendering a value near to the theoretical value (19.3 J/ml oxygen) predicted for food mainly composed of lipids and proteins. Electrical stimulation of some of the experimental lizards and snakes showed burst-off behaviour and a strong increase in anaerobic metabolism with a consequent compensation of the oxygen debt. The experiments are discussed in connection with the usual indirect calorimetry.

INTRODUCTION

In recent years no direct calorimetric experiments have been performed on lizards and snakes. All knowledge about aerobic and anaerobic metabolism of reptiles has been derived from respiratory measurements (Warburg manometry or polarography) of oxygen concentration (in the inlet and outlet air) of so-called respiratory chambers, face masks or by determining the lactate concentration in blood and body tissue of animals stressed for several minutes (ref.1, ref.2). The disadvantage of indirect calorimetry is that the form of metabolism must be known to calculate heat losses from gas exchanges.

The aim of the present study was to investigate heat production of poikilothermic reptiles depending on the species, the individual animal's weight and the environmental temperature. In this context the ability of reptiles to live under complete anoxbiosis for a certain time was of special interest (ref.3). Reptiles are able to switch to anaerobic metabolism while escaping or hunting (ref.4, ref.5). As direct calorimetry is not bound to gas exchange but detects heat production, it is an ideal method to investigate anaerobic metabolism in the animals under stress without sacrificing them, as is necessary for biochemical tests (ref.6).

Therefore, it seemed worthwhile to measure heat production of aerobic and anaerobic metabolism in lizards and snakes with a usual isoperibolic calorimeter of the Calvet type (ref.7).

METHODS AND MATERIALS

Animals

Calorimetric experiments were performed on the following lizards

- 4 Milos wall lizards (Podarcis milensis)
- 2 Common wall lizards (Podarcis muralis)
- 3 Sand lizards (Lacerta agilis)
- 3 Ocellated lizards (Lacerta lepida)
- 3 Libanon wall lizards (Lacerta laevis)
- 1 Green lizard (Lacerta viridis)

and the following snakes

- 1 Smooth snake (Coronella austriaca)
- 1 Balkan whip snake (Coluber gemonensis)
- 10 Aesculapian snakes (Elaphe longissima).

The weight of the animals ranged between 4 and 18 g. All individuals were laboratory reared and increased their weight during the experiments. Details concerning maintenance have been reported previously (ref.8, ref.9).

Instrumentation

Heat production was measured in two Calvet microcalorimeters (SETARAM/Lyon) of 100 ml vessels at changing temperatures from 18 to 35°C. With mean heat production rates of several mW and fivefold higher maximum rates the sensitivity of the recording system (Micrograph BD5; KIPP & ZONEN/Delft) was set to 2mV. However, it had to be increased at low environmental temperatures. The obtained power-time curves (P-t curves) were mechanically integrated to find the mean heat production, in some cases folded with the instrumental time constant to "desmeare" the calorimetric signal (ref.10).

The calorimetric vessels were open to air or hermetically sealed and then connected to a polarographic oxygen sensor (Monitor System, BECKMAN/Irvine) for a simultaneous determination of heat production and decrease of oxygen concentration in the air. Without larger disturbances the air in the vessel could be exchanged continuously by means of a peristaltic pump or discontinuously by a larger syringe.

The animals were electrically stimulated by two electrodes extending into the calorimetric vessel. In order to facilitate the experiments and to avoid dangerous high voltages an electrode cream (HELLIGE/Freiburg) as used for EEG and ECG was smeared on the animals. With the help of the electrode cream a stimulating pulsed voltage of 30 V was sufficient to cause an explosive movement of the animal and to keep it moving. Prior to stimulation the voltmeter was used to monitor an "appropriate" position of the animal by means of the resistance between the two electrodes.

RESULTS

Figure 1 exhibits the P-t curve of a Milos wall lizard (Podarcis milensis) and its "desmearing" by the Tian equation (ref.10).

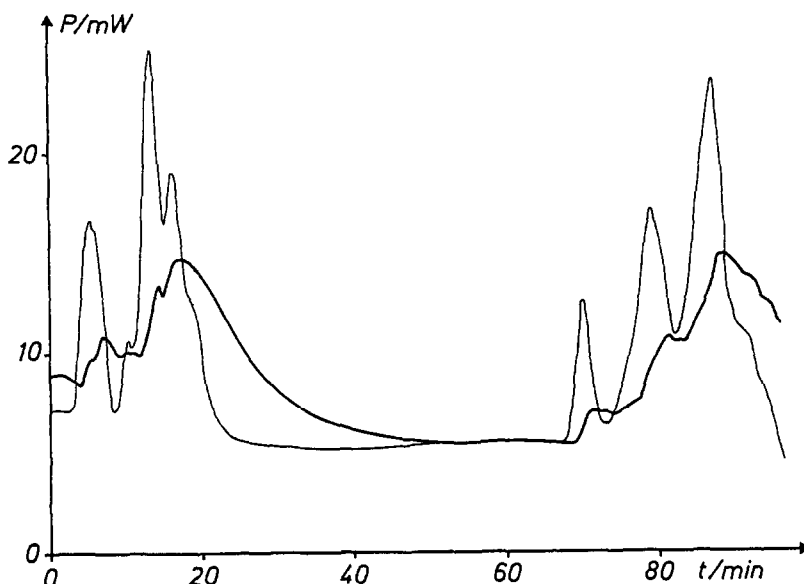


Fig. 1. Power-time curve (strong line) of a Milos wall lizard (Podarcis milensis) and the original signal (thin line) calculated by means of the Tian equation (see text).

There are periods of high activity and of rest from which the basal metabolism can be determined. Table 1 shows the mean rates of heat production for all lizards and snakes included in these investigations except those animals used for stimulation experiments. A detailed analysis of the result together with maximum and minimum rates of heat production and the correlation to oxygen consumption has recently been reported (ref.8, ref.9).

Table 1

Mean weights m (g) and mean specific rates of heat production p (mW/g) with standard deviation (mW/g) at 25°C for the different lizards and snakes in these investigations (n = number of experiments)

	n	m	p
<u>P.milensis</u>	108	4.18 ± 0.24	2.01 ± 0.43
<u>P.muralis</u>	26	2.05 ± 0.13	2.56 ± 1.00
<u>L.agilis</u>	50	7.91 ± 0.44	1.46 ± 0.12
<u>C.austriaca</u>	19	7.41 ± 0.45	0.51 ± 0.29
<u>C.gemonensis</u>	18	9.20 ± 0.29	0.59 ± 0.42
<u>E.tongissima</u>	49	14.06 ± 3.79	0.73 ± 0.26

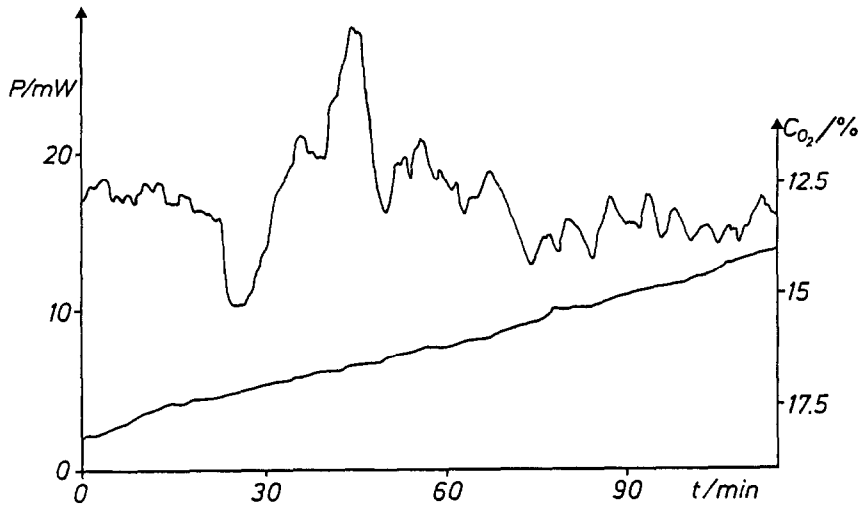


Fig. 2. Power-time curve (structured line) and change of oxygen concentration with time for an Aesculapian snake (*Elaphe longissima*) at 25°C.

Figure 2 compares the calorimetric P-t curve (structured line) with the decrease in oxygen concentration (smooth slope) for an Aesculapian snake (*Elaphe longissima*) at 25°C. The oxygen consumption as an integral method does not offer as much information as the calorimetric signal. Moreover, Fig. 2 shows that the non-stimulated, naturally occurring bursts of activity are mainly run by anaerobic degradation of glucose down to lactic acid. Usually, P-t curves of snakes are less structured than those of lizards because of the lower locomotor activities. At their preferred temperature the snakes did not move for many hours.

Lacertid lizards keep their normal metabolism down to an oxygen concentration of 16% (ref.11). In the present study the animals became more active and apparently tried to escape from the vessel if the oxygen concentration decreased below 16%. In most cases an oxycaloric coefficient of 18.6 ± 3.2 J/ml oxygen was calculated for lizards and 20.4 ± 5.8 J/ml for snakes; within the limits of error these figures correspond to the expected value of 19.3 J/ml oxygen for a food composed mainly of lipids and proteins. At very low oxygen concentrations the experimentally found values sharply increase, pointing to a predominant contribution of anaerobic fermentation to energy metabolism (ref.9).

In the stimulation experiments the two-electrode vessel allowed the animals to move around and the P-t curves are similar to those obtained without electrodes. After thermal equilibration the resistance between the electrodes was determined. Usually it is in the MΩ range and dropped to some 10 kΩ when the animal was in contact with the electrodes. This was the moment the voltage

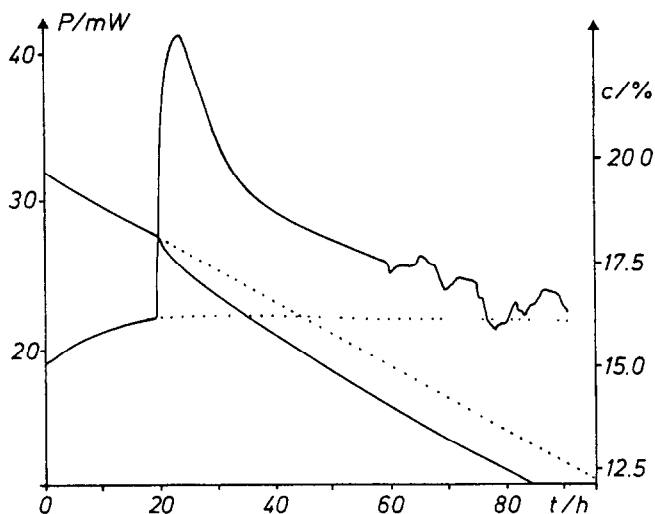


Fig. 3. Simulation of an Ocellated lizard (*Lacerta lepida*) at 30°C for 30 s with 30 V. The area between the P-t curve and the extrapolated heat production (dotted line) corresponds to 22.9 J, the maximum deviation of the oxygen slope after stimulation to 1.1 ml oxygen.

was switched on for 30 s leading to the burst of activity of the reptiles. The response is seen immediately in strong oscillations of the calorimetric and polarographic signal due to the vehement movement of the animal. Figure 3 exhibits the slopes of heat production and oxygen consumption after stimulation. Due to the thermal inertia of the instrument heat output is smeared to the typical signal of a short pulsed heat production with an approximately exponential decline to the level prior to stimulation. In contrast to observations described in the literature there is a quick response in oxygen consumption (steepness of slope) and a slow compensation of the oxygen debt. Comparing this additional oxygen consumption with the heat production by stimulation, the obtained value of 20.8 J/ml oxygen is specific for the metabolism of glucose. Further information about the aerobic and anaerobic contributions to the burst-off metabolism will be given elsewhere (ref.12).

If the duration of the stimulation is increased, a linear dependence of the additional heat production by the burst-off behaviour on this duration can be observed. Sometimes, these longer lasting stimulations are without effect because the animals find a position in which they touch only one electrode.

DISCUSSION

Except for one very early calorimetric investigation on a lacertid lizard (ref.13) no direct calorimetric experiments are found in the literature. There-

fore, it seemed interesting to compare calorimetrically determined levels of energy metabolism with those obtained from respiratory measurements. Most investigations concern reptiles that were much heavier than those used in these experiments. In the present study it was necessary to choose small specimens which would not only fit into the calorimetric vessels of 100 ml, but would also find space to move around and have enough air without the problem of a fast decrease in oxygen concentration. By means of the allometric relation between metabolism and weight (ref.1) the obtained figures for animals of different size can be compared; within the limits of error the observed values agree quite well with those cited in the literature (ref.2).

Moreover, the P-t curves of the reptiles render information about the locomotory activity; when heat production and oxygen consumption are measured simultaneously they allow deductions to the contribution of anaerobic metabolism (ref.4) to the energy output (Fig.2). As the bursts of locomotory activity are never reflected in the slopes of oxygen consumption it can be assumed that these naturally occurring, not stimulated activities are sustained by anaerobic metabolism and that the oxygen debt is compensated only over a longer period of time.

The P-t curves exhibit periods of high activities and of low levels of energy dissipation (Fig. 1 and 2). The latter ones can be attributed to the basal metabolism of the animals. The ratio of the mean to the minimum heat production renders information about the contribution of locomotory activities to the total heat output. This figure strongly depends on the species under investigation and changes from 68% for P.milensis to 79% for P.muralis for the lizards and from 74% for C.gemonensis to 81% for C.austriaca for the snakes. Within one species there are often considerable differences due to the "temperament" of the individuals, e.g. in E.longissima from 65% to 88%. In general the locomotory activities of snakes were found to be smaller than those of lizards (ref.8, ref.9).

As lizards and snakes belong to poikilothermic animals their energy metabolism strongly depends upon the environmental temperature. In most cases a parabolic dependence was observed with a flat optimum temperature slightly above 30°C (ref.8, ref. 9). For lizards the locomotory activity is more strongly influenced by temperature than the basal metabolism indicating that at lower temperatures the animals move more often or for longer times to find a place of preferred temperature (ref.8).

As direct calorimetry is a suitable means to monitor anaerobic metabolic processes in the absence of gas exchange, special attention was paid to electrically stimulated burst-offs. These burst-offs showed an immediate calorimetric response (Fig.3) with a subsequent exponential decrease to the former energetic level. The additional area between the observed P-t curve and the extrapolated heat production equals a stimulated heat production of 22.9 J. As it

was provoked within 30 s it corresponds to an additional power of 763 mW, 35 fold higher than the value of 22 mW prior to stimulation. The corresponding specific values amount to 100.1 mW/g and 2.89 mW/g, resp. These results agree with observations of Cragg (ref.14) who found a stimulation of L.viridis from 7.06 mW/g at rest to 173.7 mW/g at maximum stimulation. Fig. 3 indicates that it takes approximately 40 min for the animal to recover and to continue with its usual, non-stimulated movements in the calorimetric vessel.

The results of stimulation is not as promptly observed in oxygen consumption as in heat production (Fig.3). Nevertheless, the steepness of the slope changes immediately and attains a new steady state after approximately 20 min. The time lag may be due to physiological reasons as well as to the instrumental setup in which the larger volume of the calorimetric vessel and the small connecting channel to the polarographic electrode retards the signal. Parallel investigations of oxygen consumption of lizards and snakes in a larger container (ref.9) indicate that an immediate response in the oxygen consumption rate is observed under the chosen experimental conditions (ref.12).

Direct calorimetry proves to be a suitable tool in the investigation of the metabolism of poikilothermic animals to render information not obtainable by other methods and to avoid the sacrifice of the animal for anaerobic analyses.

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