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MICROCALORIMETRIC INVESTIGATIONS ON THE ENERGY METABOLISM OF LIZARDS

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Summary

The energy metabolism of three lizard species (*Podarcis milensis*, *Podarcis muralis* and *Lacerta agilis*) was investigated by means of microcalorimetry and polarography over the range of environmental temperatures between 17 and 35 °C encountered by active lizards during summer in Europe. In non-stimulated animals, the maximum and mean heat production rates were highest around 30 °C for all species and amounted to $3.9\pm1.1\,mW\,g^{-1}$ and $2.1\pm0.5\,mW\,g^{-1}$, respectively. Resting metabolism contributed approximately 60 % to the mean routine heat dissipation; the remainder originated from locomotory activity, 43 % of which was based on anaerobic energy metabolism. From simultaneous determinations of heat dissipation and oxygen consumption, an average oxycaloric equivalent of $18.6\pm3.2\,J\,ml^{-1}\,O_2$ was calculated, which rose to $26.6\pm7.1\,J\,ml^{-1}\,O_2$ during short bursts of locomotion.

Introduction

Anaerobic metabolism plays an essential role in reptiles during maximal activity. The rate of lactic acid formation is high during the initial stages of short-term activity, and large amounts of lactate are found in the blood and tissues of the animals during bursts of activity (Bennett, 1982). Measurements of oxygen consumption and carbon dioxide production in living animals (McDonald, 1976) or direct estimations of lactate concentrations in killed animals (Bennett and Licht, 1972; Bennett *et al.* 1984) used to be the only methods available to measure energy production. Recently, heat flow calorimeters were constructed which could measure directly the total metabolism of the biological object under investigation by its heat dissipation. The use of sensitive modern calorimeters, which compete in precision with any oxygen measuring device, in studying aerobic and anaerobic metabolism in animals has been intensively discussed in the recent literature (McLean and Tobin, 1987; Blaxter, 1989; Wieser and Gnaiger, 1989).

There are few calorimetric data on reptiles in the literature. All estimates of

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heat production originate from indirect calorimetry, i.e. from respiratory measurements and lactate determinations (Bennett and Dawson, 1976; Bennett, 1982). The aim of this study was to show that direct calorimetry combined with polarography could be an appropriate tool for obtaining more information about the metabolism of reptiles. To make conditions more natural, the animals were neither artificially stimulated nor adapted to the different experimental temperatures.

Materials and methods

Experimental animals

Three laboratory-reared male Milos wall lizards *Podarcis milensis*, two male common wall lizards *Podarcis muralis* and one male and two non-reproductive female sand lizards *Lacerta agilis* were used during the investigations. The animals were approximately 12 months old. The lizards were held in the laboratory under conditions of natural daylight and at room temperature (23 °C) for 4–6 months (January to June) before the investigations began. The lizards were kept in plastic cages (20 cm × 20 cm × 20 cm) containing a bedding of paper towels as a hiding place, and had access to food and water *ad libitum*. Their weight was measured every second day during the investigations, before and after each experiment. The animals in question were acclimated to the experimental temperature for 2 h and starved for 1 day before the experiment. Test temperatures were 17, 19, 20, 21, 22.5, 25, 27.5, 30, 32.5 and 35 °C.

Calorimetry

Two microcalorimeters of the heat conduction type (SETARAM, Lyon, France) were used for monitoring the rate of heat dissipation of the animals. The theory, construction and applications of these instruments are described in detail in the literature (Calvet and Prat, 1956, 1963; Lamprecht, 1980). The essential part of the calorimeter is a differential apparatus consisting of a working chamber and a reference chamber. The resultant calorimetric signal represents the difference in heat flows from these two chambers and thus excludes thermal disturbances from the environment. The thermal mass of the animals in the reaction vessel was compensated for by an equivalent amount of water in the reference chamber. The sensitivity of the calorimeters was 55 mV W^{-1}, with a lower limit of detection in the microwatt range. The long-term stability was better than 5 $\mu W\,day^{-1}$. The calorimeters were kept in isothermal conditions with a precision of (±0.001 °C). The calorimetric vessels, made from stainless steel, each had a volume of 100 ml and were open to the atmosphere through several holes in the top cover. In preliminary experiments the mass of the animals did not change by more than a few milligrams during a calorimetric run. Thus, possible evaporative heat losses amounted to less than 10 μW and could be neglected. During the experiments a large piece of filter paper was placed in the vessel to allow the animals to hide and climb. The lizards remained in darkness during a calorimetric run of about 6 h. Th

calorimetric signals were fed to multichannel amplifiers (type BD5, Kipp & Zonen, Delft, Netherlands) and recorded as power–time curves (P–t curves), i.e. thermal power P *versus* time t.

Respirometry

Two different polarographic systems were used to monitor continuously the oxygen consumption. (1) A Beckman monitor system (type 123 301 O_2/T, Beckman, München, Germany), which measures the partial pressure of oxygen in a closed system. This electrode was fitted to a calorimetric vessel in order to perform simultaneous determinations of oxygen consumption and heat dissipation. During these experiments the calorimetric vessel was gas-tight, so that the oxygen concentration decreased steadily. The signal was accurate to ±0.1 %. The time constant of the whole device was 20 s. (2) An oxymeter with transoxode (Dräger, Lübeck, Germany) was fitted to a glass vessel of 1100 ml volume. The digital instrument measured the oxygen pressure in mmHg, which was then converted into Pascals. This system was used to monitor the oxygen consumption in long-term experiments not synchronized to calorimetry. According to the size of the animal, the volume could be reduced to 780 ml. The respiration chamber could be placed inside a temperature-controlled incubator. To evaluate the polarographic signals for both systems, the volumes were corrected for the animal volume estimated from its mass.

Mathematical evaluation

The calorimetric device had a time constant of 8 min so that the 'raw' heat signal had to be corrected (desmeared) mathematically (Hemminger and Höhne, 1984; Randzio and Suurkuusk, 1980). After transformation (see Fig. 1) the graphs were processed to give maximum and minimum values of heat dissipation rate and mechanically integrated by a polarplanimeter to give the mean heat output. Dividing the mean heat output by time yields the mean heat dissipation rate of the animal.

The time-correction transformation, curve fittings and statistical analyses were made on a MINC-11 computer (digital, Massachusetts, USA) using the MINC BASIC V1.1 application package. All values are presented as mean±S.E.M.

Results

Heat production

Fig. 1 shows a typical power–time curve of a male *Podarcis milensis* showing large fluctuations of the heat flow signal. Minimum rates were measured from the resting animal during inactive phases. Maximum rates were observed during voluntary spontaneous movement (creeping about, standing, trying to climb). Mean rates represent routine activities including maximum and minimum values averaged over several hours. After time-correction the profile of the curve changed. Phases of enhanced heat production became more distinct and were

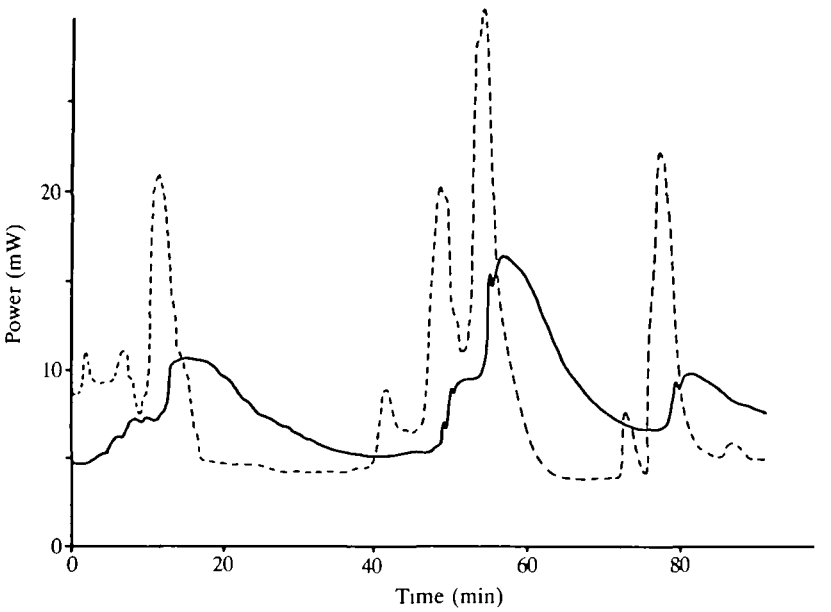

Fig. 1. Power–time curve for male lizard (*Podarcis milensis*) no. 1 at 25°C. The solid line represents the direct calorimetric signal, the dashed one the time-corrected heat signal.

clearly separated from longer periods of rest. Although the peaks in the heat production rate became higher and narrower, the total heat output (the integral of heat production rate over time) was not influenced by this manipulation. Throughout the experiment the maximum and minimum rates and the mean value were taken from these curves and divided by the live mass of the animal to give the corresponding mass-specific values (Table 1). The mass of the animals remained nearly constant throughout the whole period of investigations (approximately 1 month for each individual). The mass-specific mean heat production rate p_{mean} is the most important long-term quantity because it represents the sum of the resting metabolism and the locomotory activity, i.e. the routine rate of metabolism. This figure should correspond to the polarographically determined rates of oxygen consumption, representing mean values integrated over short fluctuations.

Assuming that p_{min} describes solely the resting metabolism, the ratio p_{min}/p_{mean} should indicate what contribution is made by the resting metabolism to the total heat output. At 25°C this contribution amounted to 56.2±5.5% for *P. milensis*, 57.1±8.9% for *P. muralis* and 66.1±6.9% for *L. agilis*. The remaining heat output was mostly due to locomotory activity occurring in short bursts and was quantified by the difference $(p_{max}-p_{min})=p_{loc}$. The relative 'overshoot' of these bursts beyond the mean rate is given by the ratio $q=p_{loc}/p_{mean}$. Table 1 shows that q depended on the species and the state of activity of the animal concerned. The difference $p_{max}-p_{min}$ is not identical with the 'scope for activity' because in the present experiments the animals were never stimulated to maximum activity.

Table 1. *Mass* (m), *mass-specific mean, maximum and minimum rates of heat production* (p_{mean}, p_{max}, p_{min}) *and the relative overshoot* $q=(p_{max}-p_{min})/(p_{mean})$ *for all lizards investigated at 25°C*

Species	Animal	*N*	Mass (g)	p_{mean} ($mW\,g^{-1}$)	p_{max} ($mW\,g^{-1}$)	p_{min} ($mW\,g^{-1}$)	*q*
Podarcis milensis	K	30	4.28±0.20	2.10	3.97	1.15	1.34
	1	30	3.73±0.24	2.37	3.86	1.45	1.02
	2	30	3.71±0.22	2.20	3.80	1.23	1.17
	S	18	4.98±0.42	1.40	2.50	0.70	1.29
	Mean			2.01±0.43	3.53±0.60	1.13±0.31	1.21±0.14
Podarcis muralis	1	16	2.09±0.14	1.82	4.25	1.05	1.76
	2	10	2.03±0.13	1.85	3.60	1.05	1.38
	Mean			1.84±0.33	3.99±0.21	1.05±0.21	1.57±0.27
Lacerta agilis	1	20	7.07±0.70	1.44	2.65	0.86	1.24
	2	12	9.24±0.31	1.58	2.58	1.16	0.99
	3	18	7.43±0.32	1.35	3.12	0.88	1.66
	Mean			1.46±0.12	2.77±0.76	0.97±0.26	1.30±0.34
	Mean of all lizards			1.79±0.35	3.37±0.66	1.06±0.22	1.32±0.26

N, number of experiments.

Oxygen consumption

Determinations of oxygen consumption were made simultaneously with calorimetry (see Fig. 3) at 25°C, in separate experiments (Fig. 2) at all temperatures. Generally, the mean rate of oxygen consumption was higher in the calorimetric vessel than in the respiration chamber. Higher rates might be due to longer periods of locomotion in the calorimeter, reflecting a situation of greater stress for the animals in the smaller calorimetric chamber (100 ml) than in the larger respiration chamber (780–1100 ml). The simultaneous measurements were made for a few hours only and no distinct changes in the slope of the oxygen concentration, such as those measured in the respiration chamber (Fig. 2), were found in the experiments in the calorimeter (Fig. 3). Fig. 2 shows the decrease of oxygen concentration over 10 h at 32.5°C for *P. milensis* no. 2 in the respiration chamber. Periods of relatively constant oxygen consumption are separated by a short transition phase at a higher rate.

The peaks in the calorimetric curves could not be assigned to changes in the oxygen concentration in the vessel, since spontaneous movements of the animals did not produce maximal rates of oxygen consumption. Escape activities, arising from the declining oxygen availability in the calorimetric experiments, took place when the oxygen tension dropped below 8.66 kPa (65 mmHg) (after 6 h) and were recognized in the calorimetric signal as uniform heat bursts. Normally the experiments were stopped before such low oxygen tensions were reached.

Oxycaloric equivalents were obtained by dividing the heat output over intervals of 5–20 min (measured by integration of the area between the slope of the heat

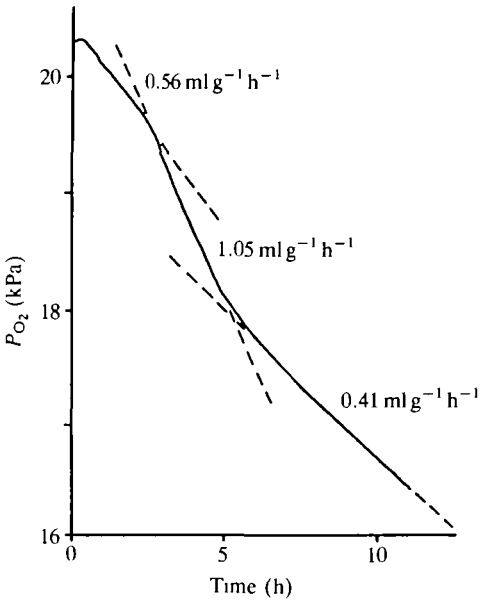

Fig. 2. Change of oxygen partial pressure with time in the respiration chamber at 32.5°C for *P. milensis* no. 2. The values given beside the trace are the rates of oxygen consumption.

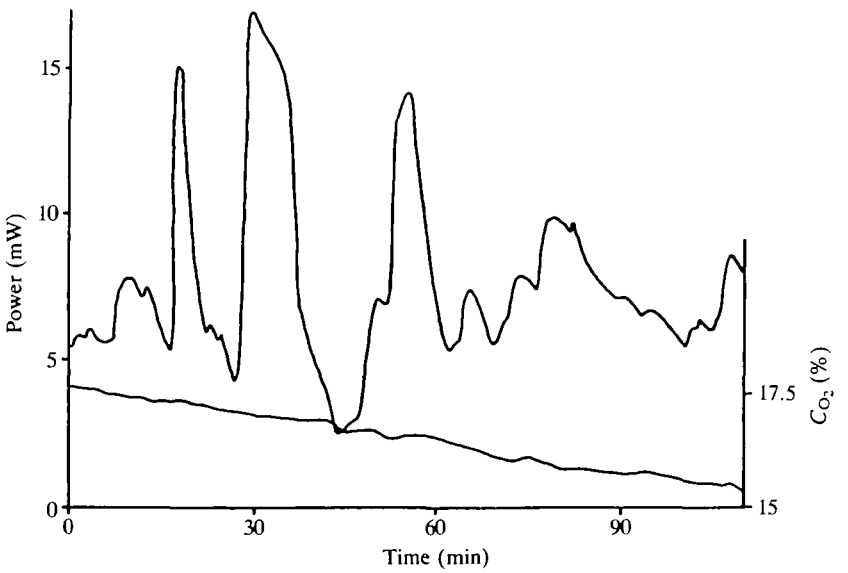

Fig. 3. Rate of heat production and oxygen consumption (given as volume per cent of oxygen in the calorimetric vessel) for *P. milensis* no. 2 at 25°C.

Table 2. *The oxycaloric equivalents during resting periods (Minimum), locomotory bursts (Maximum) and routine activity at 25°C averaged over the entire experiment lasting 5 h (Mean)*

		Oxycaloric equivalents ($J\,ml^{-1}\,O_2$)		
Species	*N*	Minimum	Maximum	Mean
Podarcis milensis	25	13.47±2.47	26.01±6.15	18.84±3.48
Podarcis muralis	7	10.93±2.42	30.43±12.23	19.00±1.26
Lacerta agilis	18	12.00±5.87	25.24±4.53	18.33±2.57
Mean of all lizards		12.53±2.61	26.62±7.08	18.63±3.17

Values are mean±S.E.M., *N*, number of measurements.

production rate and the zero line) by the amount of oxygen consumed during that interval. During heat bursts, the oxycaloric equivalent rose, during phases of rest, it dropped (Table 2). The total heat output and the total amount of oxygen consumed during an experiment (more than 5 h) smoothed out the short-term deviations and gave a mean value of $18.6\,J\,ml^{-1}\,O_2$, which is close to the predicted figure of $19.7\,J\,ml^{-1}\,O_2$ for a mixed nutrition (McDonald, 1976). No significant differences between the oxycaloric equivalents were found for the three species (Table 2).

Effects of temperature

Values measured by both calorimetry and respirometry varied with ambient temperature between 17 and 35°C. Fig. 4 demonstrates that the mass-specific rates of maximum and minimum heat production did not show the normal van't Hoff

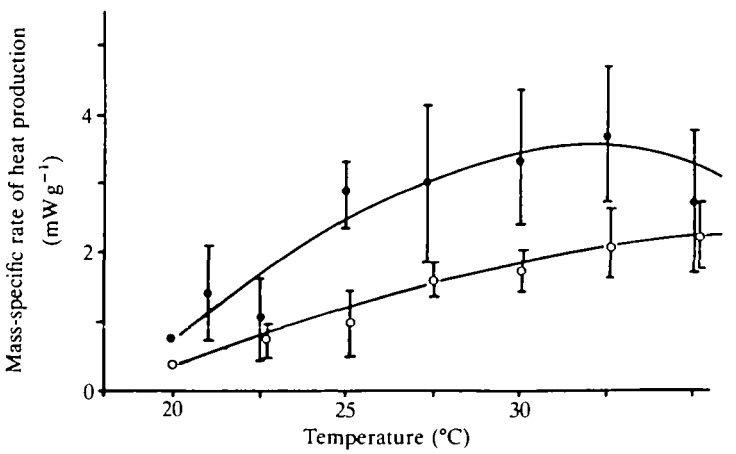

Fig. 4. Mass-specific maximum (●) and minimum (○) rates of heat production as functions of ambient temperature. Values for animals 1 and 3 (*Lacerta agilis*) are pooled for this figure. The bars give the standard deviation (N=5).

effect. A flat maximum was obtained at around 31°C. The mean rate of heat production varied with temperature in a similar manner, with a maximum at $2.1 \pm 0.5\,mW\,g^{-1}$. All curves were fitted by polynomial regression. The maxima coincided within ±5°C for all lizards. Generally (as in Fig. 4) the maximum rates increased most with temperature, showing that locomotor activity was affected more by temperature change than was resting metabolism. For *L. agilis* and *P. milensis*, the maximum rates increased twofold and for *P. muralis* 1.5-fold over the minimum rates. Within the limit of accuracy, no dependence on temperature was found for the oxycaloric equivalents.

Owing to the short exposure to the experimental temperature (2 h before and 6 h during experiments), the observed rates reflected instantaneous responses to temperature change rather than acclimated rates.

Discussion

The calorimetric signal records the heat loss of the animal and not its heat production. The differences between the rates of metabolic heat production and heat dissipation are due to heat storage in the body. Because lizards are not extensively insulated by fur, feathers or subcutaneous fat and have a high surface:volume ratio, the heat generated is dissipated rapidly (Avery, 1979). Moreover, the time-correction procedure mentioned above considers the thermal mass equivalent to the animals using the specific heat capacity of $3.43\,kJ\,kg^{-1}\,K^{-1}$ given by Bartholomew and Tucker (1963). Thus, we can state that the calorimetric signal represents the actual heat production within the animal and that the terms 'heat production' and 'heat dissipation' are, in the present case, synonymous.

The only previous calorimetric investigation on lizards was performed by Krehl and Soetbeer (1899). For a 110 g *Lacerta viridis* they observed heat production rates of $0.95\,mW\,g^{-1}$ at 25.3°C and $1.76\,mW\,g^{-1}$ at 37.0°C. The values in the present study are considerably higher. However, taking into account the allometric relationship between metabolic rate and body mass (Bennett and Dawson, 1976), the results agree well.

It is typical of lizards to engage in brief periods of burst locomotion (John-Alder *et al.* 1983). During the initial stages of activity, lizards rely exclusively on anaerobic sources of energy (Bennett and Licht, 1972). Important aspects in this connection are the anaerobic scope (maximum rate of lactate production) and the anaerobic capacity (total amount of lactate formed). Anaerobic degradation of glucose or glycogen to lactate produces an enthalpy change of $-55\,kJ\,mol^{-1}$. Taking into account the buffering capacity of the body fluid under physiological conditions, which creates an additional enthalpy change of $-25\,kJ\,mol^{-1}\,H$ (Woledge, 1972; Gnaiger, 1983), the total enthalpy change is $-80\,kJ\,mol^{-1}$. This figure, and the value of $19.7\,J\,mol^{-1}\,O_2$ predicted by McDonald (1976) are used in the following discussion to transform indirect calorimetry data taken from the literature into thermal quantities. Bennett *et al.* (1984) measured the maximum oxygen consumption and anaerobic scope after stimulation of two African lacertid,

lizards at 37 °C. *Eremias lineoocellata* showed a resting rate of oxygen consumption of $4.0\,\mu l\,g^{-1}\,min^{-1}$, which transforms to $1.27\,mW\,g^{-1}$, a maximum oxygen consumption rate of $41.5\,\mu l\,g^{-1}\,min^{-1}$ after intensive stimulation, which becomes $13.3\,mW\,g^{-1}$, and a lactate production rate of $2.56\,mg\,g^{-1}\,min^{-1}$, which transforms to $38.0\,mW\,g^{-1}$. For *Eremias lugubris*, the corresponding figures were $4.17\,\mu l\,g^{-1}\,min^{-1}$ ($1.34\,mW\,g^{-1}$), $53.7\,\mu l\,g^{-1}\,min^{-1}$ ($17.2\,mW\,g^{-1}$) and $2.12\,mg\,g^{-1}\,min^{-1}$ ($31.4\,mW\,g^{-1}$). These values emphasize the enormous short-term influence of anaerobic heat production in lizards. Similarly high values (60–90 % of the total heat production) due to anaerobic lactate production were found by Gleeson and Bennett (1982) in lizards during intensive stimulation. The present data (Table 1) of about $1\,mW\,g^{-1}$ for the resting animals agree reasonably well with those cited above. The maximum heat production values of $2.5–4.3\,mW\,g^{-1}$ found during locomotory bursts in our experiments cannot be compared with the maximum values cited above, because the lizards were never stimulated. Moderate electric shocks of 30 V and 30 s duration lead to a further 1.3- to 1.6-fold increase in heat production (Lamprecht and Matuschka, 1985; I. Lamprecht, F.-R. Matuschka and B. Schaarschmidt, in preparation). However, even taking into account this enhancement, our results would amount to only 30–50 % of those of Bennett. In contrast, Cragg (1978) interprets the work of Bartholomew and Tucker (1964) to mean that electric shocks in *Lacerta* would not lead to higher activity than spontaneous struggling does. The different values of measured heat production and external oxygen consumption (Table 2) reflect the extent to which the tissue in the animal uses anaerobic oxidation during short bursts of activity and to which oxygen is replenished during recovery periods. The anaerobic fraction of the heat production during the initial stages of locomotory bursts amounts to 43±8 % (mean of all animals), which transforms to $95.5\,\mu g$ lactate $g^{-1}\,min^{-1}$, well below the values given by Bennett *et al.* (1984) for intensively stimulated lizards.

Maximum catabolic oxygen utilization may be underestimated by measuring changes in oxygen uptake during peak activity, since no account is taken of any depletion of internal oxygen stores. The apparent anaerobic contribution to total heat dissipation would then be overestimated since, theoretically, the oxycaloric equivalent refers exclusively to internal respiration. During the resting phase following peak activity, replenishment of the oxygen stores increases the measured rate of oxygen uptake relative to the rate of cellular respiration.

Bennett and Dawson (1976) cited a critical oxygen tension of 9.31 kPa (70 mmHg) for lizards, below which it becomes difficult for them to maintain resting metabolism. During the calorimetric runs in the present study this limit was passed only a few times and only for short periods. An oxygen tension of 8.66 kPa (65 mmHg) was tolerated without any difficulty, agreeing well with the observations of Belkin (1963), who showed that lizards could survive even complete anoxia for 30 min. In contrast, Nielsen (1962) found that the activity of *Lacerta* decreased at 16.66 kPa (125 mmHg).

This paper has provided a method of measuring the total energy metabolism of

small reptiles without harming them. Clearly, more work on stimulated animals and long-term experiments are needed to analyze further the underlying anaerobic and aerobic sources of energy.

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