Thermochimica Acta, 187 (1991) 71–78
Elsevier Science Publishers B.V., Amsterdam

Heat production of frogs under normoxic and hypoxic conditions: a microcalorimetric study using a gas flow system

Carla Schulz[a], Michael Thuy, Gerhard Wegener*

Institut für Zoologie, Johannes Gutenberg-Universität, Saarstrasse 21, D-6500 Mainz, Federal Republic of Germany

[a] Present address: III. Med. Klinik/Endokrinologie - Verfügungsgebäude, Johannes Gutenberg-Universität, Obere Zahlbacher Str. 63, D-6500 Mainz, Federal Republic of Germany

* To whom correspondence and reprint requests should be sent

Abstract

Heat production of male frogs, *Rana temporaria*, was measured in a microcalorimeter through which a continuous flow of gas was passed in order to generate constant normoxic, hypoxic or anoxic conditions. The normoxic heat flow was 163 ± 37 μW/g body weight in frogs that had not been treated with curare and 149 ± 69 μW/g in animals immobilized with curare. During anoxia, frogs, whether curarized or not, decreased their heat production to about 25% of the respective normoxic control. In graded hypoxia (10% to 3% O_2), curarized frogs decreased their heat rate according to the grade of hypoxia they were subjected to.

1. INTRODUCTION

The primary purpose of energy metabolism is the production of adenosine triphosphate (ATP) which is used for powering physiological processes. ATP can be produced in aerobic pathways (using O_2 as final electron acceptor), and this mode of ATP production has substantial advantages over anaerobic energy production. From 1 mole of glucose 38 moles of ATP can be synthesized aerobically, compared to only 2 moles anaerobically. Moreover, in aerobic metabolism the main foodstuffs, carbohydrate and fat, are fully oxidized to harmless and readily excretable end products, CO_2 and H_2O, whereas anaerobic metabolism is restricted to carbohydrate and gives rise to (usually) acidic products. Yet vertebrates, particularly fresh-water fish and amphibia, may encounter oxygen deficiency in their habitats and hence must be able to cope with temporary hypoxia and anoxia.

In anaerobiosis, glycogen is the main substrate and lactic acid is the only significant end product in most vertebrates. Hence, if the aerobic turnover of ATP is to be

0040-6031/91/$03.50

maintained during anaerobiosis, this would require a rapid breakdown of glycogen to compensate for the meagre anaerobic yield of ATP and would lead to a massive production of lactate and protons. Due to the lack of metabolic energy and the decrease in pH, physiological processes would come to a halt and the animal would die. The sequelae of anaerobiosis can be delayed by decreasing the energy expenditure. Vertebrates have the capacity to decrease their metabolic rate during hypoxia, but the mechanisms by which this is brought about are poorly understood (for reviews and references, see Hochachka 1980; Hochachka and Guppy 1987; Wegener et al. 1986; Wegener 1988).

One prerequisite for studying anaerobiosis is to measure metabolic rates independent of oxygen consumption. Recently, sensitive calorimeters have been used for this purpose, but most studies have been confined to aquatic systems. We wanted to measure heat production of terrestrial animals during well-defined hypoxia/anoxia. This can be achieved by using a gas flow system. A stagnant atmosphere would continuously change due to the metabolic activity of the experimental animals.

In this work we report a method by which heat production of terrestrial organisms can be measured at high sensitivity in a microcalorimeter through which a constant flow of gas is flowing during the experiments. The system is used to follow the effects of anoxia and graded hypoxia on heat production of frogs, but it could easily be adapted for studying a variety of other physiological and ecological problems.

2. MATERIALS AND METHODS

Animals

Common frogs (*Rana temporaria*) were kept in plastic containers in the laboratory at 19°C and were fed locusts, tobacco hornworms and crickets. Male frogs of 19 to 33 g body weight were used in the experiments.

Calorimetry

A Setaram Calvet-MS 80 twin-calorimeter, equipped with circulation cells of 100 ml volume, was used in the isothermal mode. The calorimeter was calibrated with "Joule cells". A flow of gas was passed through the calorimeter at 20.8° C and at a constant rate of 600 ± 1 ml/h per cell. The gas flow was continuously measured by means of high precision flow meters (MF-200, Setaram/ Caluire, France) and the rate was readjusted, if necessary, using micro-valves (Millimite, Hoke/Mass., USA). Preliminary experiments had shown that a constant gas flow is an absolute necessity for reliable heat measurements. Another problem with gas flow systems is due to the fact that animals evaporate water which causes evaporative heat loss. To overcome this problem, 2 ml of water was added to the reference cell, and the gas was moistened before it passed through the calorimeter. With these provisions no heat effect due to evaporation was noted.

The animals were transferred to the experimental cell of the calorimeter and allowed to adapt for at least 14 hours before data were collected. Some frogs were immobilized by a subcutaneous injection of curare (tubocurarine chloride from Astra/

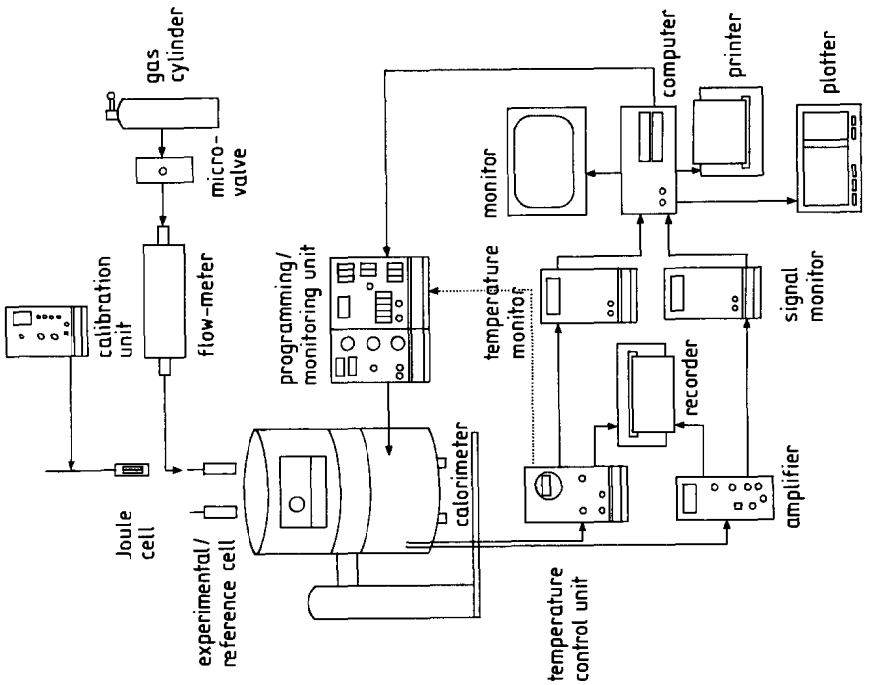

Fig. 1b: Schematic drawing of the experimental set-up

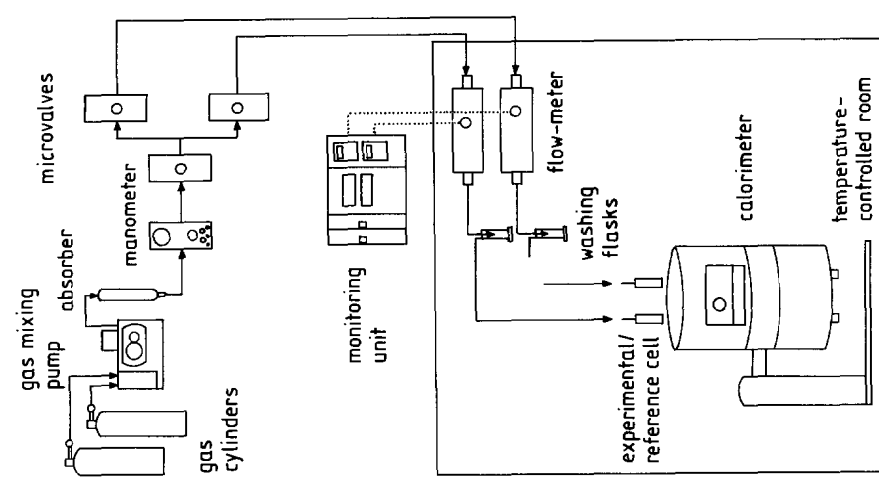

Fig. 1a: Schematic drawing of the gas flow

Bielefeld, 10 µg/g body weight) 90 min before the readings.

Synthetic air (Messer Griesheim, Frankfurt/Main), a mixture of 20% oxygen and 80% nitrogen, was used to generate normoxic conditions, while anoxic conditions were created with pure nitrogen. Graded hypoxia was produced by mixing nitrogen and oxygen using the Digamix SA 27 pump (Wösthoff/Bochum).

The experimental set-up is given in Figure 1a and 1b.

3. RESULTS

Heat production under normoxic conditions

Under normoxic conditions, frogs produced heat at a rate of 163 ± 37 µW/g body weight (mean ± SD, n=10). Under the same conditions animals immobilized with curare had a heat production of 149 ± 69 µW/g body weight (n=7).

Heat production under anoxic conditions

Frogs were kept in the calorimeter under normoxic conditions for at least 14 hours, then the gas flow was switched from air to pure nitrogen for two hours. 10-15 minutes after switching the gas flow to N_2, a precipitous decrease in heat production was noted. After about 90 min the heat production had reached a steady state which was 26 ± 9% (n=5) of the normoxic control in frogs that were not curarized (Figure 2)

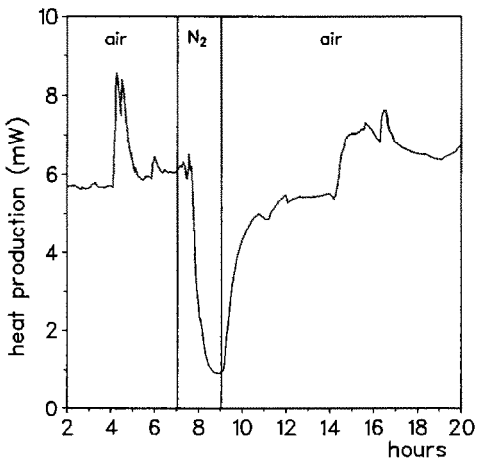

Fig. 2: Heat production of a frog (27.2 g), not treated with curare.
Peaks are due to muscular activity.

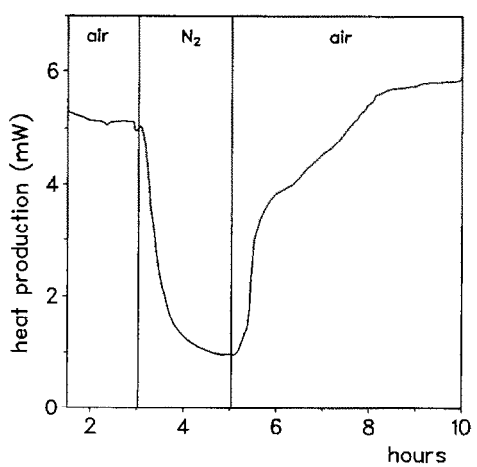

Fig. 3: Heat production of a frog (30.6 g), immobilized with curare.

and 24 ± 7% in frogs immobilized with curare (Figure 3). After two hours N_2 was replaced by synthetic air and the animals were allowed to recover in the calorimeter. Recovery was characterized by an initial steep increase in heat production which often reached a plateau above the normoxic control level.

Heat production under hypoxic conditions

The normoxic heat rate of frogs showed great individual variability. Therefore, the effect of different grades of hypoxia on heat production was expressed in relative terms using the normoxic heat rate of the individual frog as a reference. Eight frogs were used in these experiments. Each frog was immobilized with curare and its normoxic heat rate was measured and set at 100%. After 90 to 120 min the oxygen content was reduced to 10% or less. This resulted in a decrease in heat production until a new steady state value was reached after about 90 min. When a steady state was reached the oxygen content was further reduced. As with anoxia, the effect of hypoxia was noted after 10-15 min. The results of these experiments are listed in Table 1. After changing the gas flow back to air the heat rate increased rapidly and frogs recovered completely. Some frogs produced more heat during posthypoxic recovery than they had done under normoxic conditions before the experiment was started. Figure 4 shows one of the experiments in detail.

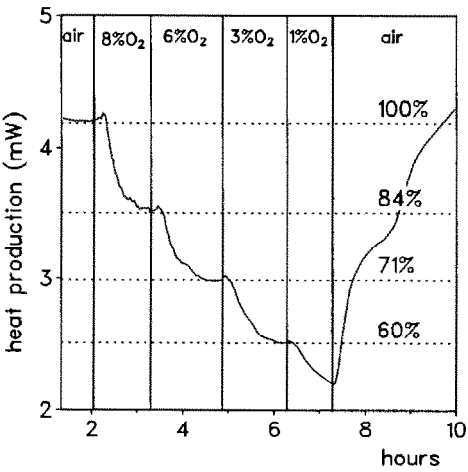

Fig. 4: Heat production of a frog (19.4g), treated with curare. The animal was subjected to different grades of hypoxia. On the right heat production rates are given in percent of the normoxic rate.

Table 1
The effect of graded hypoxia on heat production in frogs immobilized with curare

oxygen content (%)	heat production (%) of normoxic control	(n)
20	100	(8)
10	85	(1)
8	71	(6)
6	57	(5)
4	40	(2)
3	28	(2)
0	24	(3)

4. DISCUSSION

Normoxia

Under normoxic conditions, frogs immobilized with curare produced somewhat less heat than frogs not injected with curare. Curare blocks contraction of skeletal muscle and hence prevents respiratory movements. The observation that curare did not cause a significant decrease in heat rate, therefore, suggests that skin respiration was effective at the chosen temperature (20.8°C) in the relatively small frogs used in the experiments.

Anoxia

After changing the gas flow from air to nitrogen (or back to air) changes in the heat curve were first seen after 10 to 15 min. This lag depends on several factors such as the time required to change the gas in the system and the time it takes to use up the oxygen stores of the blood (cf. Jones 1972). The anoxic decrease in heat production appears to have a similar time course as the decrease in heart rate and the loss of function of parts of the brain that had been shown in previous experiments (Wegener et al. 1986, Thuy 1987).

Frogs, whether immobilized with curare or not, reduced their heat production to about 25% of the normoxic rate during anoxia. This is close to the 29% as has been measured in anoxic goldfish at 20°C (van Waversveld et al. 1989 a, b) and somewhat above the heat rate of turtles submerged in oxygen-free water, which was 15% of controls (Jackson 1968). Calorimetric studies on anoxic frogs have not yet been reported, but heat production in toads submerged in water was reported to be 20% of air breathing controls (Leivestad 1960).

The 75% decrease in heat flow upon anoxia indicates a marked decrease in energy expenditure. However, the corresponding decrease in ATP turnover can not be calculated exactly because such a calculation would require knowledge of all reactions taking place and precise information as to their thermodynamic parameters under both normoxic and hypoxic conditions. This information is not available. Some aspects of the problem have been discussed by Gnaiger (1983).

Recovery from anoxia

All frogs recovered spontaneously when air was readmitted after 2 hours of anoxia. Artificial reanimation is not required because of the efficient skin respiration. This is one of the reasons why frogs are particularly well-suited for experiments on anaerobiosis in vertebrates (for discussion, see Wegener 1988). During recovery a heat production above the normoxic control level was often maintained for several hours. Excess heat production during postanoxic recovery has recently been reported in goldfish (van Waversveld et al. 1989 b) and the mussel *Mytilus edulis* (Shick et al. 1986). Obviously, the extra heat reflects additional metabolism required for restoring pre-anoxic conditions. Restoration of metabolite concentrations to normal values requires regeneration of the phosphagen- and ATP-pools, disposal of anaerobic products and replenishment of substrates. A reduced efficiency of oxidative phosphorylation, due to anoxic damage to membranes, and the repair of damaged structures may also contribute to the increase in heat production.

Hypoxia

Because of the great individual variability in heat production of normoxic frogs the effects of graded hypoxia could not be assessed by comparing different animals. Therefore, in individual frogs the heat production was first measured in normoxia and then, in the same experiment, during hypoxia of different degrees. All frogs in the experiments were immobilized with curare to eliminate heat production due to muscular activity. This procedure allowed us to express the hypoxic relative to the normoxic heat production which was used as a reference. Heat production and oxygen content are correlated (see Table 1) suggesting that during hypoxia aerobic mechanisms contribute to ATP synthesis according to the concentration of oxygen that is available to the animals.

5. ACKNOWLEDGEMENTS

We would like to thank Mrs. Heike Stypa for excellent technical assistance. This work has been supported by grants (We 494) from the Deutsche Forschungsgemeinschaft, D-5300 Bonn, to G.W.

6. REFERENCES

Gnaiger, E. (1983) Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. J. Exp. Zool. 228: 471-490

Hochachka, P.W. (1980) Living without oxygen. Harvard University Press, Cambridge (Mass.), London

Hochachka, P.W., Guppy, M. (1987) Metabolic arrest and the control of biological time. Harvard University Press, Cambridge (Mass.), London

Jackson, D.C. (1968) Metabolic depression and oxygen depletion in the diving turtle. J. Appl. Physiol. 24: 503-509

Jones, D.R. (1972) Anaerobiosis and the oxygen debt in an anuran amphibian, *Rana esculenta (L.)* J. Comp. Physiol. 77: 356-382

Leivestad, H. (1960) The effect of prolonged submersion on the metabolism and the heat rate in the toad (*Bufo bufo*). Mat.-Naturv. Ser. 5: 1-15

Shick, J.M., Gnaiger, E., Widdows, J., Bayne, B.L., de Zwaan, A. (1986) Activity and metabolism in the mussel *Mytilus edulis L.* during intertidal Hypoxia and aerobic recovery. Physiol. Zool. 59: 627-642

Thuy, M. (1987) Wirkung von Sauerstoffmangel auf Stoffwechsel und Organfunktion bei Wirbeltieren: Eine vergleichend biochemisch-physiologische Untersuchung unter besonderer Berücksichtigung des Grasfrosches (*Rana temporaria*). Doctoral thesis, Johannes Gutenberg-Universität, D-6500 Mainz

Van Waversveld, J., Addink, A.D.F., van den Thillart, G. (1989 a) Simultaneous direct and indirect calorimetry on normoxic and anoxic goldfish. J. Exp. Biol. 142: 325-335

Van Waversveld, J., Addink, A.D.F., van den Thillart, G. (1989 b) The anaerobic energy metabolism of goldfish determined by simultaneous direct and indirect calorimetry

during anoxia and hypoxia. J. Comp. Physiol. B 159: 263-268

Wegener, G. (1988) Oxygen availability, energy metabolism, and metabolic rate in invertebrates and vertebrates. Oxygen Sensing in Tissues, H. Acker, ed., Springer Verlag, Berlin, Heidelberg, pp 13-35

Wegener, G., Michel, R., Thuy, M. (1986) Anoxia in lower vertebrates and insects: effects on brain and other organs. Zool. Beitr. 30: 103-124