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A novel 1 liter flow-through calorimeter for heat production measurements on aquatic animals without stress

A.D.F. Addink, G. van den Thillart, H. Smit and J. van Waversveld

Department of Animal Physiology, State University of Leiden, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden (The Netherlands)

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Abstract

A special custom-made 1 liter differential flow-through calorimeter (Sétaram GF108) has been adapted for flow-through heat flux measurements of aquatic animals. The lower limit of detection in a 1 liter vessel is 0.1 mW, whereas the sensitivity shows a linear correlation with the flow velocity up to at least 60 ml min^{-1}. By carefully choosing the conditions and allowing enough time for adaptation, it appears to be possible to obtain data from stress-free animals. In this way the heat production of aquatic animals as a measure of total metabolic activity can be determined under aerobic or anaerobic conditions.

During anoxia the heat production in goldfish falls to 30% of the normoxic level.

This apparatus is a major breakthrough in direct calorimetry of animals, owing to the constant conditions and to its advanced electronics.

INTRODUCTION

Literally dependent on cold, wintry days, Lavoisier and de LaPlace [1] performed experiments on oxidative processes of several chemical reagents in their ice calorimeter. As reported in 1780, they also placed a guinea-pig inside the basket of this calorimeter at an ambient temperature of 0 °C (melting ice). From the amount of melting water they calculated the heat production. The conclusion from these experiments was that animal metabolism is an oxidative process. To obtain an insight into the overall heat production of an animal, it is essential that the environment is constant. In Lavoisier's experiments on guinea-pigs, temperature stability was ensured by the melting ice, and the air inside the calorimeter was replaced two or three times per 10 hour period to prevent hypoxic or anoxic and hypercapnic conditions. However, this was not a particularly stress-free environment for the animal.

In the intervening two centuries technology has advanced tremendously, and calorimetry of warm-blooded animals (e.g. cattle) or small invertebrates has become a normal laboratory routine (Lamprecht and Schaarschmidt [2]; Gnaiger [3]). However, equipment for thermal analysis

of the metabolism of large aquatic and poikilothermal animals was not commercially available. We were interested in such measurements to quantify the energy output of facultative anaerobic vertebrates under hypoxic and anoxic conditions.

MATERIALS AND METHODS

Animals

Goldfish, *Carassius auratus* L., 8–10 g body weight, were obtained from a commercial fish dealer. The animals were acclimated to 20 °C and a 16 h light period in dechlorinated, fully aerated tapwater. They were fed daily with pelleted trout feed (Trouvit) until 1 week before the experiment.

Calorimeter

To determine the relatively low (i.e. less than 100 J h^{-1} $kg^{-0.85}$) total heat production of large, cold-blooded animals accurately, we had to develop a differential calorimeter with a detection limit of 0.1 mW l^{-1}. An inherent problem with the volume of 1 liter is the high heat capacity of water, which causes a low heat flux through the thermopiles. Advanced electronic amplification is therefore necessary. Furthermore, the surrounding water should be constant in every respect. The temperature stability should be $\pm 10^{-5}$ °C, the oxygen level needs to be maintained at a desired air saturation between 0 and 100%, and excretion products such as CO_2, NH_3 and urea should be removed.

The goal of temperature stability was attained with the aid of five thermostats and heat exchangers; oxygen supply and endproduct removal were ensured by a flow-through system (0–50 ml min^{-1}, through both the measuring and the reference vessel). The apparatus was run by a computer program for continuous monitoring during the experiments (1–3 weeks). A constant baseline was maintained over the whole period. The animals remained in excellent condition. The microcalorimetric method is non-invasive: before and after a non-toxic experiment, the same individual will produce a similar quantity of heat under controlled constant conditions.

A sensitive type of calorimeter, the GF108 (based on the Calvet principle), was constructed in France by Sétaram to our specifications with vessels of 1 liter (10 cm diameter). The vessel lids were adapted for flow-through measurements by the Fine Machinery Workshop of our zoological department. Both the vessels and the extended lids were made from stainless steel. The useful temperature ranged from 5–50 °C. The lower limit of detection of this 1 liter apparatus with water flows up to 60 ml min^{-1} appears to be 0.1 mW. This value is consistent with that for other modern small-volume (5 ml) calorimeters (Lamprecht and Schaarschmidt [2]).

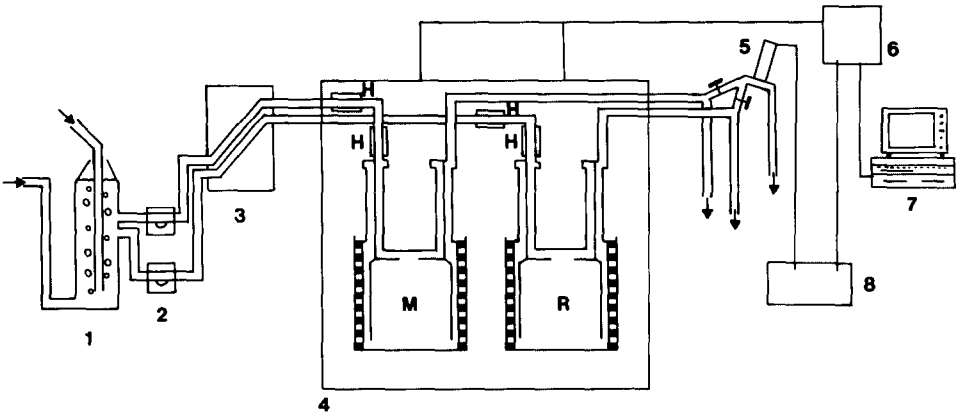

Fig. 1. Diagram of the flow-through calorimeter. 1 gas saturation; 2 two pumps; 3 thermostat with two heat exchangers; 4 microcalorimeter: M measuring vessel inside thermopiles (checkered), R reference vessel inside thermopiles (checkered), H heat exchanger connected with the two built-in thermostats; 5 oxygen or pH-electrode; 6 amplifier; 7 data acquisition; 8 recorder.

Before it enters the actual calorimeter cabinet, the water is processed through three glass gas exchangers in a thermostatted bath to obtain the desired oxygen content (Fig. 1). From here it is pumped by two parallel pumps with magnetically driven carbon cog wheels (type 2031, Verder, Vleuten, The Netherlands) of matched pumping velocity. The two parallel water streams are passed through a large-surface, semi-circular, stainless steel heat exchanger (Müller Temp-Plate, Lichtenvoorde, The Netherlands) inside the waterbath of an LKB Bioactivity Monitor for temperature stabilization ($\pm 10^{-4}$ °C). Through vacuum insulated stainless steel tubing, temperature- and gas-equilibrated water is introduced into the calorimeter. Inside the calorimeter cabinet three heat exchangers are constructed, to maintain the exact temperature inside the calorimeter. Two of them are in contact with the two thermostats of the calorimeter block, and the third is on top of the actual measuring and reference vessels, as shown in the photograph (Fig. 2). This arrangement enables counter current heat exchange between incoming and outflowing water.

The differential microvolt signal from the heatflux meters is amplified and registered simultaneously with other temperature and oxygen signals on a 320 recorder (W + W Electronic Scientific Instruments AG, Basel, Switzerland). The oxygen electrode (E 5046-0, Radiometer) is placed just outside the calorimeter cabinet. At the same time, data acquisition of the differential signal is performed by a Hewlett Packard 86B computer with software supplied by Sétaram. Calibration of the vessels is performed by means of a circular resistor (1000 Ω) mounted below the last heat exchanger in the incoming water stream, near the top of the calorimeter vessel. The constant current and voltage during the calibration time are

Fig. 2. Photograph of the stainless steel measuring vessel of capacity 1 liter and about 15 cm high. On top of this vessel is the large stainless steel lid (14 cm high) of smaller diameter containing a concentric large surface heat exchanger between incoming and outflowing water. The double flex is connected to the built-in resistor for calibration. The large divisions of the ruler standing to the left measure 5 cm each.

produced by an EJ2 Joule effect meter (Sétaram). The time constant is 35 min. The fully functional setup is placed inside a thermostatted room, maintained at least 1° C below the measuring temperature, to prevent any condensation of water.

RESULTS

Flow measurements

The performance of the calorimeter was measured at different flow rates from 0–100 ml min^{-1}. The sensitivity was fully dependent on the flow

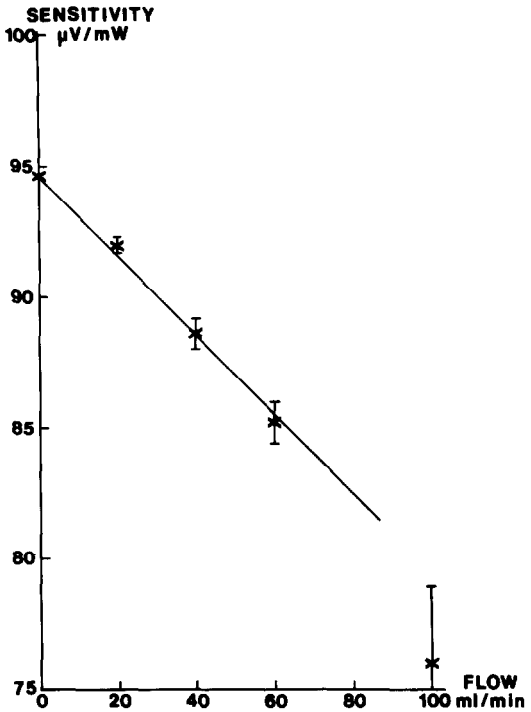

Fig. 3. Graph of the flow rate (F) through each of the vessels versus the sensitivity (S). The sensitivity as a function of the flow rate is given by $S = -0.153F + 94.60$, and the regression coefficient (r) is -0.994. Each calibration of 10 mW took 16.5 h and was repeated at least 4 times. The deviation of the mean is indicated by the vertical bars. At a flow of 40 ml min^{-1}, the mean value was 88.60 ± 0.61 μV mW^{-1} ($n = 16$).

velocity in the measuring vessel during the calibration period. Even at a flow difference of 20 ml min^{-1} between the measuring and the reference vessel, no significant deviation of the sensitivity of the measuring vessel could be observed. At flow velocities up to 60 ml min^{-1}, the sensitivity decreases linearly (Fig. 3). At higher velocities (100 ml min^{-1}), the sensitivity drops and the signal becomes unstable. Nearly all calibrations were performed over a period of 16.5 h. The obtained plateaux, as well as the baselines, were flat and reproducible at flow rates up to 60 ml min^{-1}. The baseline drift was less than 0.02 mW per week. The sensitivity of the (identical) reference vessel proved to be fully comparable.

Calorimetry of goldfish during normoxia and anoxia

During the last 25 years several direct calorimetric measurements have been made, e.g. on small invertebrate [3] and also on fish (Davies [4]; Smith et al. [5]). The handling factor was not eliminated for these fishes. It takes at least two days for goldfish to calm down (Van den Thillart [6]). To prevent the influence of circadian rhythms, the goldfish in our laboratory

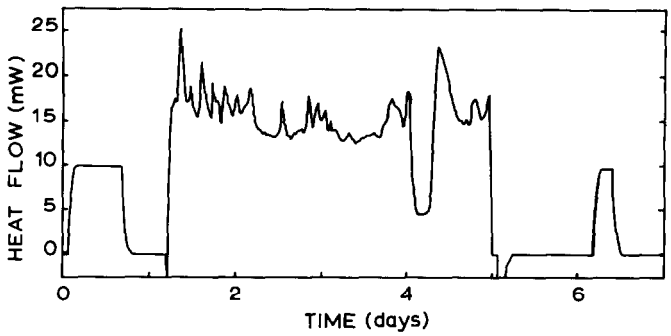

Fig. 4. Computer recording of the experiment during 7 days. At the beginning and at day 6 two calibration curves (C) and the baseline are shown . After a period of normoxia (N) from day 1 to day 4, anoxia (A) is introduced by supplying nitrogen gas for 5 h. The heat production of the standard metabolism of four goldfish drops by 70% as compared with the normoxic value before and after.

were adapted to full darkness for at least five days. As an example, the results of a biological experiment with a small group of goldfish, *Carassius auratus* L., are shown in Fig. 4. Goldfish have an anaerobic capacity, e.g. they can survive an anoxic period of 16 h at 20 °C. Instead of oxygen, goldfish (as well as crucian carp and bitterling) rely on a fermentation process, which uses acetaldehyde as an electron-acceptor (Mourik et al., [7]; van den Thillart and van Waarde [8]). The ethanol produced diffuses out of the animals, as ethanol is both fat and water soluble. As shown in the recording the baseline is straight, both at the beginning and at the end of 7 days. The two calibration curves show a sensitivity of 88 μV mW^{-1} at a water flow of 40 ml min^{-1}. These values are provided by the computer program from the recordings. The four goldfish are introduced on day 1 under normoxic conditions. The heat production of the goldfish correlates with the oxygen consumption during normoxia: 700 J h^{-1} kg$^{-0.85}$ and 35.4 ml h^{-1} kg$^{-0.85}$ of O_2, resulting in an oxycaloric value of 19.9 kJ l^{-1} of O_2. This is a likely value for oxygen consumption of normoxic starving fish (Brafield [9]). During the normoxic periods some locomotor activities of the goldfish are evident from small peaks of extra heat production (Fig. 4). Because of handling, circadian rhythm and feeding, much higher levels of oxygen consumption and heat production are reached in salmon and goldfish: more than 2000 and 1350 J h^{-1} kg$^{-0.85}$ respectively. On day 4 the water is gradually made anoxic with nitrogen over a 3 h period. The heat production of the anoxic animals declines dramatically, reaching a steady value during a further 2 h of 30% of the normoxic level.

DISCUSSION

This type of depressed metabolic activity in goldfish is characteristic of facultative anaerobic animals, as explained by Hochachka and Somero [10].

The reduction in carbohydrate demand and the fermentation process to ethanol reduce the production of acid components. This provides a longer period of survival for the fish. On returning to the normoxic situation the heat production resumes its original level.

The obtained values of total heat production are important for the interpretation of the energy metabolism of animals. The contribution of both aerobic and anaerobic pathways to the total energy output under normoxic and hypoxic conditions can be evaluated. It is possible to balance the data for heat production and energy metabolism. With biochemical determinations of intermediates of the metabolism as well as excretion products, e.g. ethanol, carbon dioxide and ammonia, van Waarde [11] and van Waversveld and co-workers [12–16] established a continuous breakdown of amino acids/proteins.

The somewhat increased glycolysis during anoxia leads to formation of acetaldehyde as an electron acceptor. Furthermore, some increase in chain length of fatty acids takes care of part of the reducing equivalents (NADH, NADPH).

Other possibilities to exploit are the testing of environmental changes, such as temperature, possible poisonous effects of noxious substances, and also water acidity. On the last subject the determinations are in progress in the described flow-through calorimeter.

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