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Construction of a low cost and highly sensitive direct heat calorimeter suitable for estimating metabolic rate in small animals

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ABSTRACT

Although the concept of a metabolic rate is readily understood, actual measurement of metabolism has proved much more difficult. The numerous strategies for estimation of metabolic rate all result in an incomplete accounting. Respirometry or gas exchange is the most widely used approach but mostly ignores the anaerobic component. Here, we describe a readily-built and low cost direct heat calorimeter that may be coupled with standard respirometry equipment to provide a more complete portrait of metabolism. The device is sensitive and provides a predictable measurement of heat flow from an organism.

1. Introduction

Metabolism is derived from the Greek word, metabole (μεταβολή), which simply means change. Metabolism refers to all of the changes that occur within an organism during a prescribed period. Obviously, the varied nature of such changes means that one cannot directly measure metabolic rate. Instead, a variety of approaches have been applied to estimate metabolic rates. Perhaps, the first modern attempt at understanding metabolism was undertaken by Sanctorio in 1590 (Withers, 1992). Sanctorio weighed himself and discovered that his food consumption did not relate to his waste production or changes in body weight. He ascribed the difference as 'insensible perspiration'. In the centuries that followed, many advances have been made in the precision and accuracy of metabolic estimates. An important advance was that of the jacketed ice bath calorimeter championed by Lavoisier in 1777. Here, an organism heats up a chamber surrounded by ice. The amount of melted ice then is proportional to metabolic expenditures. We deem such measurements as direct calorimetry (see reviews by Frankenfield, 2010; Withers, 1992). Advantages of this approach are that it may account for both aerobic and anaerobic processes. Disadvantages include that the heat may be absorbed by a warming animal and the approach is oftentimes cumbersome and difficult. Perhaps the most widely applied approach to estimate metabolic rate is that of indirect calorimetry using respirometry. In this approach, respiratory consumption of oxygen and/or production of carbon dioxide are estimated. This approach is simple, easy to perform, and relatively inexpensive. The biggest disadvantage to this approach is that it ignores the anaerobic component of metabolism. More recently, investigators have revised the traditional view that any anaerobic metabolism in mammals would be limited (Brooks, 2009; Walsberg and Hoffman, 2005). An understanding of the contributions of both aerobic and anaerobic processes is vital to a more complete picture of energetic outlays. As far as we know, there is very limited availability of direct heat calorimeters suitable for use with small rodents (Geoscience Ltd. La Jolla CA). Here, we describe the construction and optimization of a simple and low cost direct heat calorimeter.

2. Materials and methods

2.1. Materials and supplies

The calorimeter was constructed as indicated in Fig. 1. Most materials were purchased at local hardware stores or at an online auction site. Major expenses were the Sable Systems FoxBox II respirometry system, Gilson Minpuls 3 peristaltic pump, and Fluke model 189 multimeter. Many laboratories interested in examining metabolic rates currently have equivalent items. When these items are excluded, total cost of construction is $<$ $1000.

2.2. Metabolic chamber

The metabolic chamber consists of a ~4 l high-density polyethylene wide-mouth container (US Plastics Inc.). The container was cut in half horizontally for the construction process. Copper tubing (~9.5 mm outer diameter) was formed into a coil to act as

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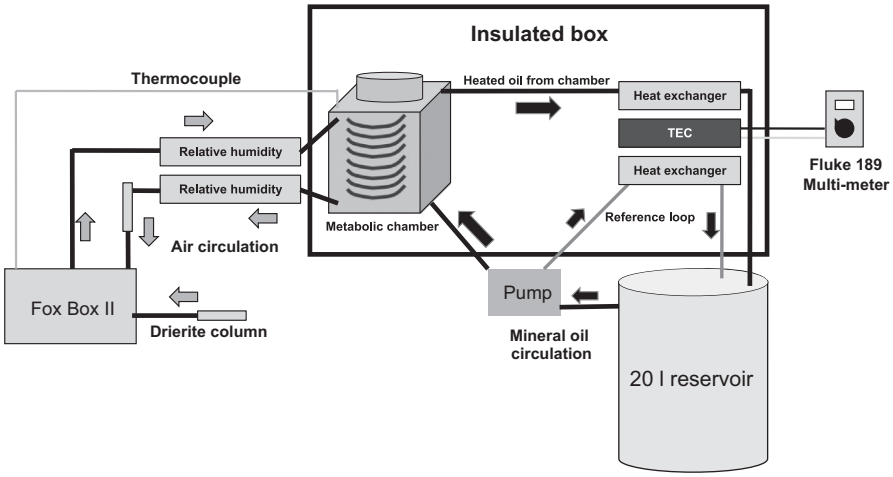

Fig. 1. Diagram of calorimetry system. TEC is the thermoelectric cooler.

a metabolic heat exchanger and inserted into the container. The container was reassembled and sealed air-tight with silicone and strips of polyethylene from a second container. The entire metabolic chamber was placed into a large foam-insulated box ($\sim 60 \times 40 \times 30$ cm^3) and all dead space was filled with Styrofoam. A Gilson Minipuls 3 peristaltic pump was used to pump mineral oil (Fisher Scientific) from an adjacent 20 l reservoir into the copper metabolic heat exchanger. Care was taken to minimize the distance between the metabolic chamber and the mineral oil reservoir. All plumbing was thermally insulated using pipe insulation from a local home building supply store.

2.3. Metabolic heat detector

The excurrent mineral oil flow from the copper metabolic heat exchanger was passed through one side of the detection device before return to the reservoir. The detection device consisted of a model 12702 thermoelectric cooler (Peltier device) sandwiched between two liquid cooled CPU heat exchangers (e.g. Koolance GPU-180-L06 water block). The thermoelectric cooler was coated with thermal paste (Koolance White Thermal Paste) to ensure good heat flow and the detection device was securely bolted together. Specifications of the thermoelectric cooler are $40 \times 40 \times 4.5$ mm^3 dimensions, maximum temperature differential of 67 °C, maximum potential of 15.2 V, and maximum cooling power of 20 W. The other side of the detection device sandwich was fed by a mineral oil reference loop that was circulated by the peristaltic pump through the 20 l reservoir. Use of this reference loop with a large heat sink proved more effective than using the incurrent mineral oil destined for the metabolic chamber due to its avoidance of partial heat passage at the Peltier device. When the heated mineral oil from the metabolic chamber circulates through the detection device, a potential is generated via the Seebeck effect. Care was taken to use a thermoelectric cooler that would result in the greatest change in potential with the least amount of heat differential i.e. a low efficiency thermoelectric cooler. After leaving the detection device, mineral oil was returned to the 20 l reservoir. A Fluke model 189 multimeter was used to measure the potential from the thermoelectric cooler. Data were logged through Fluke View Forms software at one reading per 10 s.

2.4. Respirometry

Respiratory gas exchange was measured using a Sable Systems FoxBox II. The FoxBox II delivered dried air at 300 ml min^{-1}. Data for CO_2 production and O_2 consumption were logged using Sable Systems Expedata software. The FoxBox II was also used with a thermocouple to record metabolic chamber temperature. Incurrent air from the environment and from the metabolic chamber was dried using a column filled with Drierite™ desiccant (Fig. 1).

2.5. Evaporative water loss

When used with an animal, heat loss via evaporative water loss will be determined. Digi Watchport USB humidity and temperature sensors were placed into the incurrent and excurrent air streams of the metabolic chamber. Air was dried again before being returned to the FoxBox II respirometer. Data were logged using included Digi software. The evaporative water loss when used with an animal will be determined by the difference between the two sensors.

2.6. Use of mineral oil

The 20 l reservoir was filled with mineral oil due to its low specific heat (1.67 kJ kg^{-1} K^{-1}) as compared to water (4.187 kJ kg^{-1} K^{-1}). Mineral oil also maintains rather low viscosity at low temperatures which was critical in our intended use of the calorimeter. Importantly, the use of this oil necessitated use of Santoprene tubing for the peristaltic pump. We found that after an initial conditioning period, the tubing yielded very consistent flow rates. The large thermal inertia provided by the 20 l reservoir helped to ensure stability of the temperature of the mineral oil despite modest fluctuations in ambient temperature of the environmental chamber.

2.7. Electronic signal amplifier

Initial concerns about the amplitude of signal resulted in construction of a signal amplifier. The circuitry is illustrated in Fig. 2. This amplifier was placed between the thermoelectric cooler and the logging multimeter. Later experimentation demonstrated that the amplifier was not necessary and that sufficient sensitivity was available in its absence. However, all experiments and calibrations shown here were performed with this amplifier in place. A reviewer noted that use of an OP07 amplifier would be more appropriate than the LM224 should users of a similar system require a signal amplifier.

2.8. Calibration procedures

The calorimeter was calibrated by using power resistors placed within the animal chamber. By applying a known potential to the resistor, power (watts or J s^{-1}) could be determined using Ohm's