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Construction and Use of a Calorimeter to Estimate the Anaerobic Contributions to Metabolism

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Introduction

Metabolism is derived from the Greek word metabole which translates to change. Metabolism is the sum total of chemical reactions that take place in an organism. Some reactions are exothermic (heat-producing) whereas others are endothermic (heat-absorbing). By measuring heat production (calorimetry), one can estimate metabolic rate. Historically, such measurements of direct calorimetry were difficult. As a result, most studies utilize indirect calorimetry wherein oxygen consumption and/or carbon dioxide production are measured. One limitation to this approach is that anaerobic metabolism is ignored.

No commercially-available calorimeter is available for whole animal metabolic studies. **We hypothesized that small rodent hibernators may experience significant anaerobic metabolism.** In order to empirically determine the relative contributions of anaerobic and aerobic metabolism to a hibernator's overall energetic budget, we built our own calorimeter.

Problem: Traditional approaches to estimating metabolic rate neglect the anaerobic component.

Solution: We made a calorimeter that can estimate direct heat production and therefore give a reliable estimate of the anaerobic contributions to metabolism.

Needs

Sensitivity of Calorimeter

A ground squirrel during hibernation has a body temperature that is approximately that of ambient temperature to as low as -2° C. The oxygen consumption of a 150 g ground squirrel during hibernation is ~0.5 ml $O_2 \bullet g^{-1} \bullet h^{-1}$. During torpor, ground squirrels utilize fat stores for energy. When oxidizing fats at 0.0047 kcal•ml O_2^{-1} the estimated metabolic rate during torpor is 0.35 kcal•h^{-1} or 0.4 Watts.

The calorimeter must be able to directly measure heat production of an organism whose **metabolism may be as low as 0.35 kcal•h^{-1} or 0.4 Watts.**

Measurement of O_2 consumption and CO_2 production

The estimation of anaerobic metabolism to overall metabolism requires an estimation of aerobic metabolism. The partial pressures of O_2 and CO_2 measured in the animal chamber can be used to estimate aerobic metabolism.

Since total metabolism is the sum of both aerobic and anaerobic metabolism, estimation of aerobic metabolism will allow us to estimate anaerobic metabolism.

Body temperature measurement

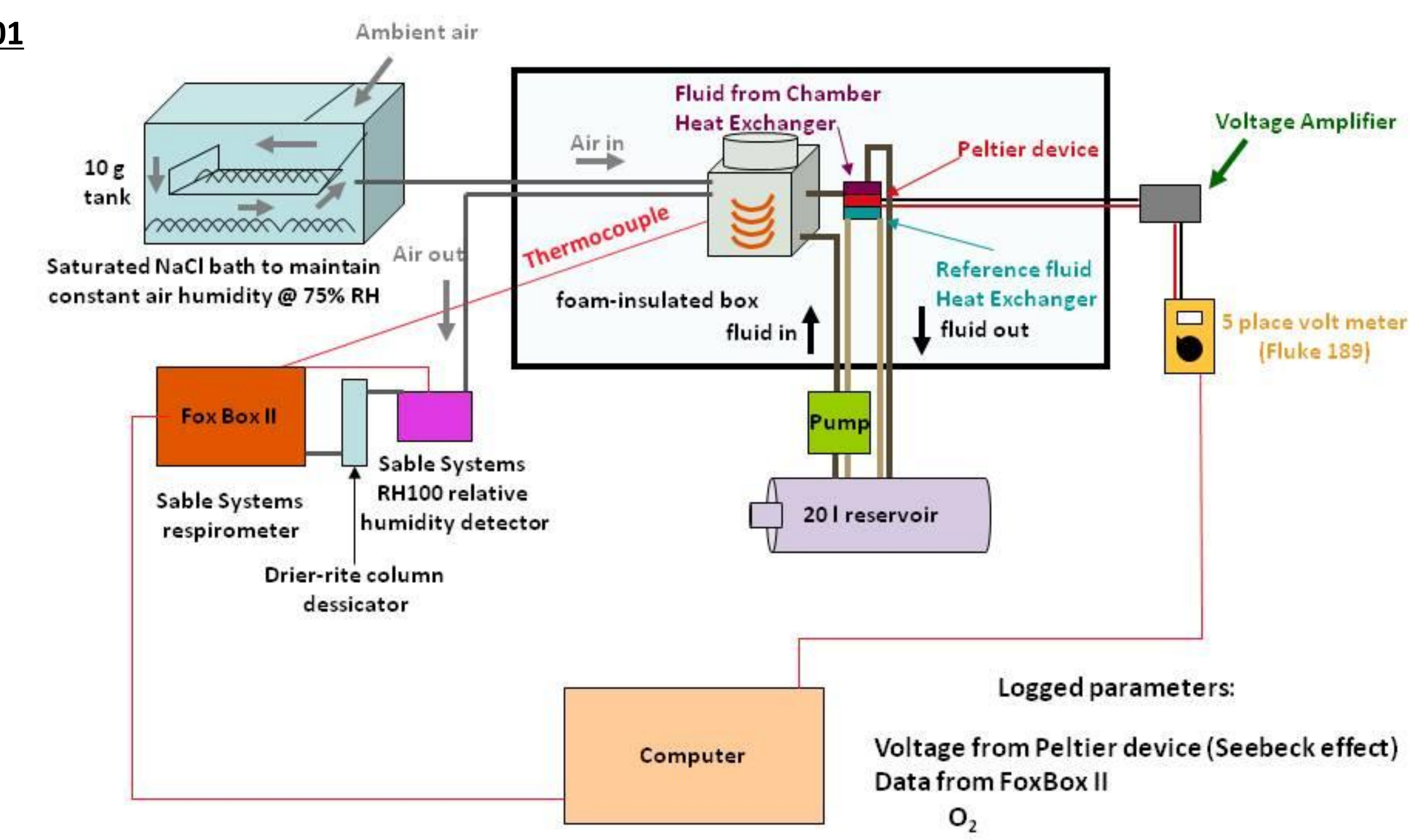
Not all heat generated from metabolism is released to the environment but is instead reabsorbed by the organism. Measurement of body temperature will allow us to estimate the amount of heat reabsorbed by the organism.

Measurement of relative humidity

Heat may also be lost through evaporative water loss. Evaporative heat loss can be estimated by measuring any change in the relative humidity of the animal chamber.

Construction and How it Works

Fig. 01

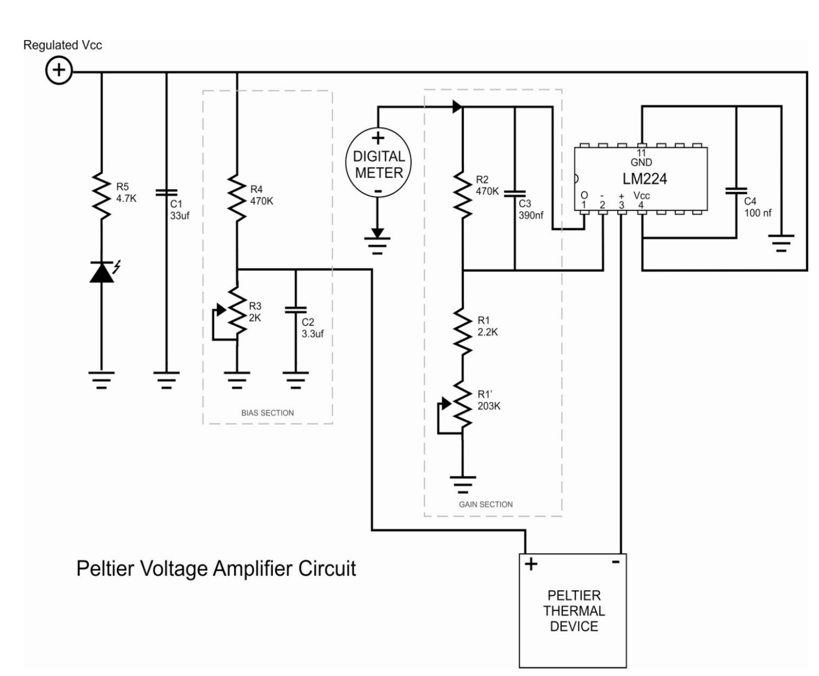


Logged parameters:

Voltage from Peltier device (Seebeck effect)
Data from FoxBox II
- O_2
- CO_2
- Flow rate
- Relative humidity (from RH100)
- Chamber temperature (from thermocouple)

Body temperature from meta-meter

Fig. 02



The Voltage Amplifier

The voltages generated by the Peltier device (Seebeck effect) were amplified by a custom made voltage amplifier. The schematic for the voltage amplifier is shown in Fig. 02.The voltage amplifier increased signal voltage by 100 fold. The amplifier is an LM224 Quad Operational Amplifier. Adjustments to the voltage gain were made by modifying the resistance value of R1 shown in Fig. 02. This operation were readily done using a potentiometer whose resistance was manually adjusted by hand. The entire circuit was soldered on an electrical board to ensure good electrical connections. The board was placed inside a grounded metal box to prevent any electromagnetic fields from causing electrical noise.

Gas Exchange

Air was pumped into the chamber from an outside container holding a saturated salt solution. Use of a saturated salt solution maintains a constant relative humidity of 75%. Any changes in relative humidity from evaporative water loss by the animal are logged by a Sable Systems RH100 humidity detector. The air was passed through a Drier-rite column which dehydrates the air before flowing into the Sable Systems FoxBox II respirometer which recorded the partial pressures of both O_2 and CO_2.

Direct heat measurement

The Peltier device relies on a heat differential to generate a voltage through what isknown as the Seebeck effect. A Peltier device or heat pump, pumps heat from one side of the device to the other and subsequently may generate a voltage in proportion to the amount of heat it pumps. Both sides of the device are interfaced to heat exchangers which contain the mineral oil that will be used to absorb and carry any heat released by the organism.

Mineral oil is used because of its ability to undergo large temperature changes with the absorption of a small amount of heat (low specific heat), and its fluidity at low temperatures. A reservoir holding 20 liters of mineral oil was maintained at a constant temperature. Mineral oil was pumped from the reservoir through copper tubing lining the animal chamber where it absorbed heat and then through one of the heat exchangers interfaced to the Peltier device. Mineral oil is simultaneously pumped from the same reservoir through the heat exchanger on the other side of the Peltier device.
Any heat absorbed by the mineral oil in the animal chamber is pumped to the mineral oil on the other side of the Peltier device. The voltages generated by the Peltier proportional to the amount of heat pumped was amplified by an op-amp circuit shown in Fig. 02. The amplified voltage was read by a Fluke 189 voltmeter and logged in a computer.

The mineral oil flowing through both heat exchangers is then returned to the reservoir where any heat that it absorbed will be dissipated. Body temperature will be logged by temperature sensitive radio telemeters that are to be implanted within the organisms. A thermocouple will monitor the chamber temperature.

Calibration

Fig. 03

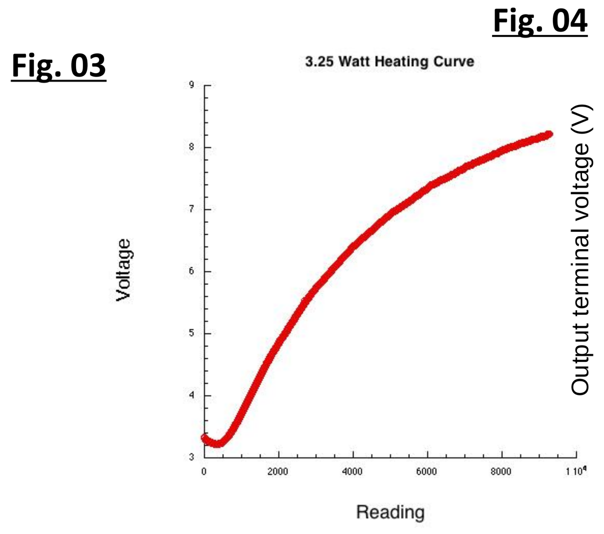
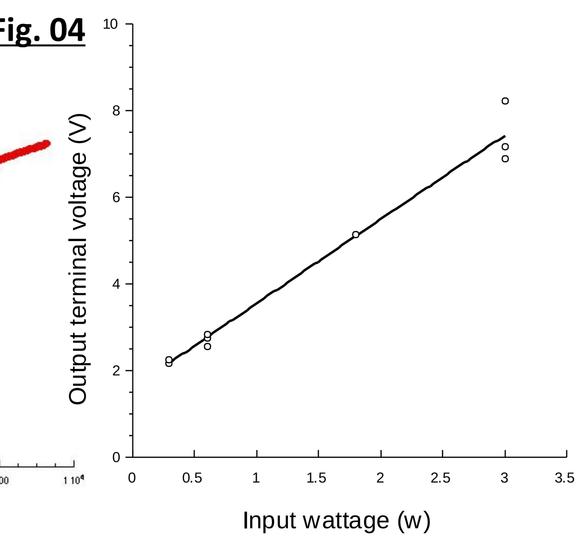


Fig. 04



Calibration of the calorimeter involves the use of a resistor placed within the animal chamber that can generate a constant wattage. The amount of heat absorbed by the mineral oil is related to this wattage. **Heat flows from one object to another at a rate proportional to their temperature difference.** The proportionality constant or time constants of the system is determined by the properties of the mineral oil and the rate at which the mineral oil flows.

Two time constants are used to interpret the data from the calorimeter. One is related to the time the mineral oil reaches absolute equilibrium, where heat can no longer be added or lost from the mineral oil, the other is related to the amount of time it takes for the mineral oil to make one complete cycle through the copper tubing.

Fluid flow rates can drastically affect the amount of time that the system can reach thermal equilibrium. Slower speeds allow for more heat absorption and faster times in reaching equilibrium, but a lowered dynamic range of the output voltages. Fig. 03 shows an example curve of voltage vs. time. The time constants can be used to estimate metabolism at any given time.

Different wattages generated within the chamber cause the mineral oil to reach different equilibrium temperatures in which no more heat can be added or lost to the mineral oil. **A sample standard curve (Fig. 04) was developed by relating output voltage generated by the Peltier device to known input watts generated by a resistor within the chamber.**

Acknowledgements

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Conclusion: **A calorimeter was constructed that was sensitive enough to estimate anaerobic contributions to metabolism.**