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The Difference Between Heat and CO2 Measurements of Metabolic Rates In Mice

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ABSTRACT

This experiment examines the differences between heat and carbon dioxide measurements of metabolic rates in mice. Metabolism is a universal term in the biological sciences that generally describes the chemical and physical changes in living organisms. The metabolism of the rodent is extremely dynamic, exhibiting marked changes in response to a variety of physical and biological factors such as body temperature, motor activity, endocrine function, reproductive state, wind velocity, altitude and nutritional state. The research paper focused on two different ways of measuring metabolism through heat and carbon dioxide studies. Metabolism was measured by placing a mouse in a chamber in a calorimeter made from styrofoam. Thermocouples were placed in the styrofoam through a hole made through the top of the container. The probes were taped to the top of the chamber above the mouse. A mouse was then placed in the chamber and the calorimeter's temperature was recorded using a microprocessor thermometer. Temperatures were recorded every fifteen seconds up to five minutes. Calibration of the heat calorimeter was as follows. A one hundred twenty-five milliliter flask of water was placed inside the calorimeter. The probes were attached to the microprocessor thermometer where temperatures of the water, air and the difference between the two were recorded every fifteen seconds up to five minutes. The calorimeter constant was then calculated using the heat loss from the water, and the change in the temperature of the calorimeter. Rate of respiration measurements were taken using a carbon dioxide analyzer(Licor model 6200). The analyzer was attached to an eighteen liter bottle where a mouse was enclosed and measurements were taken every minute for each of the ten attempts.

INTRODUCTION

Metabolism is a universal term in the biological sciences that generally describes the chemical and physical changes in living organisms (Poole, 1987). The metabolism of the rodent is extremely dynamic, exhibiting marked changes in response to a variety of physical and biological factors, such as ambient temperature, body temperature, wind velocity, altitude, nutritional state, reproductive state, endocrine function and motor activity (Christopher, 1993). Under any set of environmental conditions, the total heat produced from a rodent's metabolism is derived from its basal metabolism. Basal metabolism represents the heat produced from the sum of all catabolic and anabolic biochemical processes involved in the maintenance of respiration, circulation, muscle tone, peristalsis, body temperature and other vegetative functions (Christopher, 1993). The basal metabolic rate is defined as the metabolic rate of an organism that is resting in a thermoneutral state for fourteen to eighteen hours after eating. A major portion of a rodent's metabolism is basal but since it is difficult to create a state in which a rodent is at absolute rest but not sleeping it is easier to measure its resting metabolic rate (Foster, 1981). The resting metabolic rate (r.m.r.), which will be measured in this study, is defined as the metabolic rate of a resting animal that is not in a postabsorptive or fasting state.

There are many methods for measuring metabolism in small animals such as mice. It can be measured both directly and indirectly. In a direct method of measurement, the quantities of absorbed or evolved heat are used. An animal is placed inside the chamber of the device and the heat loss is measured by the thermoelectric detectors that are positioned throughout the inner wall of the chamber. Evaporative heat loss is measured separately by recording the difference in water vapor pressure between the influent and effluent air (Christopher, 1993). The difference in the temperatures of the influent and effluent air streams can also be determined, which represents a minute fraction of the total dry heat loss (Christopher, 1993). Assuming that there is a steady state condition in the chamber, the sum of dry heat loss and evaporative heat loss is equal to the animal's metabolic heat production (Christopher, 1993). In using an indirect method, metabolism can be estimated be measuring the rates of oxygen consumption and or carbon dioxide production over a standard time period using open or close-circuit respirometer. A respirometer is an instrument used to study the character and extent of respiration. Open-circuit systems employ a continuous flow of fresh air though the chamber containing the animal. The partial pressure of oxygen is measured in the influent air and effluent air. The change in pO2 or pCO2 after passing through the chamber multiplied by the air flow rate yields a measure of oxygen consumption or carbon dioxide production (Christopher, 1993).

Another method for measuring the metabolism in small animals has been accomplished by trying to find an experimental approach to the mechanism of weight loss. This method was used by three scientist in the 1960s. The method consisted of an apparatus that contained atmospheric pressure and partial pressure of gases being kept constant during the experimental period. Food and water intake and environmental temperature were kept constant. The factors were

altered at will. Oxygen uptake and carbon dioxide output measurements were carried out over short or long intervals of time and separate fecal and urine collections allowed accurate balance studies to be simultaneously undertaken. Experimental data obtained with the apparatus on mice indicated a diurnal variation in oxygen uptake and carbon dioxide output and showed the effects of raising environmental temperature on metabolism. The apparatus of the method consisted of a circulatory system, a manometer, and a constant water bath (Dowsett, Kekwick, Pawan, 1963). Another method used for measuring metabolism in small animals is a bomb calorimeter. A bomb calorimeter measures heat production by combusting the sample in a closed system which can neither gain heat from nor lose heat to the outside environment (McLean, 1987).

The purpose of this research is to understand more about metabolism in mice and to compare and contrast the difference between CO2 and heat measurements in metabolism.

MATERIALS AND METHODS

Twenty-one day CD 1 weanling mice from Charles River Labs were used in this experiment. Four female mice were used to test the differences between heat and CO2 measurements of metabolism. Each mouse was placed in its own cage and fed a diet(TD 95146, Harlan Teklad) consisting of 5.27% saturated fat (coconut oil). The diet lasted for about five weeks and then a pellet food was used. After about two to three weeks the mice started dying and the cause of death was determined from the food. Out of the original thirty-one mice four survived and all four mice were fed a diet that consisted of Milo, Corn and Oats. The mice were fed the diet for the remainder of the experiment and tap water was given ad libitum.

Beginning at ninety-one days of age the metabolism of each mouse was measured using the following procedure. A mouse was placed in a chamber in a calorimeter made from styrofoam. Thermocouples were placed in the styrofoam via a hole made through the top of the container. The probes were taped to the top of the chamber above the mouse. A mouse was then placed in the chamber and the calorimeter's temperature was recorded using a microprocessor thermometer. Temperatures were recorded every fifteen seconds up to five minutes. Calibration of the heat calorimeter was as follows. A one hundred twenty- five milliliter flask of water was placed inside the calorimeter. A temperature probe was placed inside the flask and to the top of the calorimeter. The probes were attached to the microprocessor thermometer where temperatures of the water, air, and the difference between the two were recorded every fifteen seconds up to five minutes. The calorimeter constant was then calculated using the heat loss from the water, and the change in the temperature of the calorimeter.

Rate of respiration measurements were taken using a CO2 analyzer(Licor model 6200). The analyzer was attached to an eighteen liter bottle where a mouse was enclosed and measurements were taken every minute for each of the ten attempts.

RESULTS

The results for the two different types of measurements are related in the following figures. Figure 1 describes the average rate of respiration verse the average carbon dioxide concentrations taken for all four mice during the carbon dioxide measurements. Figure 2 describes the heat given off by the mice measured in calories versus time. Figure 3 is a survivorship curve describing the relationship of the number of mice that died throughout the time before the actual experiment.

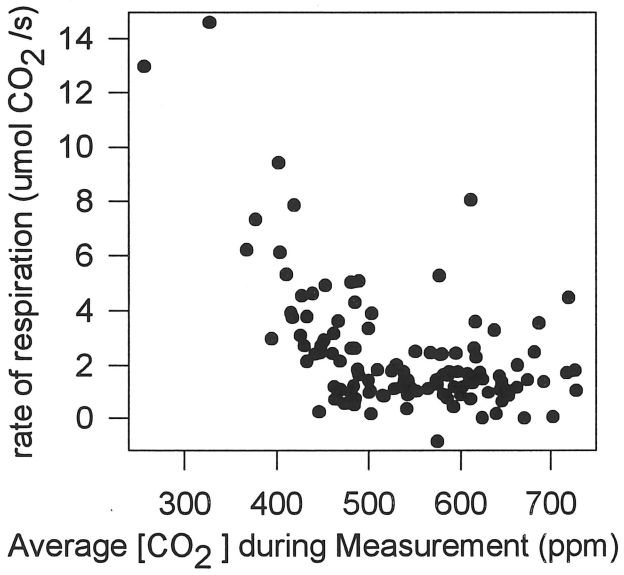

Figure 1. Comparison of the Rate of Respiration vs. Average CO_2 Concentrations.

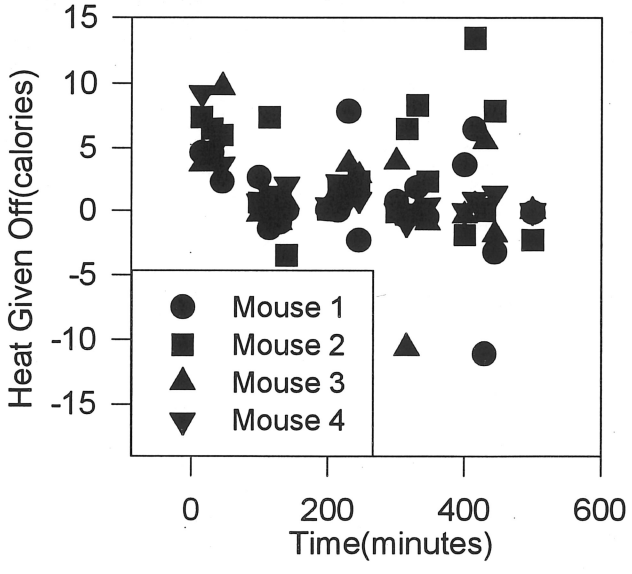

Figure 2. Comparison of Time vs. Rate of Respiration

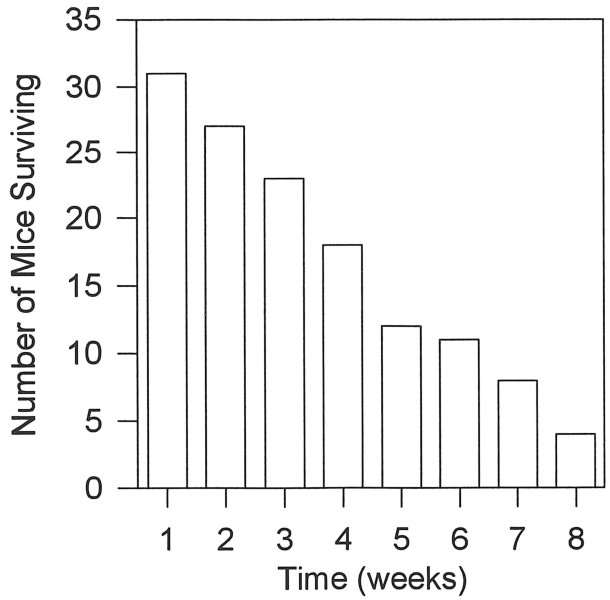

Figure 3. Results of Survivorship Curve of Mice vs. Time.

DISCUSSION

Figure 1 describes the average carbon dioxide concentrations given off by each mouse, ranging from 400 to 600 parts per million. These high levels of carbon dioxide were caused by a high diffusion and respiration rate by the mice. Also the analyzer's scrubber, which removes carbon dioxide, was not able to remove carbon dioxide fast enough before the next measurement was made. A pump was used to aid the analyzer's scrubber but the concentration was never scrubbed low enough. Also in figure 1 are the points of high respiration and low carbon dioxide measurements. These points were because the respiration rate was high but the rate at which the mice were able to diffuse the carbon dioxide out of their system was low because of the already high amount that was in the bottle.

Figure 2 describes the amount of heat that was given off by the mice versus time. The amount of heat can be converted to the number of moles of carbon dioxide given off by each mouse(70kcal = 1mol Co2). The graph shows that the relative amount of carbon dioxide was relatively low at the start of the run but as different runs took place the levels of carbon dioxide increased and remained high throughout the experiment. This was because the calorimeter was not equipped with a ventilation system and no means was provided for removing the carbon dioxide given off by the mice.

Figure 3 is a survivorship curve made for all 31 mice before the experiment began. Each mouse was fed a low fat powder diet that was depleted in three weeks. A pellet diet was then used as a substitute and was discovered to be old. After two weeks several mice died due to the diet. Blood samples were then taken from the dead mice and roundworm parasites were found in the blood. The diet was switched again and the remaining mice were fed a diet of milo, corn and oats. The Figure shows the number of mice that died over the number of weeks.

In closing, both studies proved to be effective ways of measuring metabolism. Both proved that the rate at which a mouse diffuses and respires carbon dioxide is great compared to their body composition and the fact that they were at rest. The metabolic rate of a mouse and a human both depend on a number of factors such as food intake and exercise. Over all, humans have higher metabolic rates than mice because humans have bigger bodies and more energy is required for everday activity than is needed for mice.

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