Myothermic Calibration for Skeletal Muscle

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Abstract
A physical and mathematical method is presented for the calibration of myothermic apparatus and calculation of the specific heat capacity of skeletal muscle. Artificial agar "muscles" heated by electrical energy were utilized. By cutting the agar to approximate the dimensions and mass of murine hindlimb muscles a constantan/silver wire wound thermopile was calibrated to within 0.5% of its thermoelectric output. The system was then used to measure the specific heat capacity of murine soleus muscle, which was 3.73 ± 0.1 (Joules/gram/degree centrigrade).
Key words: myothermic, calibration, thermopile, muscle specific heat capacity.

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The measurement of evolved heat is a valuable tool for evaluating the energetic cost of various muscular contractile states: for example, resting [2], shortening under load and maintaining a load [6,8]. The heat produced during efflux of calcium ions from the sarcoplasmic reticulum can also be measured [9]. Myothermic techniques can be adapted for whole muscles, groups of fascicles or individual fibres and can be applied in clinical and diagnostic situations. These typically involve the application of thermopiles, devices consisting of many pairs of thermocouples which give rise to a thermoelectric output. The thermopile design used in the present work was based on that of Ricchiuti and Mommaertz [7] and consisted of constantan/silver thermocouples pressed to a thickness of 50 μm. The thermojunctions were insulated with a thin layer (10 μm) of PVDC copolymer and mounted in a gold plated brass supporting frame. The size of the thermopile was 1.5 cm in length and 0.4 cm in width. This methodology can be adapted to any similar thermopile or thermocouple system.

For most thermopile systems to be accurate, the specific heat and mass of both the thermopile and muscle being studied need to be known. In the past, wide use has been made of a value derived for amphibian muscle by Hill in 1931 [4]. This value may not be applicable to all preparations particularly since the accuracy and sensitivity of thermopiles have improved considerably over the past few decades. In this paper a method is described to not only accurately calibrate and check wire wound thermopiles but to calculate the specific heat capacity of any muscle preparation that is to be myothermically examined.

Materials and Methods.

Heat loss calculation

The raw thermoelectric output of a Ricchiuti (constantan/silver wire wound) type thermopile was amplified by a nanovolt amplifier (Astrodata 120) and all frequencies over 25 Hz were filtered out [5]. The heat loss from the muscle to its surroundings is approximately exponential and has a different time constant of decay for each new muscle preparation. The time constant of heat loss decay was calculated at the beginning of each experiment by passing a burst of 100 kHz current through the muscle via stimulating electrodes and measuring the half time of the decay. The decay of the thermoelectric output is described by the equation

$$V = Vm\, e^{-t/T} \qquad (1)$$

where Vm = output at time = 0, V = output at t seconds and T = time constant. For each preparation the time constant for exponential decay was obtained by dividing the half time by the natural logarithm of 2.

The exponential heat loss can be corrected for by passing the thermoelectric output through an electronic integrating device. The operating principle is that the raw thermoelectric output is added to its integral multiplied by an appropriate rate constant for the exponential heat loss. The time constant for each particular rate of heat loss was selected by varying the resistance of the integrating circuit. This is represented by

$$Vo = Vi + K\int_0^t Vi\, dt \qquad (2)$$

where K (the rate constant) = 1/T , Vo = output from the heat loss corrector and Vi = input to the heat loss corrector [10].

The output of the heat loss corrector is a constant signal equal to the initial thermoelectric output. When a muscle is actively producing heat, the output from the corrector will be an increasing signal which will reach a plateau only after active heat production ceases.

Thermopile sensitivity

The thermopile was placed in an ice/water mixture and the output measured by a microvolt stable linear chart recorder (Brush, mark 280). Once the thermopile had attained thermal equilibrium with its surroundings it was rapidly plunged into water at room temperature (measured by an electronic ther-

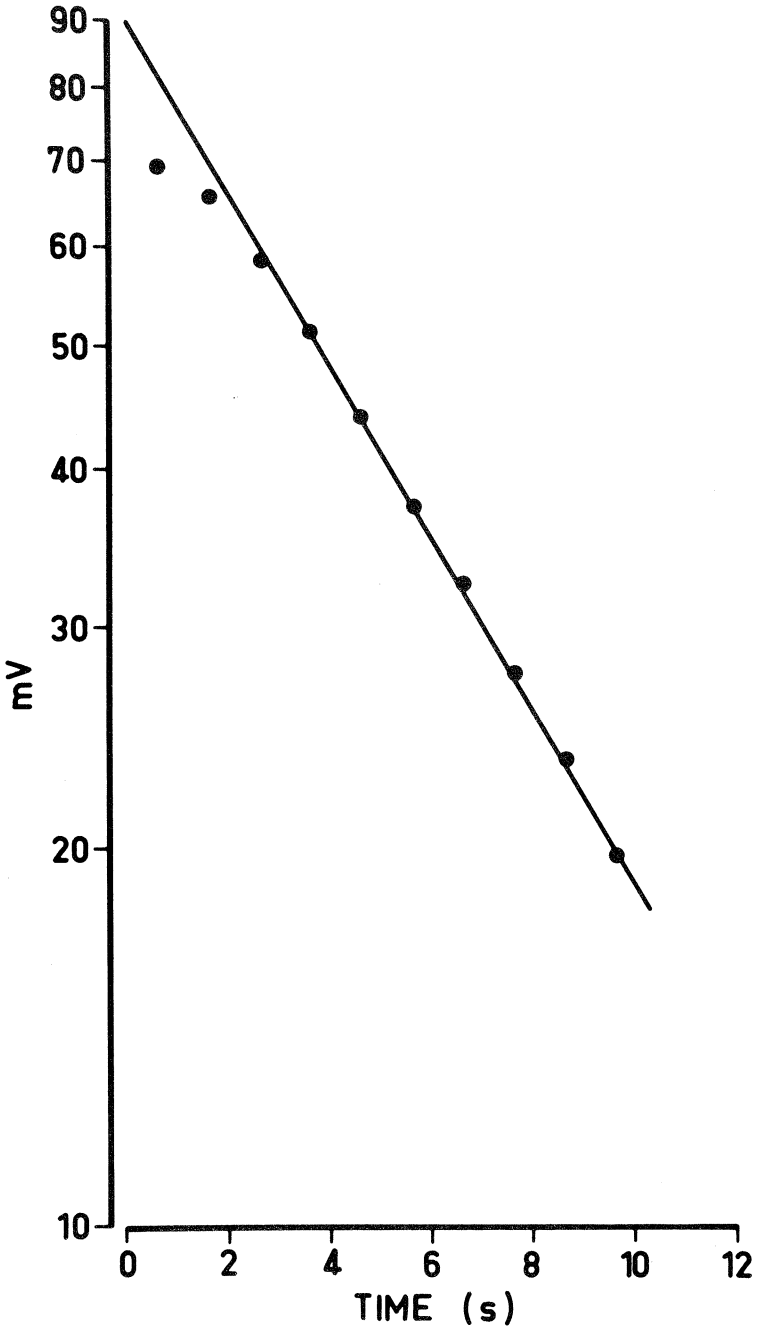

Figure 1. In order to determine the precise thermoelectric output generated by a theoretically instantaneous change in temperature from 0 °C to room temperature (20.45 °C), a logarithmic plot of the thermoelectric output from the thermopile versus time was prepared. Extrapolation back to zero time gives a value of 90.0 mV for the thermoelectric output due to an instantaneous switch from 0 °C to 20.45 °C.

mometer as 20.45 °C), during which time the thermoelectric output was being continuously monitored over the temperature change from 0 °C to a final temperature of 20.45 °C. Both solutions were stirred to prevent any local thermal effects. The thermoelectric decay was plotted logarithmically against time, and then extrapolated back to zero (Figure 1). This enabled a calculation of true thermoelectric output for an instantaneous change in temperature. For this thermopile, a change of 20.45 °C produced an output of 90.0 mV. Its overall sensitivity therefore is 4.40 mV per °C.

Thermopile calibration

Calibration was done by discharging into the muscle preparation a capacitor that had been charged to a known voltage. This method has been used successfully by Aubert [1] and Wendt and Gibbs [11,12], although some early difficulties were reported by Wilkie [13] and Woledge [14]. To verify the accuracy of this method, experiments were devised using artificial "muscles" of differing weights made from 3% agar in Krebs Henseleit solution. The concentrations of the various ionic species in the agar muscles were (in mM): NaCl 118.0, KCl 4.75, $CaCl_2$, KH_2PO_4 1.18, $MgSO_4$ 1.8 and $NaHCO_3$ 28.4. The agar was dissolved in ionic solution at 70 °C, poured into cylindrical perspex moulds and allowed to cool. Once removed, the moulds were cut to produce masses varying between 3.0 and 8.0 mg. The agar "muscles" were attached to the surface of the thermopile by capillary attraction and electrical contact was made by means of two platinum electrodes attached to the thermopile support frame by a miniature hinge. The whole thermopile was sealed in a glass envelope and maintained at 27 °C by submersion in a thermostatically controlled water bath. This novel method circumvented uneven heating and local heat currents resulting from the biological variability and inhomogeniety of real muscle tissue. This enabled a more precise examination of the calibration method. Typically, the capacitor does not discharge completely but contains a residual charge of between 1 to 5%. This is due to polarization between the platinum electrodes and the "muscles". This was overcome by discharging the capacitor through a high speed relay which reversed the discharge polarity every 20 msec. The energy of the capacitor discharge is given by

$$E = 1/2\ CV^2 \qquad (3)$$

where V = charged voltage and C = capacitance in farads. The temperature change of the muscles can be calculated by

$$\Delta T = (Df\ .\ Cs) / (Sn\ .\ G) \qquad (4)$$

where Df = chart deflection (divisions), Cs = chart sensitivity (mV/division), Sn = thermopile sensitivity (mV/°C) and G = amplifier gain. The overall increase in heat (Q) is given by

$$Q = 1/2\ CV^2 = MmCm\Delta T + MpCp\Delta T \qquad (5)$$

where MmCm are the muscle mass and specific heat capacity respectively, and MpCp are the thermopile mass and specific heat capacity respectively. Therefore

$$Q/\Delta T = MmCm + MpCp \qquad (6)$$

A plot of $Q/\Delta T$ vs muscle mass will (theoretically) be linear; MpCp can be calculated from the intercept and the slope will be equal to Cm. Thus the linearity of the above plot will give a direct index of the accuracy of the heat calibration of any thermopile being investigated. The calculation of MpCp is also vital for subsequent use of the thermopile in order to measure the heat output during muscular contraction (refer to equation 10 below).

Myothermic calibration of agar "muscles"

To obtain data to produce the above plot and therefore to ultimately confirm the accuracy of the calibration method, agar "muscles" were used. These were cut to various masses so as to provide a range of datum points for plotting. The total mass of each agar "muscle" never exceeded 15 mg and the length was always less than the active zone of the thermopile. A glass envelope surrounded the thermopile and this was filled with air saturated with water vapor to prevent the agar drying out and changing its heat capacity. The ratio of heat produced to temperature change was calculated for each muscle using two discharge voltages, 100 and 150 volts. Each voltage was discharged five times and any results that failed to agree within 0.5% were rejected.

Calculation of specific heat capacity of skeletal muscle

A prime objective of this work is to estimate accurately the specific heat capacity of mammalian skeletal muscle such that this value may be used (instead of values for other muscle types) for future myothermic investigations.

To convert the temperature changes detected by the thermopile into heat units, an accurate value for the muscle specific heat is necessary. To determine the most appropriate value (in this case for murine skeletal muscle) equation 5 was rewritten as

$$Cm = (1/2\ CV^2 - \Delta T.MpCp) / Mm.\ \Delta T \qquad (7)$$

where $1/2\ CV^2$ = the energy liberated into the muscle by a capacitor discharge, MpCp is the product of the mass and specific heat capacity of the thermopile previously determined as 26.04 ± 1.93 mJ/°C, Mm = mass of the muscle and Cm = specific heat capacity of the muscle. To determine Cm, 5 soleus muscles were removed from the hindlimbs of adult Swiss mice. The animals were killed by cervical dislocation and both hindlimbs removes and bathed in oxygenated Krebs Henseleit solution. The soleus muscles were dissected free and kept under light tension prior to use. The muscles were positioned on the active regions of a Ricchiuti (wire wound) thermopile by attachment with 6/0 silk suture and a series of 10 measurements made using the capacitor discharge method previously described.

Measurement of heat output

In an experimental situation, the capacitor discharge method can be used to determine the proportion of heat output actually measured by the thermopile, after first rendering a muscle inexcitable by either bathing in high potassium solution (100 mM) or electrocution with 100 V pulses [3,11,12]. The increase in heat (Qm) as measured by the thermopile can be calculated from the following formula:

$$Qm = \Delta T\ (MmCm + MpCp + MsCs) \qquad (8)$$

where Mm, Ms and Mp are the masses of the muscle, adhering solution (which must be assessed by blotting and weighing), and the thermopile respectively. ΔT is obtained using equation 4 and an "efficiency factor" for the thermopile can be calculated from

$$F = 1/2\ CV^2 / Qm \qquad (9)$$

Therefore the heat (Q) produced during an experiment with contracting muscle tissue expressed in Joules/g of muscle is

$$Q = (\Delta T - \Delta Ts)\ .\ F[(MmCm)+(MpCp)+(MsCs)]\ /Mm \qquad (10)$$

where ΔTs is the heat produced as a result of the electric stimulation.

Results

Agar "muscles"

The thermoelectric output as a result of capacitor discharge into 12 artificial agar "muscles" cut to the approximate dimensions of mouse hindlimb flexor and extensor muscles is plotted in Figure 2. The standard error of the mean (SEM) was calculated for each different muscle size but was in each case too small to be represented i.e. less than 1.0 millijoules (mJ)/°C. Linear regression analysis yielded a slope (specific heat capacity) of 4.08 ± 0.34 Joules/g/°C and an intercept (MpCp) of 26.04 ± 1.93 mJ/°C for R = 0.99. As the agar pieces were composed of 97.0% water it was anticipated that the value of the specific heat should approximate that of water i.e. 4.184 Joules/g/°C.

Specific heat capacity of skeletal muscle

To obtain the specific heat capacity of skeletal muscle, the specific heat was calculated from equation 7 as 3.73 ± 0.1

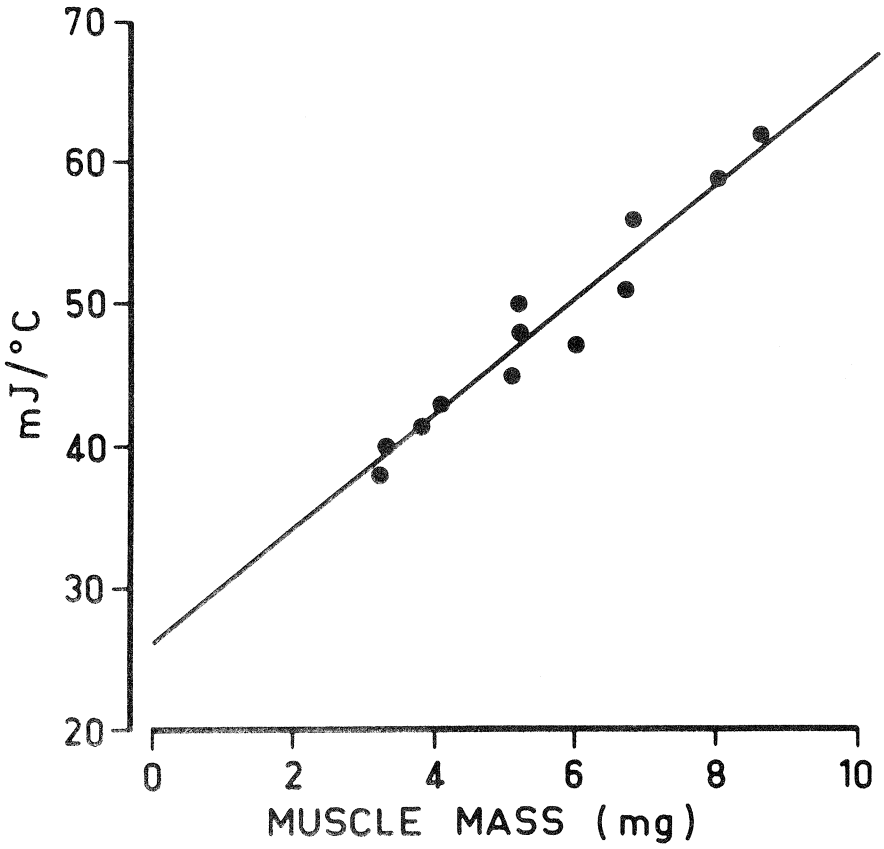

Figure 2. Myothermic calibration using agar "muscles". A series of 12 measurements was made discharging a capacitor into agar "muscles" of different masses. A straight line was fitted by linear regression (r = 0.9367), and produced a slope of 4.08 ± 0.34 Joules/g/°C with an intercept of 26.04 ± 1.93 mJ/°C.The linear relationship confirms the accuracy of the calibration method for the thermopile being examined and enabled the calculation of the product of the mass and specific heat capacity of the thermopile which is necessary for calculating actual muscle heat evolved during experiments with real muscle tissue.

Joules/g/°C ± S.E.M. This compares with a value for amphibian skeletal muscle of 3.684 Joules/g/°C [4].

Discussion

Many other myothermic calibration techniques require that the thermoelectric output of each junction type be known accurately. This, summed over the total number of junctions produced the overall sensitivity of the thermopile [5]. This method cannot be adopted for electroplated thermopiles as the thermoelectric junctions are not uniform. As the agar "muscles" are composed of 97% water, the value of the calculated specific heat capacity should approximate that for water which is 4.1840 Joules/g/°C. As this was indeed the case. The calculated value of 4.08 ± 0.34 Joules/g/°C corresponds closely, and it was concluded that this method of calibration is to within 1% of the thermoelectric output for this type of wire wound thermopile, and that this method is a valid one for use with muscles comparable in dimension and mass to the soleus muscles of the mouse. The specific heat capacity for mammalian skeletal muscle, calculated as 3.73 ± 0.1 Joules/g/°C, is comparable with a value of 3.684 Joules/g/°C for amphibian skeletal muscle that has been used by many researchers for various myothermic calibration techniques [4].

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