Journal of Thermal Analysis, Vol. 30 (1985) 1403—1413

A POOR MAN'S CALORIMETER (PMC) FOR SMALL ANIMALS

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A simple calorimeter is described which consists of a cooling/warming box as used for picnic equipment. The volume of this calorimeter is 8 dm^3, the sensitivity is 19.2 mV/W, and the time constant is 580 s. As such an instrument is designed for animals weighing some 100 g, a signal of 10 to 50 mV can be expected, which can easily be monitored with the usual laboratory recorders. The long-time baseline drift is sufficiently small when the calorimeter is placed in a wooden box with Styropore insulation. Experiments were run for 1 to 15 h with various animals, among them chinese hamsters, hedgehogs, turtles and rats. The price of the box is appr. $ 100.

Although measurements of animal thermogenesis were among the earliest uses of calorimetry (by means of the Laplace/Lavoisier ice calorimeter), only a few calorimetric experiments on middle-sized animals are cited in the literature. There are two reasons for this:

(1) While small animals weighing a few grams can be investigated in the usual batch calorimeters, e.g. of the Calvet type [1–3], and special "whole body calorimeters" have been constructed for men and domestic animals [4–8], no instruments are at hand for animals weighing several hundred grams (e.g. rats, hamsters, guinea-pigs).

(2) From the experiments of Rubner and others [7] at the beginning of this century, it became clear that there is a strong proportionality between oxygen consumption, carbon dioxide production and heat dissipation. As a consequence, the cumbersome "direct" calorimetry was replaced by the easier "indirect" calorimetry and the monitored gas metabolism was converted into terms of heat production.

In recent years it has become well known that there may be considerable differences between the heat productions calculated from the gaseous metabolism (indirect calorimetry) and those measured calorimetrically (direct calorimetry). On the one hand, less dissipating heat is observed than expected from oxygen consumption. Zotin and Lamprecht [9, 10] developed a theory of the "bound dissipation function", and discussed its connection to linear and non-linear irreversible processes and to the ontogenetic and phylogenetic development of organisms, a theory giving rise to vehement controversy [9, 11]. On the other hand, animals such as amphibia can live for prolonged times (minutes to hours) under anaerobic conditions, obtaining energy from the anaerobic glycolysis of sugars (down to lactate) [12—14]. In these cases, indirect calorimetric data are useless, because far more heat is dissipated from the animal than can be expected by theoretical calculation. Only direct calorimetry renders a true picture of the momentary metabolic turnover.

Indirect calorimetry on smaller animals is easily performed with face masks [15] or by enclosing the whole animal in a gas-tight box [16]. In both cases a stream of air passes through the set-up and the difference between the oxygen and carbon dioxide concentrations of the inflow and outflow are monitored.

The respiratory quotient RQ, the ratio of carbon dioxide production to oxygen consumption, gives information about the substrates metabolized by the animals. On this basis, the heat dissipation can be calculated which theoretically amounts to 21 J/ml O_2 during the oxidation of carbohydrates.

With direct calorimetry, difficulties arise due to the thermal insulation, the long-time stability of the instrumental base-line, and thermal disturbances by evaporating heat loss and air flow.

If larger animals are investigated, a mean heat production of several mW/g can be expected and the net production is close to 1 W, so that with the usual calorimetric sensitivities signals up to 100 mV will be obtained. Further, the basal metabolism is small relative to the metabolic level of active motion, so that strong fluctuations in the heat dissipation occur due to changing periods of resting and activity. Compared with these figures, some of the physical disturbances mentioned above are small and can be neglected and even a simple calorimetric set-up may be helpful in the evaluation of energetic data from smaller animals.

In this paper we report on the application of a simple cooling/warming box as a small animal calorimeter and its equipment with additional sensors and a mini-computer.

Experimental

Instrument

The applied calorimeter is a cooling box (type "Sunny Cool" Universal-Camping-Box; Nr. 010 185; Quelle International/Fuerth, West Germany) which normally runs with a power supply of 12 V, e.g. a car battery (Fig. 1). Its main part is a Peltier element at the bottom of the box, carrying a metallic cross with four arms running up the four sides of the box. According to the polarity of the DC current, the Peltier element cools down or heats up. The walls and the cover of the box are made from plastic, the cover carrying an O-ring so that the box can be closed airtight after some modifications.

In order to use the box as a calorimeter, the Peltier element serves as a heat flux sensor by means of the Seebeck effect. Typical data for the heat conductance λ and thermoelectric voltage α of the Peltier elements at room temperature are 0.2 W/K and 14 mV/K, respectively. By Fourier's law of heat conductance, one may expect approximately 70 mV at a heat flow of 1 W through the element. Since not all the heat produced in the calorimeter is dissipated in this way, the sensitivity should be considerably smaller.

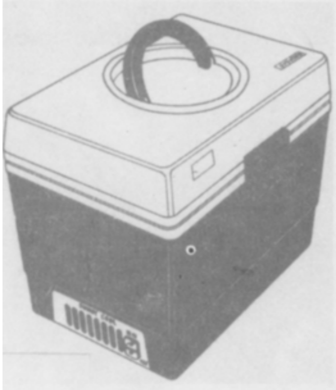

Fig. 1 Sketch of the Sunny Cool box which was used as a PMC calorimeter

The available space within the box is 8 dm^3, so that smaller animals such as rats, guinea-pigs or hamsters can be housed over several hours without any problems. With typical oxygen consumption rates of less than 2 ml/h.g wet weight, the oxygen concentration decrease is slow enough to be harmless to the animals and to have no influence on their metabolism.

As there is no active temperature regulation in the calorimeter, it runs at ambient temperature, i.e. possibly from +5° (cool room) up to 40° (incubation room). This is just the physiologically interesting range of temperature for the energetic metabolism of animals.

If the room temperature is not controlled within special limits, more or less pronounced fluctuations appear in the base-line of the calorimetric output. An easy way to minimize these is to put the calorimeter into a wooden box internally insulated with plates of Styropore (20 to 30 mm thick). The fluctuations then become smaller than 5% at a recording sensitivity of 10 mV.

With this arrangement, the temperature of the direct surroundings of the calorimeter is kept constant and the calorimeter should be considered to be an isoperibolic heat flow instrument according to the classification by Hemminger and Hoehne [17].

Sensitivity

The sensitivity of the calorimeter was determined by Joule heating. A resistor of approx. 1500 Ω, glued into the hole of a small metallic block, was placed on the bottom of the box or on top of a perpex cylinder so that it was situated in the middle of the box. Figure 2 shows the dependence of the electric signal upon the position of the heater. Only if the heater is situated on the bottom is there a linear relationship between power input and voltage signal rendering a sensitivity of

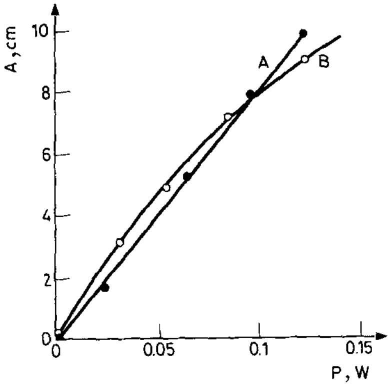

Fig. 2 Dependence of the thermoelectric signal upon the power input to the calorimeter. *A:* heating block directly at the bottom of the instrument; *B:* heating block on a perpex cylinder in the center of the instrument

$$\varepsilon = 19.2 \pm 0.3 \text{ mV/W}$$

Due to the construction of the cooling device, different positions on the bottom of the box were expected to render different sensitivities. However, within the limits of error, all points were of equal sensitivity, in contrast to the time constants, which varied considerably.

Time constants

An important parameter of every calorimeter is its time constant(s), because this is responsible for the deformation of the original heat signal by the different diffusion modes in the instrument. Only with slowly changing heat production rates and small time constants does the electric signal give a true picture of the energy metabolism of the animal under research. As locomotive activities are of short duration, the time constant is of considerable influence. Again, it may be neglected if, with integration over a longer experimental period, only the total heat output of the animal is determined.

The time constant of the instrument is a function of the heat capacity and the thermal conductivity of the calorimeter [18]. Mathematical derivation leads to the well-known equation of Tian [1, 2]. This shows that the actual heat production rate is proportional to the sum of the electric signal and the product of its derivative times the time constant. It expresses the dynamic response of the instrument to heat production.

If not only the heat diffusion through the flux-meter but also that from the heat source to the calorimeter has to be taken into account, a second time constant comes into play and the second derivative of the signal is included in the expanded Tian equation [18].

To evaluate the dynamic behaviour of the calorimeter, a special curve-fitting program with two exponential terms (i.e. two time constants) was developed. A heat pulse of 20 s was applied, and the response was stored on-line on a computer and then processed by a gradient procedure to find the minimum deviation between the experimental points and the fitted curve. In a typical run the minimum was obtained after 8 to 10 iterations and the two time constants were printed out. In no case was the difference between the experimental and the theoretical curves larger than 2%. Figure 3 shows such a run and the fitting by the computer.

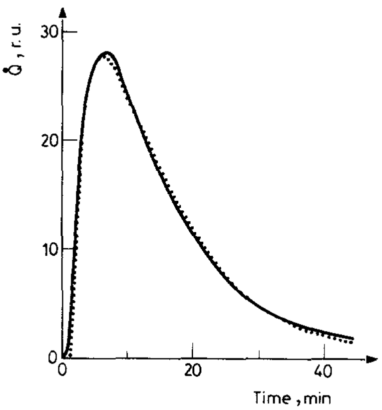

Fig. 3 Experimentally obtained calibration curve (solid line) and mathematical fitting (dotted line) as described in the text

For the determination of the time constants of this calorimeter, the bottom was divided into 3 lines of 5 fields, each being symmetrically arranged to the centre. Due to its shortest distance to the Peltier element, this point should show the smallest time constant. This was confirmed in the experiments by a time constant of 460 s.

Table 1 Characteristic figures of the PMC Calorimeter

Type	single system	
Detector	heat flow	
Method of operation	isoperibolic	
Volume	8000	ml
Temperature range	5 to 40	°C
Sensitivity	19.2	mV/W
Time constant 1	581	s
Time constant 2	154	s
Price	100	$

Along the central line it was 488 ± 27 s, and in the corners it was 635 ± 43 s. Taking the mean of all points renders a first time constant of

$$\tau_1 = 581 \pm 81 \text{ s}$$

The second time constant should depend only upon the configuration of the heat source, and no longer on the geometry of the calorimeter. Therefore, approximately the same value was expected at every position in the calorimeter. Nevertheless, a smaller value was obtained along the axis than in the corners or in the other positions. As the experimental deviations were rather large, only one common second time constant is given:

$$\tau_2 = 154 \pm 96 \text{ s}$$

For the following corrections of the calorimetrically obtained power—time curves, only the first time constant was used as a specific parameter of the instrument.

Results

The calorimeter was tested with several small animals, such as guinea-pigs, hamsters, water turtles and hedgehogs. Three examples of these investigations will be given.

A male gold hamster (Mesocricetus auratus), weighing 125 g was placed in a standard metallic breeding cage, which just fitted into the working space of the calorimeter. The bottom was covered with litter as usual, and sometimes an open glass dish containing silica gel for the absorption of moisture was placed beside the cage. Several runs were performed during day and night (Fig. 4). The results are

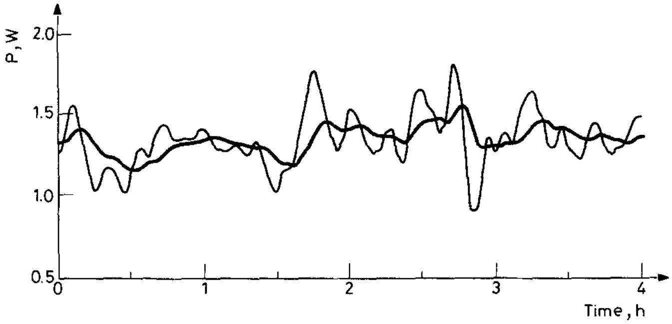

Fig. 4 Experimental (strong line) and time-corrected (thin line) power-time curve of a male hamster during daytime (experiment no. 5)

Table 2 Heat production of a Hamster (Mesocricetus auratus)

Exp.No.	Temp., °C	Time	Durat., h	Q, kJ	dQ/dt, W	q_{mean}, mW/g	q_{max}, mW/g	q_{min}, mW/g
1	23	d	9.83	37.19	1.05	8.40	10.7	7.56
2	23.5	d	10.00	36.97	1.03	8.22	10.5	7.90
3	21.5	n	11.95	48.33	1.12	8.99	11.8	6.76
4	22.5	n	12.50	44.49	0.99	7.91	9.9	6.31
5	22.5	d	9.83	42.89	1.21	9.70	11.3	8.15
6	23.5	n	14.83	48.57	0.91	7.28	9.7	5.84
mean					1.05	8.42	10.7	7.09
SD					0.10	0.84	0.80	0.93

given in Table 2. The obtained data coincide well with data from the literature: $dq/dt = 8.78$ cal/h/g = 10.2 mW/g for an adult hamster (Ochrotymus nuttali) [19]. The experiments seemed not to stress the animals (observation at the end of the experiment; there were no strong fluctuations in the power—time curve). Calculation of the oxygen consumption during these prolonged runs yields a value of appr. 230 ml O_2/h, which corresponds to 7.2 h for total consumption of the oxygen. As there was no significant change in heat production during the whole span of the investigation, oxygen in the calorimeter must have been replaced by diffusion.

In another experiment, a young hedgehog Erinaceus europaeus) was used to test the calorimeter. This animal had to be kept in the house during winter as its weight (170 g) was too low for outside hibernation. On several occasions it was put into the calorimeter on a thin layer of paper like that in its usual box at home. The hedgehog was sometimes active during the experiment, but it was normally sleeping when the calorimeter was opened after 1 hour of measurement. The data collected in this manner are compiled in Table 3. As the hedgehog was not adult but still growing, the specific heat productions decreased, in accordance with the theory [10].

Fig. 5 Experimental power-time curve of a water turtle in 3 liters of water during daytime

Table 3 Heat production of a young, growing hedgehog (Erinaceus europaeus)

Exp. No.	Temp., °C	Time	Weight, g	dQ/dt, W	q_{mean}, mW/g	q_{max}, mW/g
1	23	n	170	1.31	7.71	10.5
2	23	n	230	1.70	7.39	7.81
3	23	n	290	1.46	5.02	5.36

In a few runs an adult water turtle (Pseudemys scripta elegans) was investigated in the calorimeter partly filled with 3 dm^3 of water. The aim of these experiments was to test the response of the instrument when the heat capacity increased due to the experimental conditions. Figure 5 exhibits a power *vs.* time curve of a turtle at 20.5° during daytime. The slope is even richer in structures than in the case of the hamster. This is probably due to the agitation of the water by the animal.

Discussion

A calorimeter has been introduced which is extremely cheap, and easy to handle, but nevertheless sensitive enough for all types of experiments on smaller animals. The whole set-up is commercially available for appr. $100 and is ready for operation when a usual laboratory recorder is at hand.

The sensitivity of 19.2 mV/W of this simple construction is surprisingly high compared with that of sophisticated expensive instruments (appr. 60 mV/W). As the heat output of the animals in question is at least about 0.5 W, the signal of the calorimeter is 10 to 100 mV, which can easily be recorded.

The base-line stability is brought to a value better than 0.5% full-scale deflection by insulating the calorimeter in a wooden box with Styropore plates along the inside walls. As no temperature regulation is used and therefore no electric supply is needed, it is well suited for field studies (or even expeditions) provided that the obtained data are directly monitored on a battery-driven recorder or on a tape, or—a super poor man's calorimeter (SPMC)—registered every minute by hand from a voltmeter.

One disadvantage of the instrument—in common with all calorimeters of larger volume—is the large time constant, which smears the signal and cancels out short time peaks. As these are not likely to occur with larger animals, except for sudden locomotive activities, the time constant mainly influences the length of time to the final establishment of an approximately constant signal. By a subsequent computer processing of the rough data, the original structures can be recovered. Moreover, the length of the measurement can be considerably shortened, thereby lowering the stress for the animals under investigation. The ideal solution for laboratory experiments would be the on-line coupling of the calorimeter to a computer. As the deconvolution program after the Tian equation is really simple, a small personal computer would be sufficient provided that an AC/DC converter at the input and a DC/AC converter at the output to the recorder are available. Such a system should be realizable for less than $400. Besides the temporal signal processing, the computer can be used to integrate the heat output over time and determine the mean heat dissipation of the animal, which is the real parameter of interest.

The calorimeter can easily be coupled to other monitoring systems. If it is ensured that the cover of the instrument is air-tightly connected to its body, oxygen consumption rates can be measured with an oxygen electrode. In this manner, direct and indirect calorimetry are combined, rendering more information on the metabolism than the mere sum of them [3]. Under these circumstances it is necessary to limit the experiment in time, so that no critical oxygen concentrations arise in the system.

It is well known that animals show different activities and hence different metabolic levels when living in the dark or in light. When determining both levels of heat production, it is necessary to illuminate the calorimeter. The easiest way to do so is to install a cold light source outside and couple flexible optical fibres into the instrument. The additional heat transported to the system is so small that it can be neglected in the thermal output. Nevertheless, the illumination is strong enough to provoke or cancel the activities of the experimental animal. Such investigations were run with hamsters and with turtles.

Another advantage of the calorimeter is its larger size, so that populations of smaller mammals such as mice, for instance, can be investigated. Questions may be answered as to the heat production of a single animal or several animals in a group, or as to crowding effects.

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Zusammenfassung — Es wird ein einfaches Kalorimeter beschrieben, das aus einer Kühl/Wärme-Box für Campingausrüstungen besteht. Das Volumen dieses Kalorimeters beträgt 8 l, die Empfindlichkeit 19,2 mV/Watt und die Zeitkonstante 580 Sekunden. Da solch ein Instrument für Tiere mit einigen 100 g Lebendgewicht bestimmt ist, kann ein Signal von 10–50 mV erwartet und von den in Laboratorien allgemein verwendeten Schreibern aufgezeichnet werden. Die Langzeitdrift der Grundlinie ist genügend klein, wenn das Kalorimeter in einer Holzkiste mit Styropor-Isolierung untergebracht ist. Experimente wurden über Zeiträume von 1–15 Stunden mit verschiedenen Tieren ausgeführt, darunter Goldhamster, Igel, und Ratten. Der Preis der Box beträgt etwa 100 $.

Резюме — Описан простой калориметр, состоящий из бытовой коробки нагрева-охлаждения. объём такого калориметра достигает 8 л, чувствительность составляет 19,2 мВ/ватт и постоянная времени — 580 сек. Значение сигнала этой аппаратуры, предназначенной для животных с весом до 100 г, составляет 10–50 мВ и легко записывается обычными лабораторными самописцами. Дрейф нулевой линии достаточно мал, когда калориметр помещен в деревянный ящик с теплоизоляцией. Проведены опыты длительностью от 1 до 15 часов с такими животными, как китайские хомяки, ежи, черепахи и крысы. Стоимость калориметра составляет приблизительно 100 долларов.