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Dedicated to Prof. Antonius Kettrup on the occasion of his 60th birthday

DIRECT CAROUSEL FLIGHT CALORIMETER FOR METABOLIC INVESTIGATIONS OF SMALL INSECTS

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Abstract

An isoperibolic heat flow calorimeter is described for the determination of heat production rates during the tethered flight of small insects such as flies, honeybees or hornets. The insects are fixed with their thoraces to one arm of a low-friction carousel. A sensor counts the number of revolutions per time and determines the speed of flight. Wing sound is monitored by a microphone with an audio recorder, so that wing beat frequencies and hence locomotor activities can be determined. Different illumination means are incorporated to guarantee the illumination levels necessary for flight.

Keywords: direct calorimetry, energy metabolism, flight, illumination, insects

Introduction

The energy metabolism of living organisms is of the utmost interest for physiologists. It even stood as godfather at the cradle of calorimetry [1]. While the standard or resting metabolism of an animal is easy to define and to determine, states of activity vary considerably depending on the external parameters. For many animals, the highest level of activity that limits the metabolic scope is approximately 10 times higher than the resting metabolism [2]. Exceptions are flying organisms and especially insects. Under the conditions of flight, the metabolic rate is more than 20-fold for honeybees, for example [3–6]. The highest energy turnover rates are observed during the hovering flights of humming birds and some insects (e.g. moths), since no support is then given by the surrounding medium, in contrast with the situation during running, swimming or forward flight. Under such circumstances, the rates are increased up to 100-fold [2, 7].

The difficulties involved in determinations of the heat output during activity states arise from different sources. Such states often appear irregularly, in the form of bursts, without a steady state for an exact evaluation. The animals have

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to be kept motivated [8] to perform special tasks with a high turnover. This may be attained by long training, as for investigations on a treadmill, for instance, or by continuously stimulating them by mechanical or electrical irritation, but this may lead to artificial conditions [3]. Rewards can be a means to overcome these problems. In the special case of insect flight, it is often difficult to initiate flight and keep it going [9], although many insects are excellent flyers over long distances or long times [6].

The energy turnover during rest or activity can be determined by different methods. These are often classified as 'direct' and 'indirect' calorimetry methods. Direct calorimetry applies isothermal/isoperibol heat flow calorimeters in most cases, with a few exceptions of the use of quasi-adiabatic or true adiabatic instruments, while the spectrum of indirect approaches is very broad. The most common procedure is to determine oxygen consumption and carbon dioxide production. These are monitored by the classical manometric Warburg method [5], by paramagnetic or optical sensors and gas-specific electrodes. Their ratio, the respiratory quotient RQ, provides information about the substrate consumed during the metabolism. A value of RQ=1.00, RQ=0.83 or RQ=0.71 indicates that carbohydrates, proteins or fats, respectively, are utilized as fuel. The fat metabolism, with a heat output of up to 39 kJ g^{-1}, affords the highest amount of energy per gram and is thus observed particularly in long-distance flyers or hovering insects (e.g. moths [10]). Carbohydrates and proteins provide only 16 and 13 kJ g^{-1} respectively, i.e. less than 40% of the level for fat. A carbohydrate metabolism with an RQ=1.00 is typical for honeybees; RQs between 0.71 and 1.00 are characteristic for hornets [unpublished data].

Other methods of indirect calorimetry include the uptake and consumption of radioactively labeled compounds, very often ^{14}C glucose [11], the determination of metabolites in tissue and/or blood/hemolymph [10], or the measurement of heart beat rates [12]. A typical approach with insects is 'exhaustion flight' [5, 11], which is readily applicable when the type of metabolism and the kind of energy substrate are known. An insect is kept flying, e.g. tethered to a carousel, until all of its energy resources ('fuels') are consumed. It is then fed with a known amount of energy in the form of a glucose or mixed glucose/protein solution and stimulated to fly again until exhausted. This can be done so effectively that, for example, honeybees will die within a few minutes if they are not fed again immediately [13]. The amount of energy provided divided by the flight duration yields the energy consumption rate and hence the flight metabolism.

An even more indirect physical method is the 'grab-and-stab' procedure often applied to measure the energy metabolism of insect flight (see e.g. [14, 15]). An insect is caught during flight, and within a few seconds a very thin thermocouple (installed inside a hypodermic needle) is implanted into its thorax, the main site of heat production. The temperature differences between the thorax and the ambient air are recorded, and the heat loss is calculated from cooling curves evaluated via the heating-up of dead specimens [14]. A more sophisticated approach in which there is no direct contact works with infrared thermocameras to determine the heat distribution in the bodies of flying insects [16, 17].

It was briefly mentioned above that insects need stimulation to fly. Light plays an important role in this. There are species that nearly never fly in the dark under natural conditions (e.g. most honeybees), whereas others (e.g. hornets) fly by both day and night, even at very low illumination levels [personal observations]. Yet others fly exclusively in the dark (e.g. moths). Moreover, tethered insects have to receive optical information [9], such as a changing horizon or surface below them, simulating flight motion. A further impulse to fly is given by the tarsal reflex, when a piece of matter between the legs is suddenly removed. To keep insects flying requires a mechanical sensation of their efforts, so that a draught is sometimes more important than the optical information flux [9]. An 'intrinsic' stimulus to fly is given by the personal motivation of the insect [8, 9, 18]. Foraging bees caught at departure from their hive are better suited for investigations than those returning from foraging or those with hive activities.

Metabolic flight experiments are performed in several different ways: (i) free, (ii) tethered or (iii) fixed flight. Free flight investigations use the 'grab-and-stab' method plus thermometry [14, 15, 19], or enclose the animals in large jars [14], where they are trained to fly for several minutes without touching the wall [8]. The decrease in oxygen concentration and/or the increase in carbon dioxide are monitored as functions of time. Many experiments have been performed with insects flying in a temperature-controlled room and tethered by a thermocouple [19], fastened to a carousel and connected with a thermocouple to a recorder [14], or placed in a large vessel for manometric determinations [7, 20]. Highly sophisticated experiments have been performed by Nachtigall and his group on tethered animals in a windtunnel [21–25], these providing several physiological parameters of the insect simultaneously. Tethering is always performed by glueing a selected support to the thorax. Fixed flights are run without any spatial movement of the animal, while the other parameters remain as described above.

When we started to construct the carousel flight calorimeter, we had in mind all the earlier forms of investigations mentioned above. Since the flight energetics of some insects are strongly dependent upon temperature (honeybees never fly below 10°C or above 43°C, for example), the working temperature of the new instrument had to cover the physiological range between 10 and 45°C. This was attained with an air bath thermostat surrounding the calorimeter proper. To prove the influence of light and to determine the dependence of the flight speed on the illumination level, different kinds of illumination had to be incorporated. Determinations of flight speed by electric/mechanical counting of the number of revolutions of the carousel per minute and determinations of the activity level of the animal by acoustical monitoring of the wing beat frequencies are performed simultaneously.

Set-up of the calorimeter

Calorimetric cabinet and thermostats

The whole calorimetric set-up is placed in a climatized cabinet, which works in a temperature range down to about 15°C, so that experimental conditions above 17°C can be obtained. For special investigations on insects flying during cold days at winter temperatures, the location can be changed to a cold room at 6 to 8°C, the working range being extended in this way by more than 10°C.

The calorimeter proper is housed in an air bath thermostat operating at between 18 and 42°C in its usual mode. It originates from an LKB flow calorimeter (type 10700, LKB, Bromma/Sweden). Its inner volume of 380×300×400 mm, or 46 L, determines the size of the calorimeter. The air bath is connected to a cooling pre-thermostat (type D1, Haake, Berlin/Germany).

Carousel calorimeter

The calorimeter consists of an inner cylindrical aluminum container 180 mm in diameter and 110 mm in height, with a 10 mm wall. It is connected along its periphery via 8 vertical, and at its bottom via 2 horizontal Peltier heat flux sensors to an outer square-based aluminum container measuring 235×235×167 mm with walls 12 mm thick. The top of the cylinder is closed by a corresponding aluminum or perspex lid without Peltier elements. The heat sensors measuring 40×40×4 mm (type TEC1-12705) are connected in series and transmit their heat signals to a chart recorder (typical setting 10 mV and 0.6 cm min^{-1}) or to a computer for registration and further evaluation. The outer cube, with a mass of about 10 kg, serves as the heat sink during measurements; it is insulated from the air bath by a 20 mm layer of styropor and a final 1 mm aluminum shield.

A calibration resistance of 1003 Ω is placed along the central support of the carousel and connected to an outside constant power supply. If necessary, before and after each experiment, a short ballistic calibration may be performed to check the sensitivity and time constant of the instrument. In most cases, the rather long time constant is of no importance, since steady-state values during flight are used for energetic calculations only.

Carousel

The heart of the calorimeter is a central two-armed low-friction carousel. Its radius of 62 mm leaves 28 mm available for the wings of the flying insect, so that it never touches a wall or any other obstacle. The distance flown per turn is 390 mm. Two sharp ended needles, one below and one above the arms, support the carousel in two ball-shaped, adjustable-bottomed glass tubes, affording an optimum between minimum friction and maximum slide. The insect is fixed to one arm of the carousel by means of a small plastic tube (see below). The central support of the arms activates an inductive proximity switch, which counts the number of revolutions per minute mechanically and electrically, and hence the speed of flight. For continuous registration of the speed, the electronic signals are converted to pulses of equal height and length but varying frequency, and subsequently integrated, yielding a voltage proportional to the insect's speed.

Illumination

Since some insects (e.g. most honeybee species) do not fly in the dark, the inside of the calorimeter is illuminated via the eight arms of a bundle of light guides ending just 30 mm above the bottom of the calorimeter. This is approximately the height at which insects fly. The bundle of light guides is connected to a cold light source (Cold light projector type 81482, Karl Storz/Tuttlingen, Germany) through an infrared absorbing filter to minimize the heat input into the calorimeter. At the maximum intensity of 150 W of the light source, a calorimeter signal shift of –0.17 mV is observed, corresponding to –2.7 mW, while the heat output of flying hornets amounts to about 50 to 100 mW. If the maximum illumination of around 120 lx is still too low to allow for the flight of insects, one or two fluorescence 'snap lights' 5 mm in diameter and 38 mm in length (type 05595, snap light) are placed on the bottom of the calorimeter, providing an additional illumination of 50 lx. These commercial lights for nocturnal fishing produce a negligible heat flow of $P<0.1$ mV. They have a lifetime of more than 24 h, with an initial drop in intensity and afterwards a long plateau phase.

Some experiments indicated that such illumination levels are still too low to initiate and sustain flight activities. Therefore, two light guides 5 mm in diameter, connected to a cold light source (Intralux 150 h, Volpi/Schlieren, Switzerland), were placed vertically through the air bath, the calorimeter insulation and the aluminum lid, up to a few millimeters above the perspex cover of the inside. Under these conditions, the illumination was increased to 300 lx, combined with an additional heat input of +5.2 mW.

Preparation of the insects for flight

The insect under investigation is fixed to the carousel by means of a small plastic screw (diameter 2.8 mm and length 100 mg for hornets; diameter 1.7 mm and length 10 mm for honeybees) glued to the thorax. This screw is inserted into a rubber tube attached to the active arm of the carousel. The lengths of the screw and tube are prepared in such a way that the wings of the insect never touch the suspension above it or the walls, to avoid any irritation during flight.

Mass determination

Before and after flight, the insects are weighed to the nearest 0.1 mg by means of a mechanical balance (type 414, Sauter/Ebingen, Germany). This is an approach to determine the fuel consumption during the experiment and to compare it with the calorimetric energy output. Any simultaneous water loss by evaporation has to be taken into account.

Registration, observation and evaluation

All electrical signals from the experiment are led to a PC with an appropriate registration program. The calorimetric signal of several mV is amplified 1000-fold by a DC amplifier (type C3050C, Knick/Berlin, Germany) to fit into the registration level of the computer. The flight speed and gas sensor signals can be monitored directly without further amplification. Parallel to the PC, a two-channel recorder is used to register the heat production rate and the flight speed, to give an instantaneous and complete overview of the experiment. Further, a counter shows the total number of revolutions, and provides qualitative information about the speed through the sequence of its acoustic 'clicks'.

A condenser microphone (Sony ECM-144, Japan) is incorporated into the calorimeter for the simultaneous perception and recording (Sony, Digital Audio Tape-Corder TCD-D8, Japan) of sound generation during flight, a later determination of the wing beat frequency, and hence the flight intensity. The audiograms are decoded by means of computer software (AVI Soft) for sound frequency analysis. These values can be compared with similar data reported by other authors, and with their indirect calorimetric determinations of flight metabolism (see Introduction).

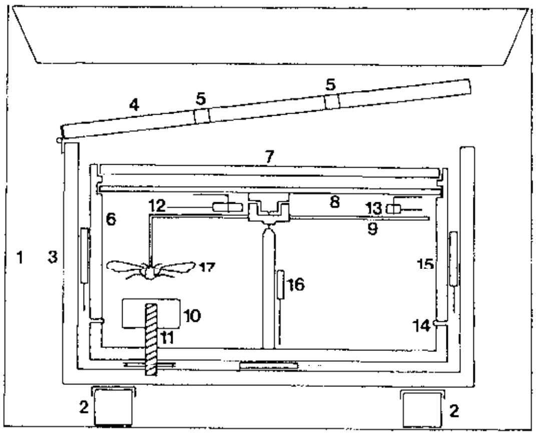

Fig. 1 Schematic sketch of the calorimetric set-up (not to scale!). 1 – LKB 10700 air bath with insulating calorimetric support (2); 3 – cubic aluminum heat sink with turnable lid (4) and two holes (5) for external light guides; 6 – cylindrical calorimeter wall; 7 – calorimetric lid made from metal or perspex; 8 – carousel support; 9 – flight carousel; 10 – starting table with lowering screw (11); 12 – inductive proximity switch; 13 – microphone; 14 – internal light guides; 15 – Peltier elements in series; 16 – calibration heater; 17 – insect

Results and discussion

Figure 1 shows an out-of-scale sketch of the complete calorimetric set-up with the thermostated air bath, the calorimeter proper and the different accessories. Figures 2 to 4 illustrate the mechanical parts of the calorimeter and the manner of their assembly. The most important device is the carousel, which has such a low friction that all tested insects were easily able to start its movement and keep it moving (if they were motivated to fly at all in the calorimeter). It is known from the literature, from personal communications and from our own experience with a windtunnel that only 30 to 50% animals undertake to fly on carousels or in windtunnels.

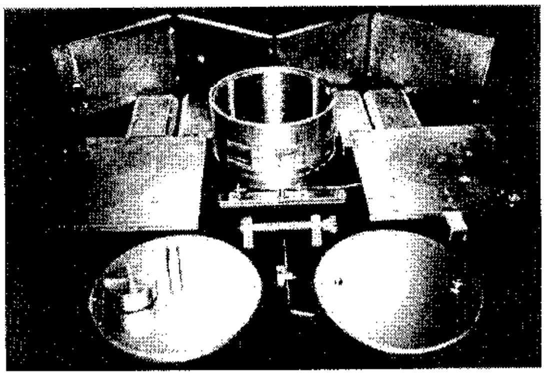

Fig. 2 Disassembled aluminum parts of the calorimeter with the cylindrical calorimeter proper in the center and its bottom (front left with carousel support and table) and lid (front right), the carousel (front center) and its upper support (in front of the cylinder), with microphone, support and inductive proximity switch (from left to right)

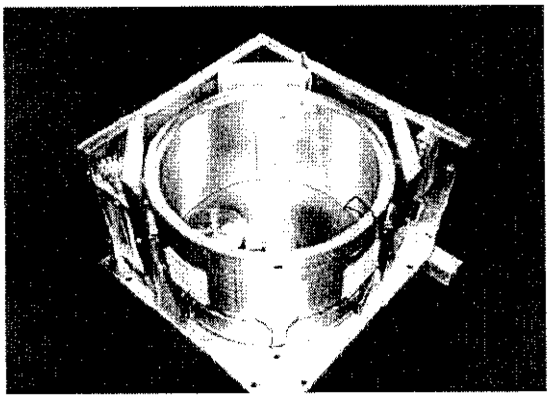

Fig. 3 Half-assembled calorimeter, showing three white Peltier elements in front, the carousel support in the center, and the starting table with its lowering device (left)

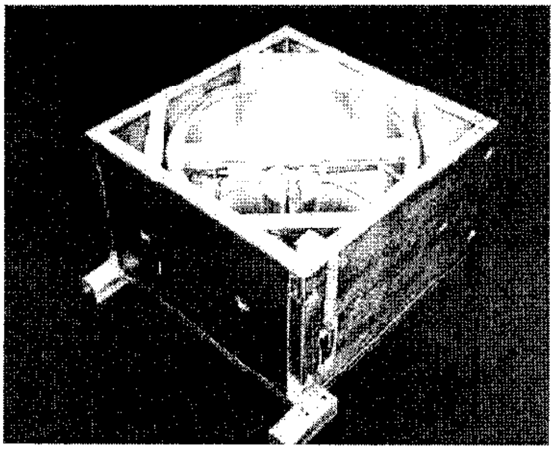

Fig. 4 Completely assembled calorimeter without lids, showing the carousel support with microphone (left) and inductive proximity switch (right), the carousel and the starting table (behind)

Calibration runs with the instrument yielded a sensitivity of 63.6 mV W^{-1} and a time constant of 14.3 min. This relatively slow response is without importance, since only longer-lasting steady states of flight are of interest, which are easily observed via the flight speed. Short-time actions play no role in these investigations. Moreover, the application of a Fourier transform of the calorimetric signal (see e.g. [26]) or of the Tian equation with one or two time constants [27] may afford pictures of the true power-time curves of the flying animal. The sensitivity is high enough, and corresponds to that of modern heat flow instruments. With power outputs in the range 50 to 100 mW during flight, signals up to 10 mV are typical. They are directly monitored on a two-channel recorder or fed after preamplification to a computer for registration and further evaluation. The most important characteristic data of the instrument are compiled in Table 1.

The carousel flight calorimeter has so far been successfully tested with hornets (*Vespa crabro*). Table 2 shows the first results of these investigations. All hornets had been caught from relocated nests, which were placed in artificial wooden nest boxes (for details, see [28]). The heat production rates of hornet drones and workers show no significant differences. This also holds true for the mean values of mass specific heat production rates and body mas (Mann-Whitney U-test, $\alpha=0.05$). The findings are confirmed by rather high values for standard deviations. The scatter in the heat production rates may be due to different motivational states in the hornets. The highest heat production rate of drones at 30°C was 253 mW g^{-1}, as compared with 291.2 mW g^{-1} for workers. Depending upon the insect flying, different light conditions had to be guaranteed to sustain flight, and various tricks had to be applied to start flying. For hornets, the easiest way was to fix the insect on the carousel under full outside illumination, admin-

Table 1 Characteristic data of the calorimeter

External dimensions	235×235×167 mm (L×B×H)
Internal dimensions	180×110 mm (Ø×H)
Internal volume	2800 cm^3
Mass of the whole calorimeter	10234 g
Thickness of the outer wall	12 mm
Thickness of the internal cylinder	10 mm
Arm length of the carousel	62 mm
Number of Peltier elements	8 at the walls 2 at the bottom
Type of Peltier elements	TECI-12705
Calibration heater	1003 Ω
Sensitivity of the calorimeter	63.6 mV W^{-1}
Time constant of the calorimeter	14.3 min

Table 2 Heat production rates of flying hornets at 30°C

Caste	P/mW	M/mg	p/mW g^{-1}	n
Drone	64.4±17.7	535.7±132.0	128.2±51.1	20
Worker	63.5±24.6	401.6±75.6	162.8±68.9	13

P = heat production rate, M = body mass, p = mass specific heat production rate, n = number of experiments. '±' values are standard deviations

ister the tarsal reflex to induce flight, and then close the calorimeter. With this procedure, the time of thermal equilibration fell in the flying period and accordingly was not a pre-flight period, as intended. Energetic evaluation was started when a steady state of heat output was obtained after about 15 min at 30°C. Flight times often exceeded 1 h, so that this lost registration time did not play a significant role, but further experiments are planned to achieve an improvement of the performance.

Figures 5a and b present two typical flight curves of male hornets weighing 620.6 and 537.7 mg, in periods of slowly increasing or decreasing speed. In these experiments, the speed was determined by mechanically counting the number of revolutions per time. The graphs show merely part of the full experiments, which lasted longer than the indicated times. Before and after the run, the baseline was established; it remained unchanged during this period. Unfortunately, an indirect estimation of the metabolism by means of the mass loss, and thus the consumption of the food substrate was impossible. A large proportion of the mass loss during the experimental period was due to defecation, which became visible in

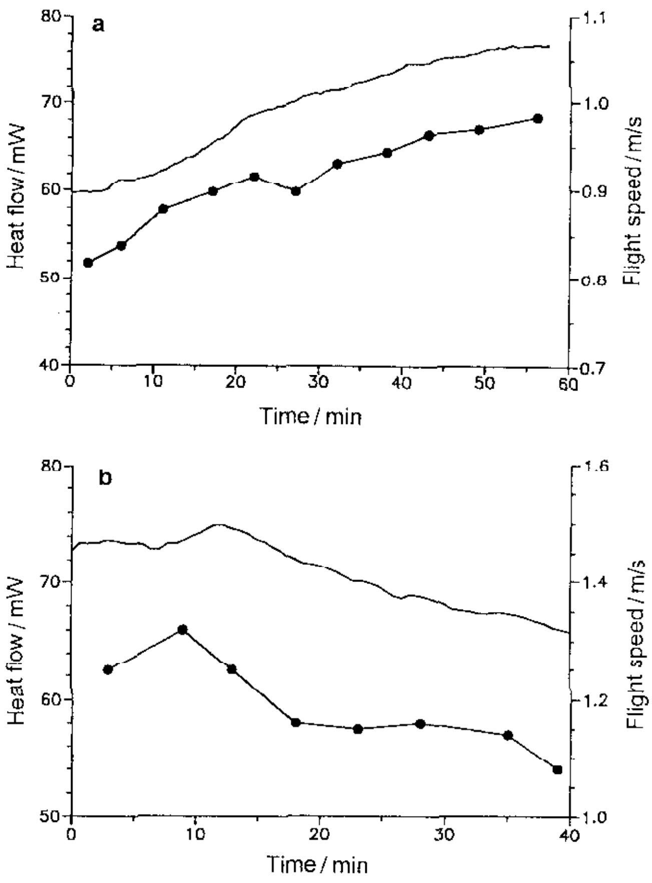

Fig. 5 Two carousel registrations with increasing (a) and decreasing (b) flight speed. The solid lines represent the heat flows, and the dotted lines the mechanically determined flight speeds of two hornets weighing 620.6 and 537.7 mg, respectively, at 30°C and an illumination by the internal light guides and two additional snap lights. A speed of 1.2 m s^{-1} corresponds to 180 revolutions per min. The time denoted '0' in the graphs corresponds to the start of the speed determination, and not to the beginning of the whole experiment

the calorimetric signal as small endothermic peaks. Periods in which these peaks occurred were excluded from the evaluation.

Future prospects

The present paper describes orientational experiments with the first direct calorimeter for flying insects. Fundamental investigations are performed concerning heat production rates during flight and their dependence on the flight time, temperature, illumination levels and modes of preparation of the animals, and further simultaneous determinations are briefly discussed. In a subsequent step, indirect calorimetry will be carried out by monitoring changes in oxygen and carbon dioxide concentrations during flight, with hermetic sealing of the carousel volume and the application of special gas sensors. The thorax temperature will be measured by means of thermocouples and low-friction sliding contacts at the carousel support. Future flight stimulation by tarsal reflex may be achieved by means of a trapdoor mechanism in the calorimeter. A small video camera may observe the physiological state of the insect before and after flight and during any intermissions.

Not all of these possibilities are already incorporated in the new prototype, but they can be included more or less easily after preliminary testing experiments. The new experimental approach presented in this paper for the measurement of metabolic rates may yield further insight into physiological mechanisms of insect flight, and into the energy budget of social insect colonies.

* * *

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