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Calorimetric investigations on activity states and development of holometabolous insects

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Abstract

Holometabolous insects are an extraordinarily species-rich animal taxon, characterized by a clear distinction between larvae, pupae and adults in their lifecycles. From a calorimetrist's point of view, they are interesting study objects because larvae and adults often have very different lifestyles. Some common patterns of the time course of metabolic rates are observed during calorimetric investigations. Larvae have high metabolic rates after hatching from the egg, up to 160 mW/g in the wax moth *Galleria mellonella*. Prior to pupation, metabolism is strongly reduced. During metamorphosis, the pupal instars typically exhibit a U-shaped pattern of heat production. Insect adults have low resting metabolic rates, close to the pupal metabolism. Metabolic rates of flying insects are up to 50 times higher than resting metabolism.

Keywords: Insect metabolism; Insect development; Insect flight; Calorimetry

1. Introduction

Holometabolous insects represent a very large group within the taxon of insects. The holometabola are characterized by (i) a clear separation between larvae, pupae and adults, (ii) moulting from larvae to pupae and (iii) a complete metamorphosis to the adult during the pupal instar without any energy uptake. Nearly all holometabolous insects, e.g. flies, bees, ants, wasps, butterflies and beetles, can fly (one of the few exeptions are fleas, which are secondarily wingless) and have an enormous ecological as well as economical influence on life, especially human life, on earth. One of the keys to their evolutionary success is the fact that larvae and adults often live in very different habitats. One example is mosquitos, where the larvae develop under water and feed on nanoplankton (very small unicellular organisms) whereas the adults can fly, are completely terrestial, and feed on plant nectar or vertebrate blood. With seperate habitats, larvae can exploit different food resources (an adult mosquito would not be able to feed from small organisms in water) and will, therefore, avoid any competition for food energy with adults.

As the course of development in holometabolous insects consists of three very different lifeforms, the development of metabolic rates should be expected to be far from having a linear relationship with age or body mass. The division of the holometabolous lifecycle into three parts causes a partitioning of tasks

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such as rapid and efficient accumulation of biomass and energy reserves (larvae), metamorphosis of biomass into the adult lifeform (pupae), and reproduction (adults). Therefore, from a calorimetrist's point of view, holometabolous insects represent a unique taxon for investigations on the energetics of the very different aspects of animal growth, activities and reproduction.

This paper summarizes some of the calorimetric investigations of our working group concerning metabolic rates of holometabolous insects. As the definition of activity states plays an important role in the measurement of metabolic rates (and thus heat production rates) in animals, we will focus on this topic. Our investigations included a manifold of different species (i.e., butterflies, beetles, honeybees and hornets). Nevertheless, we will seperate our article not by species, but by developmental stages in order to look for general patterns in the development of heat production rates in holometabolous insects.

2. Experimental

If not otherwise mentioned, we investigated all chosen insects in 15 and 100 ml vessels of Calvet-type microcalorimeters (Setaram, Lyon, France). The sensivity of the instruments ranged from 11 up to 55 μV/mW, the temperature varied between 10 and 35°C and the measuring vessel could be ventilated, enabling a simultaneous determination of gas exchange. Depending on size, single or groups of individuals were investigated.

2.1. Eggs

The insect lifecycle starts with the development of embryos in the eggs. Unfortunately, we have little or no knowledge about the heat production rates of insect eggs, although it would be interesting to see how metabolic rates change during embryonic development from a unicellular to a multicellular organism. The heat production rates of eggs of the wax moth *Galleria mellonella*, a parasite in honeybee colonies, amounts to 0.3 mW/egg or 3 mW/g [1] at an ambient temperature (T_A) of 30°C. This is somewhat higher than the heat production rates of flour beetle (*Tribolium confusum*) eggs, which amount to 1.8 mW/g at the same T_A [2]. Similar to small larvae, eggs are often very sensitive to dessication. This, together with their small size, is the main obstacle for calorimetric investigations.

2.2. Larvae

Holometabolous insect larvae are mainly designed as feeding automatons. They accumulate biomass for body construction of the adult, and store food reserves for the construction of the adult body during metamorphosis. Larvae exhibit very different lifestyles compared to adults. Most calorimetric studies on insect development have been done on two familiar laboratory insects which are easy to raise and cultivate: the mealworm *Tenebrio molitor* (despite its name, a beetle) and the greater wax moth *Galleria mellonella* (which belongs to the butterflies).

We first consider some theoretical predictions for the time course of heat production rates during larval development. After hatching from the egg, the larvae should have rather high specific heat production rates, as their body mass is low and the first goal for a larva should be a rapid growth, which means high rates of catabolism. The heat production rates should then decrease, because the amount of tissue which is inactive in catabolism increases, e.g. the fat body. After reaching a certain body size, the larva begins to store food reserves when sufficient food can be taken up per unit time. The specific heat production rates of the larval instar should be lowest shortly before pupation, when body growth has ceased and energy must be saved for the process of metamorphosis.

Calorimetric experiments on *Tenebrio molitor* revealed the expected patterns of heat production. After the hatch, heat production rates began to increase rapidly and reached a peak with values of up to 18 mW/g after about 3–4 days. The heat production rates then continuously decreased over a period of about 80 days. In the last week prior to pupation, the heat production rates were low but stable with values of 2–3 mW/g [3]. A similar pattern is observed during development of the honeybee *Apis mellifera*, although the time needed for development from egg to pupa amounts to only 9 days and is thus 10 times shorter than the larval development of *Tenebrio*. During these 9 days, a honeybee worker larva increases its body

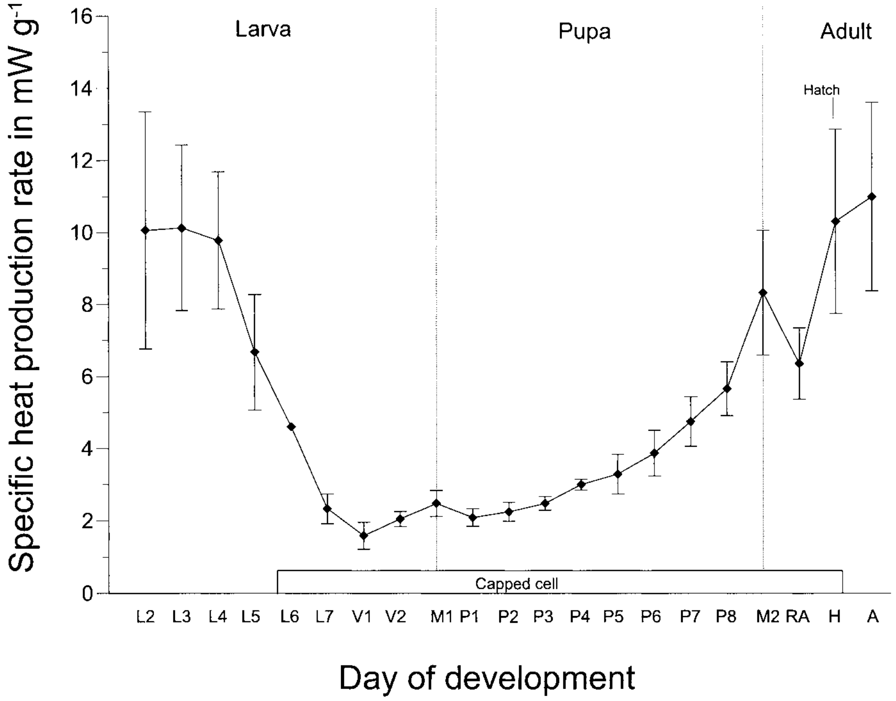

Fig. 1. Specific heat production rates of the honeybee *Apis mellifera* during development. Bars indicate standard deviations. Abbreviations: L2, L3...L7: 2, 3...7th day of larval development; V1, V2: 1 and 2 day of the prepupal stage; Ml: moult from larval instar to pupal instar (pupation); M2: moult from pupal instar to adultus; P1–P8: 1–8 day of pupal development; RA: resting adult between moult to adult and hatch from the capped cell; H: hatching adult: A: young adult (age<24 h).

mass, 540-fold from 0.32 mg in the first larval instar up to 173 mg in the last (seventh) instar. The specific heat production rates are lower than those reported for *Tenebrio* and amount to about 10 mW/g during the first 4 days of development. During the next 3 days, heat production rates decrease to a value of 2 mW/g and remain at this level for the last 2 days prior to pupation (Fig. 1). Honeybee larva are fed with a nourishing fluid secreted by nursing worker bees. This fluid is rich in proteins and carbohydrates, and developmental time can be shortened by this kind of very intense brood care. Interestingly, the brood cells of *Apis mellifera* are sealed (‘capped’) with a wax lid on the 6th day of larval development and opened again by the freshly developed young adult. As a consequence, worker bee larvae are without external food supply for 4 days. In this time, the specific heat production rates are at a low level [4].

Heat production rates during the larval development of *Galleria mellonella* show an unexpected pattern with very high heat production rates in the 4th and 5th larval instar (L_{4+5}) [1]. From the first larval instar on, heat production rates of wax moth larvae are unusually high with values from 50 to 60 mW/g in L_1 to L_3 -larvae. The heat rate continues to increase until the end of the fifth larval instar with values up to 160 mW/g. After this maximum, heat production decreases steadily, and prepupae (i.e., last larval instar, cocooned and preparing for pupation) have heat production rates of only 5 mW/g [5]. The energy content of wax moth larvae, determined by means of combustion calorimetry, gives no hint as to why growing wax moths have such extraordinarily high metabolic rates. The energy content remains relatively unchanged during the growth process with values ranging from 27.5 J/mg in the first instars up to 32 J/mg before pupation [5], but is considerably higher than in honeybees, where early larvae have an energy content of about 18 J/mg, a value which increases during development up to about 25 J/mg in the last larval instar [4]. *Galleria* *mellonella* is a parasite in honeybee colonies, where the larvae feed on wax, pollen, brood and other organic matter, but until now, neither the diet nor other traits in the lifecycle of the wax moth provide us with an explanation for heat production rates during larval development which are close to those of flying adult moths (see below).

One should be aware that heat production rates measured during the development of the holometabolous insects mentioned here do not represent resting metabolic rates. Resting metabolism is defined as the metabolism of an inactive, non-sleeping, non-digesting and non-growing animal. Although we do not know anything about sleep in insect larvae, they certainly grow and digest, and most of the time they are active. Therefore, it is impossible to measure resting metabolism in insect larvae.

2.3. Pupae

During the pupal stage of development, holometabolous insects undergo metamorphosis when all body structures are built more or less completely new, so that one may call this phase a 'second embryogenesis'. Insect metamorphosis can be roughly divided into two phases. In the first, all larval structures are digested and degraded, and in the second the final adult body is newly constructed from imaginal cells (cells which remain undifferentiated during larval development and play a crucial role in adult growth). Again, we can make predictions about the development of heat production rates during metamorphosis before looking at our experimental results. After pupation, the metabolic rates should decrease slowly as organs and tissues are deconstructed and partly digested, until the imaginal cells divide and start to build the adult. With continuing construction of the adult body, the metabolic rates should increase until the adult moults and sheds the cuticle of the pupa.

As most pupae are motionless and no pupa takes up food, they have been favorite subjects for respiratory as well as direct calorimetric studies. Although unusual in its larval metabolism, *Galleria mellonella* fits theoretical prediction of an U-shaped pattern of heat production rates during metamorphosis (Fig. 2). A deviation from this pattern can be found in honeybees, where after 2 days of no change, heat production rates increase steadily until the final moult from pupa to the adult. A reason for the differences in the patterns of heat production rates between *Apis* and *Galleria* can be found in the different lifestyles of the larvae: wax moth larvae are freely moving and feeding, and their body structure may be more complex than that of honeybee larvae, which are being fed by adult workers and do not move outside their cells. The life of a honeybee larva needs only very few special adapta-

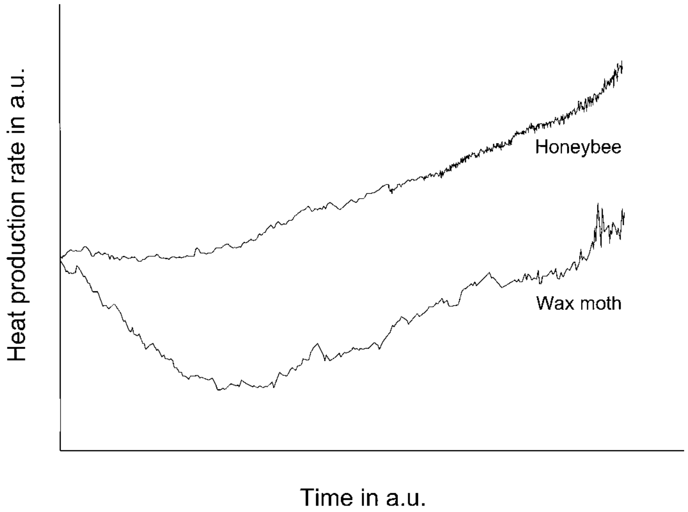

Fig. 2. Heat production rates of pupae of a wax moth (*Galleria mellonella*) and a honeybee worker (*Apis mellifera*). For a better comparison of the structure of the calorimeter curves, time units as well as heat flux units are omitted in this figure. Note that the duration of the pupal phase is different for honeybees (8 days) and wax moths (11 days). Heat production rate and time are given in arbitrary units (a.u.).

tions compared to wax moths. Therefore, the larval body degradation during metamorphosis requires less energy in the beginning of the metamorphosis, whereas in wax moths, more complex body structures have to be degraded and as a consequence the metabolic rates are higher.

The common picture of an insect pupa as a motionless being is incorrect in some cases. Pupae of the mealworm *Tenebrio molitor*, for instance, show clearly visible body movements during metamorphosis, which can also be observed as exothermic peaks in the calorimetric curves. These movements probably ventilate the trachees of the pupae [6].

Generally, the specific metabolic rates are lowest throughout the lifecycles of holometabolous insects during the process of metamorphosis. This can be explained by the fact that pupae spend little energy in locomotion, which plays a major part in the energetic budgets of adult holometabolous insects, as we will see in the next section.

2.4. Adults

Most direct calorimetric measurements on holometabolous insects have been made on the adult lifestage. As we do not want to give a complete review of all studies on adult insects, we focus only on the differences between resting metabolic rates and metabolic rates during activities of the insects. Although resting metabolism is not easy to define in some insects, such as bees, bumblebees or hornets, which seldom rest and have almost no internal food reserves, it is even more complicated to define the activity states of insects. Two activities we discuss in this section are flying and alarm behaviour, which can be provoked with specific alarm pheromone substances in bees, hornets and wasps. But first we will concentrate on resting metabolism.

2.4.1. Resting metabolism and diapause

True resting metabolism, as defined above in the section on larvae, can be found only in insects which have internal food reserves. An ideal candidate is the wax moth, because the adult moths have only degenerated mouthparts, do not take up food, and live exclusively on internal food reserves. Wax moths exhibit a circadian pattern of activities with periods of rest of about 12–14 h [5]. The resting metabolic rates come close to values for metabolism during metamorphosis and amount to 5.6 mW/g.

Many holometabolous insects undergo a diapause (controlled state of rest and inactivity during unfavourable conditions, e.g. overwintering) during their lifecycle. Diapause can occur in eggs, larvae, pupae and adults, but we concentrate on the latter. Although diapause truly is a state of rest in insects, diapause metabolism is not necessarily equal to resting metabolism. During winter the physiology of a hibernating insect changes considerably (e.g., when it experiences sub-zero temperatures), and metabolism is greatly reduced. Low heat production rates can be expected, but we concentrate on another interesting phenomenon: during diapause, insects risk dessication. Their cuticle with an outer wax layer usually prevents most water loss, but while ventilating their tracheae, insects lose water through respiration. To avoid this, they open their tracheae periodically, a phenomenon known as discontinuous ventilation (DV) [7]. In some insects, discontinuous ventilation produces a pattern of regular fluctuations which can be seen in the calorimetric curves (Fig. 3). When the tracheae are closed (C-phase), the calorimetric curve shows a maximum. Subsequently, the tracheal opening (stomata) begins to flutter (F-phase), and some CO_2, together with water, is being exhaled. This phase can be observed in the calorimetric curve as a slow decrease of the heat production rate due to the endothermic effect of water evaporation. When the insect opens its stomata completely and begins to ventilate the tracheal system (O-phase), more water and CO_2 is being released, which can be seen as a steep decrease of the heat flux signal of the calorimeter, as most water evaporates into the measuring chamber. In general, heat production rates are low during diapause as the insects live exclusively on stored energy sources. Thus, the metabolic rates of overwintering hornet queens range from 0.35 mW/g at TA=10°C up to 1.36 mW/g at TA=20°C [8].

2.4.2. Flight metabolism

To compare resting metabolism with that of maximum activity, we constructed a calorimetric device for the measurement of heat production rates of flying insects [9]. In principle, the calorimeter chamber was constructed as an aluminium cylinder (height 11 cm, diameter 18 cm) with an incorporated carousel. The

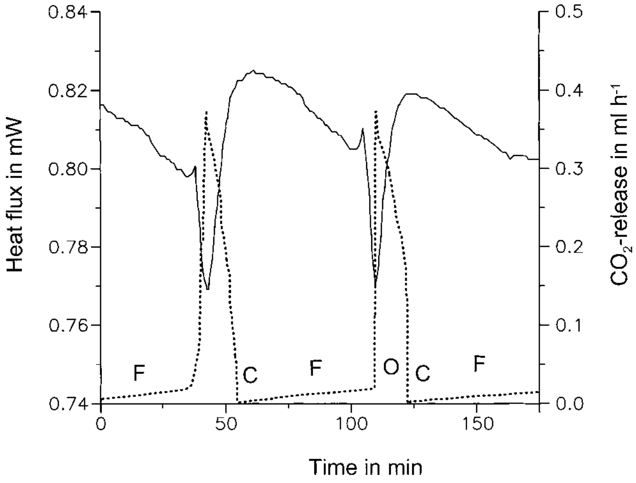

Fig. 3. Periodic structures in the calorimetric curves of a hibernating hornet queen (*Vespa crabro*) at T_A=15°C, caused by discontinuous ventilation. Full line: heat flux, dotted line: CO_2-release; C: *closed*-phase, F: *flutter*-phase, O: *open*-phase. For details see text.

insects were fixed (with removable glue) to one carousel arm and started to fly. The calorimeter chamber could be illuminated via glas fibres connected to a cold light source. Depending on the varying experimental setup used in our studies, up to three parameters could be recorded: heat production rates at various ambient temperatures, flight speed and (in some cases) wing beat frequencies. A detailed description of the method is given elsewhere [9,10,11].

Before we present detailed results, we again can make some theoretical predictions. As small insects have a small body mass, inertia plays no role during locomotion. Consequently, they cannot glide during flight, but have to continuously move their wings. For this reason, transport costs are high for insects compared to other flying animals like birds or bats, and their wing beat frequencies can be up to 500 Hz. The metabolic rate of an insect during flight may be roughly estimated (at least to an order-of-magnitude) when morphological parameters such as weight and wing span are known. Using C.J. Pennycuick's set of formulas for theoretical predictions of power requirements for animal flight [12], the minimum mechanical power (P_{MIN}) for a male wax moth with an average weight of 84 mg and a wing span of 40 mm comes to 0.011 W or 130 mW/g. It can be assumed that muscular efficiency is about 20%, so that high specific heat production rates up to several hundred milliwatts per gram body mass may be expected.

Indeed, the specific metabolic rates of flying wax moths are up to 50 times higher than the specific resting metabolic rates of adult wax moths rendering values of 277 mW/g for males and 181 mW/g for females at T_A=20°C and 230 mW/g for males and 101 mW/g for females at T_A=30°C, indicating that heat production during flight is dependent on ambient temperature. Flying hornet drones had lower specific heat production rates than wax moths with values of 119 mW/g at T_A=20°C and 70 mW/g at T_A=35°C, demonstrating a similar temperature-dependence of metabolism on T_A [10]. Although it seems to be useful to divide the heat production rate P by the total body mass in order to compare with the metabolic rates of larvae, this does not reflect the true metabolic rates of the heat generating tissues. By far the most heat during insect flight is produced by the flight muscles, which are found exclusively in the thorax. The thorax of holometabolous insects is almost completely filled with musculature. Therefore, if we want to compare the flight metabolism of male and female moths, we have to consider that the different sexes have a different proportionality between thorax mass and total body mass (the ratio for this is 35.4% for males and 23.3% for females in wax moths, due to a more heavy abdomen, filled with ovaries). To avoid artifacts caused by this different allometry, we divide the heat production rates by thorax mass (which we define here as mass of thorax without legs and wings). The

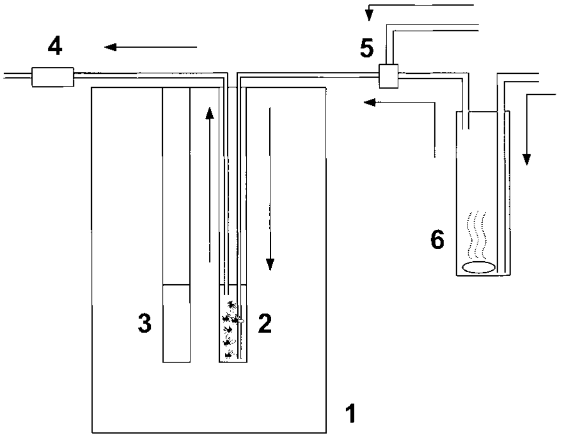

Fig. 4. Sketch of the experimental setup for measurement of heat production rates of social insects under the influence of alarm pheromones. Not drawn to scale. Arrows indicate the direction of the air flow. 1: calorimeter; 2: reaction vessel (100 ml) with bees, wasps or hornets; 3: reference vessel; 4: air pump; 5: valve for switch from normal air to air with pheromone; 6: wash flask with filter paper soaked with pheromone.

specific heat production rate of wax moths at T_A=30°C then amounts to 872 mW/g *thorax mass* for males at an average flight speed of 1 m/s [11].

2.4.3. Alarm pheromones

A different method of provoking defined activity states of adult insects can be employed in some social insects. Bees, wasps and hornets show high metabolic rates under the influence of their alarm pheromones which induce agressive behaviour. In principle, two methods can be used to investigate the metabolic reaction of social insects to such alarm pheromones by means of direct calorimetry. If we want to measure the reaction of a single wasp or bee and of small groups of them to the pheromones, a reaction vessel of 100 ml is sufficient. A slow air current is led through the reaction vessel, and at some defined point, the pheromone can be added to the air (Fig. 4). Usually, the reaction is seen immediately in the calorimetric curve as a steep increase of the heat production rate (Fig. 5). If we want to follow the reaction of a complete colony of bees or wasps under the influence of pheromones, we use large-volume calorimeters specially designed for these purposes. A detailed description of the calorimeters may be found elsewhere [13]. When a defined amount of pheromone substance is injected into the interior of the measuring chamber, the reaction of the colony is seen in the calorimetric signal. In honeybees (*Apis mellifera carnica*), the metabolic rates increase by about 30% up to values of 85 mW/g, depending on the pheromone substance [14].

2.5. Moulting

Other interesting structures in the calorimetric curves can be seen during moulting (ecdysis; the larva

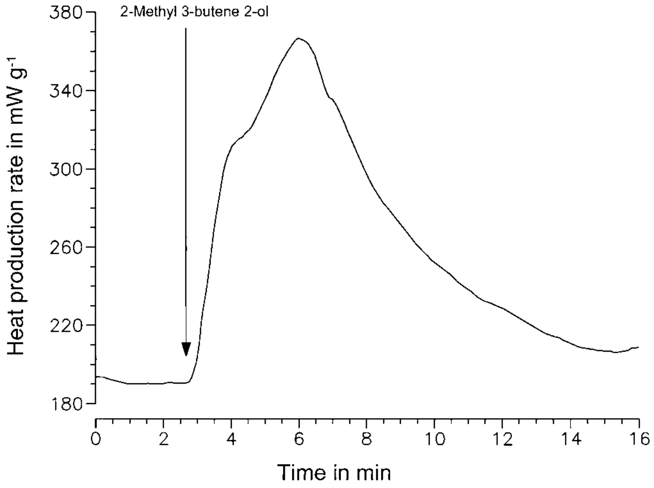

Fig. 5. Increase of the heat production rate of a group of eight hornets (*Vespa crabro*) workers under the influence of their main alarm pheromone component, 2-methyl 3-butene 2-ol. The arrow indicates the application of the pheromone to the hornets.

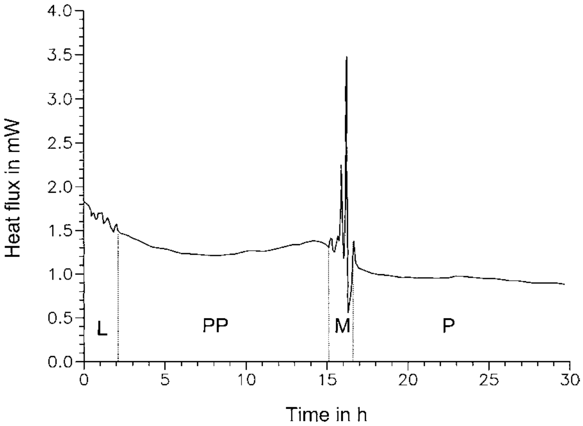

Fig. 6. Heat production rate of a wax moth (*Galleria mellonella*) during moult from larva to pupa (pupation). L: larval instar; PP: prepupa; M: moult; P: pupal instar.

or pupa sheds the old cuticle and emerges with a new, at first soft, cuticle which hardens later). During larval moults, metabolism is greatly reduce but no special structures connected to ecdysis can be observed in the power-time-curves [1]. In contrast, pupation, i.e. the emergence of adults from the old pupal cuticle are clearly visible as one or two strong exothermic peaks followed by a short endothermic peak (Fig. 6). The exothermic signals result from strong locomotor activities when the insect breaks free from the old cuticle. The endothermic signal stems from evaporation of exuvial fluid, lying between the old and the new cuticle and being released during the moulting event.

3. Conclusions

Holometabolous insects are probably the most influential group in the animal kingdom. With a multitude of different habitats and lifestyles, they offer unique possibilities to study the impact of ecological adaptations, physical constraints and developmental processes on the energy metabolism of organisms. A variety of interesting effects can be observed during calorimetric investigations. Some insect larvae are completely aquatic during development, others experience hypoxic conditions. Creative use and modification of calorimeters will open the doors to a previously underpopulated field in calorimetry. The aim of future studies will not only be the search for characteristic common patterns in metabolism and deviations from them, but to look also at causal explanations for these patterns.

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