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Metabolic cost of ventilating movements in pupae of Tenebrio molitor and Galleria mellonella studied by direct calorimetry

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Abstract

The energetic cost of periodically occurring rhythmic body movements in pupae of the mealworm *Tenebrio molitor* and the wax moth *Galleria mellonella* was estimated by the use of direct calorimetry. The motions affect tracheal ventilation and/or accelerate hemolymph circulation.

The contribution of muscular activity to the total specific heat production rate in 46 to 52 h old pupae of *Tenebrio molitor* and *Galleria mellonella* was 1.24% and 0.9%, respectively. The energetic cost of stereotyped body motions serving diverse functions formed a negligible part (less than 3%) of the total energy consumed during pupal development.

Keywords: Direct calorimetry; *Galleria*; Insects; Locomotion; Metabolism; *Tenebrio*

1. Introduction

Direct calorimetry monitors the heat production rate of living organisms giving direct information about their aerobic and anaerobic metabolism. It has been demonstrated for the mealworm *Tenebrio molitor* that direct calorimetry is well suited for describing the development and ageing of insects [1]. Monitoring heat production over a period of many days in adult cockroaches *Blatella germanica* revealed regular peaks on calorimetric power–time curves owing to the metabolism or locomotion of animals [2].

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Body motions must be checked at the same time in order to determine correctly the basal level of metabolism (i.e. standard metabolism). For the simultaneous recording of heat production rate and locomotion of insects a combination of direct calorimetry and acoustic monitoring was used [3] as well as a combination of calorimetry and endoscopy of small animals [4]. Carbon dioxide production and heat flow in single insects were also simultaneously recorded [5]. In some insects the tracheae are ventilated periodically when the spiracles are opened and release of carbon dioxide occurs [6, 7]. Rhythms of cyclic carbon dioxide release in insects were first recorded by direct calorimetry in the cockroach *Blatella germanica* [2].

In the pupal stage of insects when locomotion, nutrition and excretion are absent, the standard level of metabolism can be readily distinguished from the metabolic level during the activity of skeletal muscles (metabolic rate during activity). In a number of pupae including those of *T. molitor* and *G. mellonella* periods of more or less regular abdominal rhythmic movements promote gas exchange and/or hemolymph circulation [8–11] and even seem to support heartbeat [12]. Recently the periodicity and pattern of abdominal ventilating movements were examined in *G. mellonella* and *T. molitor* by use of a respirometer combined with a twin setup calorimeter [13–16]. These movements are not real abdominal pumping but rather the up–down pulsation of the abdomen which externally is often not perceivable.

So far the metabolic cost of tracheal ventilation in insect pupae has been studied very rarely (if at all). In the present report the energetic cost of respiratory movements in *T. molitor* and *G. mellonella* pupae is investigated by direct calorimetry.

2. Materials and methods

2.1. Animals

Yellow mealworms (*Tenebrio molitor*) were reared at 27°C in constant darkness on flour with the addition of 1% dried yeast. Greater wax moths (*Galleria mellonella*) were bred on a semi-artificial diet at 30°C and 70 ± 5% r.h. in constant darkness [17, 18].

Easily excitable individuals with chaotic skeletal muscle activity were eliminated from experiments by preliminary actographic tests [14].

2.2. Calorimetric measurements

Simple calorimeters in a twin setup were constructed of vessels made from copper foil and connected with four thermocouples (copper-constantan) [19]. The volume of both the animal and the reference vessel was 0.25 ml [15] while the sensitivity of the calorimeters amounted to 12 to 22 $\mu V/mW^{-1}$ for the different instruments and the detection limit to 2 μW (twice background noise). The calorimeter temperature was the same as in rearing chambers, i.e. 30 or 27°C.

The insects were placed into the calorimeter at the stage of pharate pupae approximately 1d before larval–pupal ecdysis (the moulting at the transition from the larval to the pupal stage). The moments of larval–pupal and pupal–adult ecdyses were seen on the calorimetric recordings as high endothermic downward peaks due to the liberation of exuvial fluids [15, 16]. Thus, the pupal age was known from the recordings as well as the exact duration of the pupal stage (i.e. the time interval between the two ecdyses).

3. Results

We determined from respirometric measurements whether the rhythmic abdominal up–down movements of the *Galleria* pupae act as ventilating movements or not. The simultaneous recordings of the respirometer and the calorimeter revealed [20] that at the moment of CO_2 release a peak of heat flow occurred (Figs. 1 and 2). During muscular ventilation the peaks of CO_2 in *G. mellonella* disappeared owing to a sudden increase of the heat flow rate (Fig. 1). On the other hand, respirometric recordings showed that the rhythm of cyclic gas exchange was not disturbed. Only the downward peaks of CO_2 production were reduced or became absent, indicating opened or semi-opened spiracles (Fig. 1). Thus, rhythmic body movements in these pupae were regarded as muscular ventilation of tracheae.

The pattern of ventilating movements showed some specific differences between the two insects under investigation. In *G. mellonella* pupae the strength of contractions of intersegmental skeletal muscles during the activity period increased gradually up to the peak maximum and then decreased gradually. This pattern is reflected on calorimetric

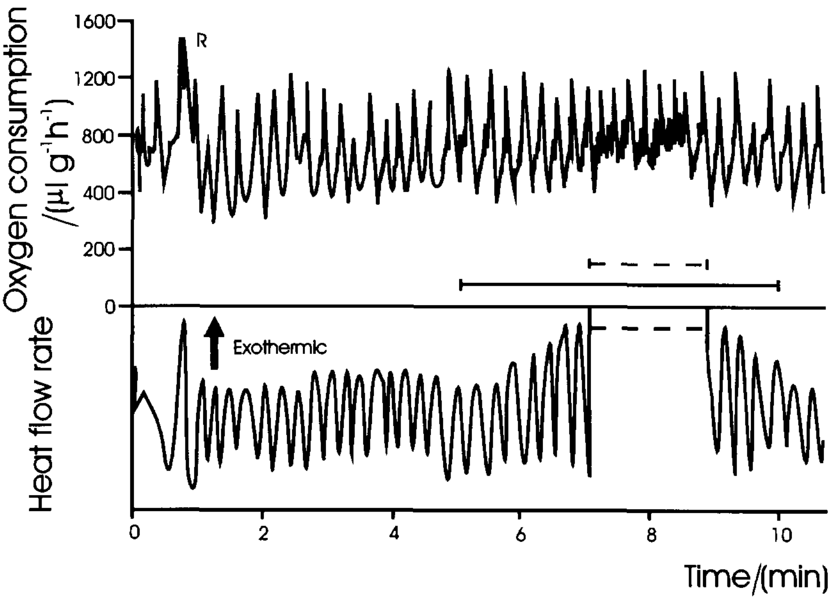

Fig. 1. Gas exchange cycles in *Galleria mellonella* pupae simultaneously recorded with a respirometer (above) and a calorimeter (below). The horizontal solid bar indicates the whole period of muscular ventilation, broken bars show the period of most vigorous muscular contractions:R-a bout abdominal rotation.

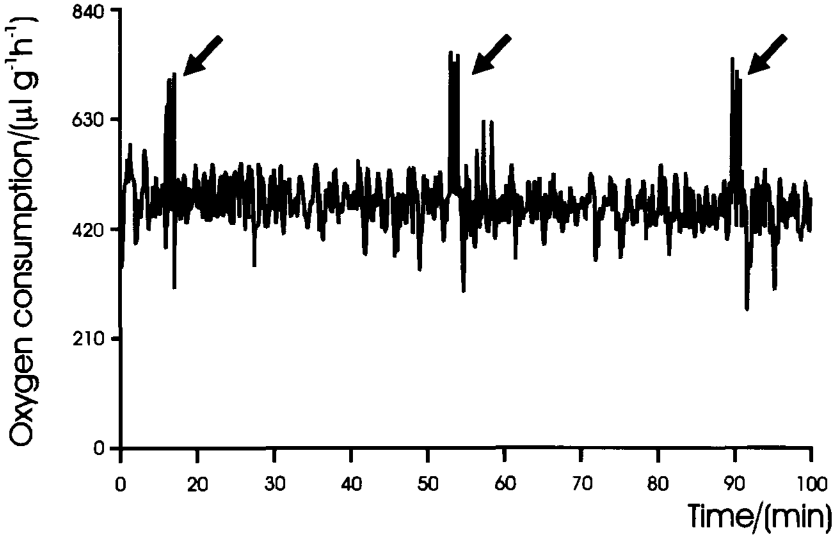

Fig. 2. Respirometric recording of chaotic gas-exchange cycles and three bouts of muscular ventilation (arrows) in a *Tenebrio molitor* pupa.

recordings as more or less symmetrical peaks (Fig. 3). In contrast, *T. molitor* pupae mostly exhibited the strongest muscular contractions at the beginning of the ventilating bout while later the contractions became weaker (Fig. 3).

In *G. mellonella* pupae rotating movements of the abdomen occurred in addition to rhythmic up–down motions. The rotations consisted of two to three strokes lasting

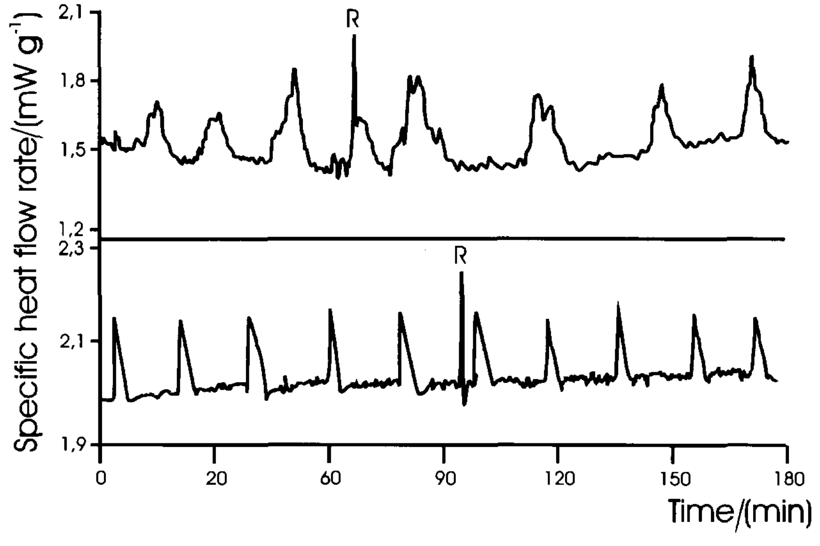

Fig. 3. Periodically occurring muscular activity in pupae of *Galleria mellonella* (above) and *Tenebrio molitor* (below) recorded with a twin calorimeter: R-peaks due to rotating or twisting movements.

Table 1
Data on heat production rate (mean ± SD) in pupae of *Tenebrio molitor* at 27°C and *Galleria mellonella* at 30°C (the contribution of ventilating movements calculated from the calorimetric recordings is given in brackets).

	Galleria mellonella	*Tenebrio molitor*
Heat flow rate[a]/μW	$232 \pm 26 (2.1 \pm 0.3)$	$177 \pm 18 (2.2 \pm 0.2)$
Specific heat flow rate[a]/(μW mg^{-1})	$1.76 \pm 0.3 (0.017)$	1.61(0.2)
Specific heat production per hour at 46 to 52 h*/(mJ mg^{-1})	$6.33 \pm 0.8 (0.06)$	$5.78 \pm 0.06 (0.07)$
Specific total heat production during pupal development(118 ± 4 h)/(mJ mg^{-1})	$956 \pm 78 (12 \pm 1.6)$	$1150 \pm 103 (30 \pm 2.6)$
Ventilating part of heat production rate*/%	0.94	1.24
Ventilating part of total heat production/%	1.25	2.6
Initial body mass of pupa/mg	115 ± 5	110 ± 5
Body mass loss per day/%	2.2 ± 0.07	1.15 ± 0.03

[a] Measured at 46 to 52 h of pupal development.

normally 5 to 8 s and resulted in high thermal peaks (Figs. 1 and 2). These usually occurred at the beginning and/or at the end of muscular activity periods. This specific type of body movement served also as tracheal ventilation resulting in a deep air intake stroke at the end of a rotating bout. *T. molitor* pupae exhibited rare irregular twisting movements noted also on calorimetric recordings as high peaks (Fig. 2). Neither of the tracheal ventilating types disturbed the main rhythm of cyclic gas exchange (i.e. time intervals between subsequent cyclic CO_2 releases) but the amount of cyclically released CO_2 was reduced.

No significant increase in the oxygen consumption level was observed on respirometric recordings of *G. mellonella* pupae during rhythmic abdominal up–down motions (Fig. 1). However, calorimetric recordings exhibited distinct peaks of elevated heat flow rate, although the contribution of ventilation to the total heat production was small (Table 1).

The most regular periods of muscular activity in pupae occurred during the lowest level of the U-shaped metabolic curve (from 46 to 52 h of pupal development). At this time, the energetic cost of active tracheal ventilation was smaller than before the adult insect emerged. Then pupae exhibited frequent chaotic wriggling movements with a higher energetic cost (Table 1) during the last 8 to 10 h before pupal–adult ecdysis. Thus, the standard level of metabolism could not be estimated [16].

4. Discussion

Pupae of *G. mellonella* and *T. molitor* normally exhibit regular periods of ventilating motions which are seen in calorimetric recordings as exothermic peaks superimposed on a constant plateau. Thus, the level of standard metabolism is well distinguished from that of muscular ventilating or locomotive activities. It is evident that the same motions in *G. mellonella* and *T. molitor* pupae act not only as tracheal ventilation but also accelerate the hemolymph circulation and obviously support the heartbeat directly [12].

The part of muscular ventilation at 46 to 52 h of pupal development (minimum level of the U-shaped metabolic curve) made up about 1% of total heat production. Therefore, the energetic cost of stereotyped body movements serving diverse functions formed a negligible part of the total energy consumed during pupal development as determined by direct calorimetry (Table 1). Economy of energy use is well comprehensible in the pupal stage.

According to preliminary data [14, 15], periodically occurring muscular ventilation in the adult colorado beetle *Leptinotarsa decemlineata* and the weevil *Hylobius abietis* raises the heat flow rate by 12 to 18% over the standard level of metabolism. In contrast, adults showed cyclic CO_2 release at the level of standard metabolism during rest. In our view regular exothermic peaks recorded by direct calorimetry in adult insects [2] may in some cases be the result of muscular ventilation of the tracheae.

A highly regular cyclic CO_2 release occurred in *G. mellonella* pupae resulting in upward peaks on calorimetric recordings. We suppose that these exothermic peaks were due to the warming effect of CO_2 bursts from spiracles. In some lepidopteran pupae (e.g. large cabbage-worm *Pieris brassicae*) cyclic CO_2 release results in endothermic peaks as a result of the relatively large amount water vapour liberated with the CO_2 [21].

It is concluded from our results that the use of direct calorimetry enables simultaneous study of the periodicity of muscular activity, its energetic cost and the cyclic nature of external gas exchange in the pupal stage.

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