*Journal of Thermal Analysis and Calorimetry, Vol. 85 (2006) 3, 581–584*

# THERMOGENESIS OF *ARTEMIA* IN POST-DORMANT DEVELOPMENT*

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Isothermal microcalorimeter was modified to measure slow and weak thermal dissipation for developmental and evolutionary biology. The calorimeter can detect the continuous thermal dissipation of the ampoule at $P$=20 nW and fast thermal events with $E$=0.1 μJ. Baseline was stabilized to be ±10 nW over a week. By using this calorimeter, post-dormant development of a single larva of *Artemia franciscana* (Great Salt Lake) was measured as a function of incubation time at $T$ = 293.17 K. Characteristic thermogenesis was observed at emergence, hatching and growth. The energy of yolk in a cyst was evaluated to be 38 mJ in average on the basis of the total thermogenesis of a larva.

***Keywords:*** *Artemia franciscana, post-dormant development, thermogenesis*

## Introduction

Recent developmental biology has revealed that molecular mechanisms contribute to characteristic phenotype in specific animal. Genes encoded in DNA are materialized in turn under the right and sophisticated mechanisms to induce differentiation of cells, which are called as body plans. On the other hand, the view of evolution suggests that any phenotype has never been planed in nature but just resulted to come off. Therefore, even after the details of the molecular mechanisms are clarified, we still have a fundamental question how and why they have gotten such sophisticated molecular mechanisms in their evolution. In fact, the molecular mechanisms are adapted to inner environment inside a cell, while the body adapts itself to outer world. Namely, body and molecular mechanisms have adapted themselves to different environment. However, both adaptations are determined by balance of energy and material. Therefore, energy balance must be an important measure, which would relate the evolution of the molecular mechanisms to that of body.

Calorimetry can measure both enthalpy changes of biochemical reactions in vitro and thermogenesis of body, with which the energy balance of body can be evaluated. Recently, the author has successfully modified a commercial isothermal microcalorimeter (Thermometric, LKB-2277) to measure weak thermogenesis of a single larva in early stages from an egg. The present study shows that the single-body thermal measurement of *Artemia* in post-dormant development [1, 2]. Encysted gastrula of *Artemia* emerges even after a decadelong dry anoxic storage [3]. The dormant gastrula seems to be a kind of glassy state, in which trehalose vitrifies the whole cells [3–5]. Once the cyst is immersed in salt water, trehalose is hydrolyzed to glycerol. The osmotic pressure of glycerol has been suggested to help rupture of the cyst shell [6]. In umbrella state after the emergence, the animal is enveloped in egg membrane. Larva, nauplius, swims by using a pair of long antennae after hatching. This post-dormant development is not synchronized, so that it is necessary to measure a single cyst in order to observe thermogenesis depending on the developmental stage.

## Experimental

Dry cysts of *Artemia franciscana* (Great Salt Lake) were kindly supplied from Japan Pet Drugs Co., Ltd. Artificial seawater (ASW) was made of 19 g $L^{-1}$ of artificial sea salt, Marine Art SF-1 (Tomita Pharmaceutical Co., Ltd.) with antibiotics, penicilline G potassium salt and streptomycin sulfate. A dry cyst is encapsulated in a glass ampoule (LKB 2277-303) of 3.3 mL in volume with the 0.1 mL ASW for calorimetry. The air sealed in the ampoule supplies enough amount of oxygen to the animal for respiration. The glass ampoules were sealed with a screw cap made of aluminum alloy for quick thermalization and permitting repeated use, which is necessary in order to get an exactly reproducible baseline (Fig. 1). Rubber cap can not be utilized because it prevents from high-pre-

* Constribution number 101 from the Research Center for Molecular Thermodynamics

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*1388–6150/$20.00*

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**Table 1** Incubation time of thermally and microscopically observed events (in day)

| events | microscopy | calorimetry | | | | | |
|---|---|---|---|---|---|---|---|
| temperature | 296–299 K | 293 K | | | | | |
| – | – | #1 | #2 | #3 | #4 | #5 | #6 |
| onset (thermal) | – | 1.33 | 0.16 | 0.98 | 0.00 | 0.66 | 0.41 |
| emergence | 0.77 | 1.97 | 0.73 | 1.38 | 1.14 | 1.43 | 0.96 |
| hatching | 1.03 | 2.41 | 1.15 | 1.90 | 1.68 | 1.92 | 1.46 |
| 1st molting | 1.69 | 3.44 | 2.08 | 2.87 | 2.64 | 2.91 | 2.77 |
| 2nd molting | 2.33 | 4.11 | 2.97 | 3.62 | 3.40 | 3.84 | – |
| 3rd molting | 3.23 | 5.42 | 4.01 | – | 4.64 | – | 4.63 |
| end (thermal) | 5.20 (dying) | 8.35 | 6.23 | 8.05 | 7.56 | 8.18 | 8.00 |

cision measurement by its thermal dissipation lasting for a day after crimp.

The calorimeter was equipped with an ampoule measuring cylinder (LKB 2277-201). The temperature of thermostat was regulated to be 293.17 K. The output of thermopiles was measured by a digital nanovoltmeter (Keithley 2182). The signal was fed every second into a personal computer to be accumulated for 120 s. The average of 120 outputs and their standard deviation were recorded. The measuring cylinder was kept under a steady dry argon gas flow at the rate of 1 $cm^3$ $s^{-1}$, in order to reduce effect of humidity. The calorimeter was caged in a box regulated at 298.2±0.1 K. Under such modifications, the calorimeter detects the slow thermal dissipation of the ampoule at $P$=20 nW or a heat pulse of $E$=0.1 μJ. Baseline was stabilized to be ±10 nW over a week. The thermal power was calibrated with a calibration heater equipped in the measuring cylinder.

The video picture was taken for a week at 296–299 K in order to observe the post-dormant development of the animal. A dry cyst was loaded in a droplet of ASW on a microscope. The droplet of 3 mm in diameter and 0.4 mm in thickness was sealed between two slide glasses to avoid vaporization.

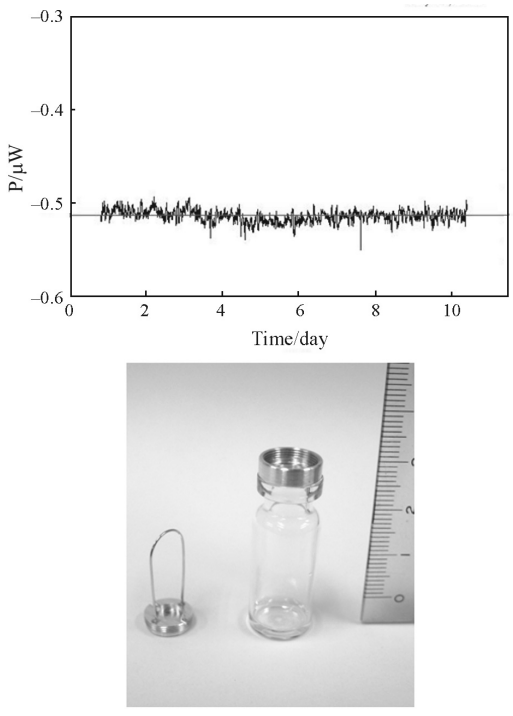


**Fig. 1** Baseline for calorimetry at $T$=293 K. Ampoules with 0.1 mL ASW were loaded into the measuring cylinder. Standard deviation was 7 nW. Photo shows the glass ampoule with aluminum alloy cap

## Results and discussion

Figure 1 shows the baseline which was obtained by measuring thermal signal of the ampoule with 0.1 mL ASW. Standard deviation of the thermal signal was 7 nW. Thermal disturbance was ceased in two hours after inserting the ampoules into the calorimeter.

Figure 2 shows a typical thermogenesis of *Artemia franciscana*. A dry cyst of 0.22 mm in diameter was loaded in ASW at $t$=0 day. The horizontal line is the baseline, which indicates zero thermal dissipation. Thermogenesis rose soon after loading in this experiment. The onset of thermogenesis distributes from $t$=0 to 1.5 day, depending on individual cysts. The first significant peak was found at $t$=1.14 day, followed by the second sharp peak at $t$=1.68 day. In the other experiments, the ampoule was unloaded to observe the animal at these peaks. Then these peaks were confirmed to be observed at the incubation time near emergence (rupture of the cyst shell) and hatching, respectively. The figure shows that a long thermogenesis with several peaks lasts to $t$=7.6 day.

By taking the video picture, the variation of length of the animal was measured as shown in Fig. 3, which clearly shows stepwise increases of the length at emergence, hatching, and 3 molting events. The

**Table 2** Thermogenesis of larvae (in mJ)

| | #1 | #2 | #3 | #4 | #5 | #6 | average |
|---|---|---|---|---|---|---|---|
| total (onset to end) | 40 | 40 | 38 | 42 | 32 | 37 | 38 |
| onset to 3rd molting | 29 | 27 | – | 30 | – | 26 | 28 |
| emergence | 0.55 | 0.39 | 0.71 | 0.84 | 0.61 | 0.36 | 0.58 |
| hatching | 0.18 | 0.32 | 0.48 | 0.39 | 0.35 | 0.35 | 0.35 |

line before emergence indicates the diameter of the cyst. A steep increase was also found before and after the first molting. It is known that the animal takes food after the third molting [3]. However, in the present study, no food was supplied to the animal, which immediately suffered from starvation after the third molting. Actually, the length shrank one day after the third molting. At $t$=5 days, the forth molting was observed. However, the animal could not complete it. The growth rate is the maximum in the vicinity of the first molting. Therefore, the thermal peak at $t$=2.64 days would be related to the first molting.

Figure 4 shows a linear relationship between the time of the microscopically observed events and that of the thermal peaks. Thus, the two peaks after the first molting are attributed to the growth around the second and third molting. After the third molting peak, the thermogenesis steeply decreases to zero at $t$=7.6 days. This indicates that the larva completely looses thermal activity in 3 days under starvation at $T$=293 K. Calorimetry has been done 6 times. Table 1 summarizes the incubation time of the peaks, which were assigned by using linear relationship similar to Fig. 4.

Table 2 summarizes the amount of thermogenesis. Total thermal dissipation in 8 days was 38 mJ in average. This amount is exactly the energy of yolk prepared for post-dormant development. The amount of energy consumed from the onset to the third molting was 28 mJ in average, 75% of the total yolk energy. Therefore, surplus energy of the yolk is estimated to be 25%. They can not be tolerant of starvation beyond a couple of days. *Artemia* lives in a rich environment with abundant food. No other animal competes for food in the hypersaline lake they live

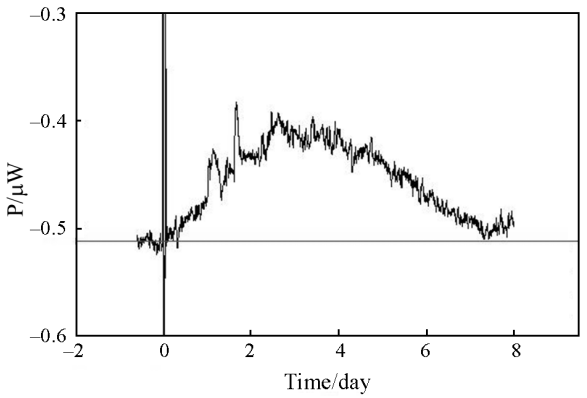


**Fig. 2** Thermogenesis of a single larva of *Artemia franciscana* (Great Salt Lake) at $T$=293 K. A dry cyst of 0.22 mm in diameter was encapsulated at $t$=0 day with 0.1 mL ASW in the glass ampoule

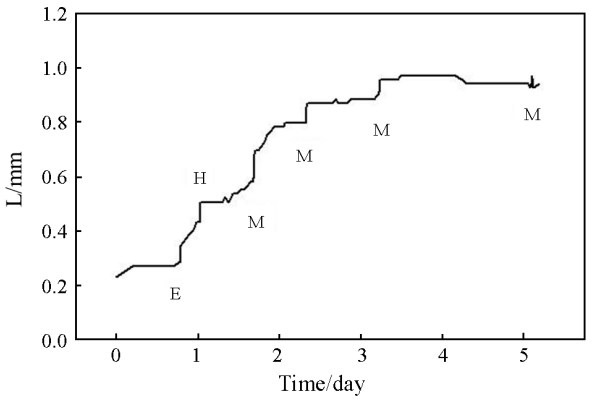


**Fig. 3** Variation of head-to-tail length of a larva as a function of incubation time at $T$=296–299 K. A dry cyst was immersed in ASW at $t$=0 day. A line before emergence (rupture of the cyst shell) indicates the diameter of the cyst. E – emergence, H – hatching, M – molting

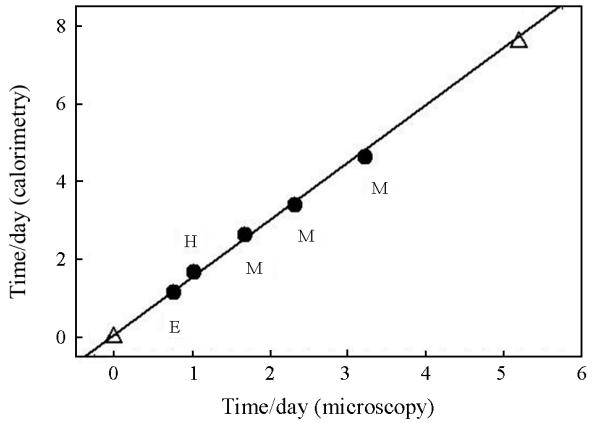


**Fig. 4** Linear relationship between thermally and microscopically observed events. E – emergence, H – hatching, M – molting. Open triangles indicate the onset and the end of thermogenesis. Microscopy and calorimetry were carried out at $T$=296–299 K and $T$=293 K, respectively. Temperature accelerated growth under microscope

[7]. At glance, it would be likely interpreted that such an environment would allow eggs to minimize the surplus energy, because the larva can immediately meet food. However, the same condition allows female to accumulate surplus in eggs as well. Actually, Shirai and Kuroiwa found that egg of starfish, *Asterina pectinifera*, has surplus up to 86% of the total mass. They discussed the surplus energy of yolk in relation to degeneration of larval era of echinoderms [8]. Therefore, another reason should be found in order to explain the amount of yolk in the cyst of *Artemia.*

Owing to literature [9], the amount of trehalose has been estimated to be 0.6 μg, 15 mass% of the dry cyst. However, this amount is too short to supply the total thermal dissipation, because the heat of combustion of trehalose is evaluated to be 10 mJ on the basis of those of carbohydrate [10]. Therefore, other high-energy material such as lipids must be a major energy resource in the yolk for the post-dormant development of this animal. Thus, the major role of trehalose is confirmed to be not energy resource but preservation of embryonic body in the dry cyst [3–5]. The concentration of glycerol increases in egg membrane during emergence [6]. This suggests that the sharp peak at hatching is possibly heat of dilution of glycerol. However, the dilution of glycerol is endothermic [11] and the amount of glycerol is too small to give the peak. Therefore, the peak at hatching is related to metabolism of the animal.

## Acknowledgement

The author thanks Prof. Y. Nakaoka for permission to use his microscope and digital video recorder.

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DOI: 10.1007/s10973-006-7634-y