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Microcalorimetric studies on *Tetrahymena pyriformis* Part 1. Growth metabolic power and thermal equations [α]

Xie Chang-Li [a,1], Sun Day-Ung [a], Song Zhau-Hua [a], Qu Song-Sheng [a], Guo Yu [b] and Li Bing [b]

[a] *Department of Chemistry, Wuhan University, Wuhan (People's Republic of China)*
[b] *Department of Biology, Wuhan University, Wuhan (People's Republic of China)*

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Abstract

The thermograms of metabolic growth of *Tetrahymena pyriformis* have been obtained by the microcalorimetric method. From these thermograms it can be established that the thermokinetic equations for the growth metabolism are $P_t = P_0 e^{k_m t}$ or $\ln P_t = 0.7913 + 0.008919t$ and $C_t = C_0 e^{k_g t}$ or $\ln C_t = 8.8836 + 0.003369t$ where k_m is the metabolic rate constant, k_g the multiplication rate constant, P the power output, t the time and C the population density.

The experimental results indicate that the relation between population density and its power output can be characterized by the following thermal equations: $P = -91.422 + 0.006518C$ and $\bar{P} = -34.5026 + 3.6734 \ln C$, where $\bar{P}$ is the power output by unit population density. Therefore we can calculate the metabolic rate constant, $k = 0.008919$ min, the average generation time, $\bar{G} = 199.2$ min, and the power output of a single cell, $\bar{P}_{1/2} = 3.18$ nW per cell.

INTRODUCTION

It has recently been demonstrated that the microcalorimetric method can be used for fundamental studies of the growth metabolism of cells [1,2]. The metabolic thermograms, continuously monitored with a microcalorimeter, can provide information on the metabolism, and are very significant for the study of cell metabolic processes. For example, we can establish some relationship between the bio-thermokinetics and thermodynamics of cell metabolism.

Tetrahymena pyriformis belongs to the protozoa family and is widely distributed. In general it is collected from polluted water, and can be

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[1] Author to whom correspondence should be addressed.

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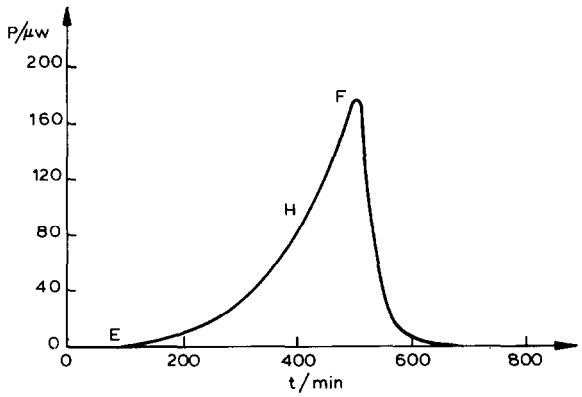

Fig. 1. Growth thermogenesis curve of *Tetrahymena pyriformis* (25 °C)

separately cultured into a bacteria-free species because it is easily cultured and preserved in the laboratory. *T. pyriformis* is a eukaryotic monocellular animal, i.e. an animal with only one cell. It can be used as a biological indicator in freshwater biology and environment pollution studies. At the same time it has been used widely as a "test animal" to monitor and evaluate toxicants, nutrients, antibiotics, anti-cancer medicaments etc. [3].

We studied the metabolic processes of *T. pyriformis* in culture media and recorded the thermograms (see Fig. 1). From these thermograms it can be established that the thermokinetic equation for the growth metabolism (log phase) is $dP/dt = k_m P$, with order of metabolism $n = 1$, or

$$\ln P_t = 0.7913 + 0.008919t \qquad P_t = P_0 e^{k_m t} \tag{1}$$

where k_m is the metabolic rate constant, P the power output and t the time. The experimental results also indicate that the relationship can be characterized by the following thermal equations:

$$P = -91.422 + 0.006518C \qquad \text{or} \qquad P = a + KC \tag{2}$$

$$\overline{P} = -34.5026 + 3.6734 \ln C \tag{3}$$

where $\overline{P}$ is the power output of unit population density and C is the population density. By this microcalorimetric method we can calculate the rate constant, $k_m = 0.008919\ \text{min}^{-1}$, and the average generation time parameter of energy metabolism, $\overline{P}_{1/2} = 3.18$ nW per cell. These results are very significant in biology and thermochemistry.

INSTRUMENT AND MATERIALS

Instrument

An LKB-2277 Bioactivity Monitor was used to obtain the metabolic thermograms of *T. pyriformis*. The performance of this instrument and the details of its construction have been described previously [4,5].

Materials

Tetrahymena pyriformis (BJ4, mononuclear) was provided by the Department of Biology, Beijing University. The culture medium was a solution containing the nutrients peptone (1 wt.%), beef extract (0.1 wt.%) and glucose (0.5 wt.%).

EXPERIMENTAL METHOD

The bacteria-free *T. pyriformis* species, which had been cultured pure, was added to 80 ml of liquid medium at a population density of about 4000 cells ml^{-1}, and cultured at 25 °C by the cycle–flow method. A schematic representation of the experimental apparatus has been given previously [4]. The preparation was monitored and its thermogenesis curve was obtained.

When the pen of the chart recorder starts rising, this indicates that the *T. pyriformis* cells are entering an exponential growth state. A sample (3.0 ml) was removed at this stage, 1 ml of 1% formaldehyde solution was added to kill the organism, and the population density was measured with a haemocytometer.

RESULTS

The metabolic thermogenesis curve is shown in Fig. 1, and the corresponding P (μW) and C (cells ml^{-1}) vs. t (min) data (log phase EF) are given in Table 1.

TABLE 1

$P-t$ and $C-t$ data for growth metabolism of *Tetrahymena pyriformis* (25 °C)

t(min)	P(μW)	ln P	C(cells per 0.6 ml) [a]	ln C	$\bar{P}$(nW per cell per 0.6 ml) [b]
200	13.7	2.617	14700	9.595	0.93
250	18.6	2.923	16300	9.699	1.14
300	30.2	3.407	19500	0.878	1.55
350	51.9	3.949	23200	10.052	2.24
400	91.6	4.517	27800	10.233	3.29
450	121.7	4.802	33000	10.404	3.69
500	172.8	5.152	39300	10.579	4.40

[a] Flow cell volume, 0.6 ml.
[b] $\bar{P} = P/C$.

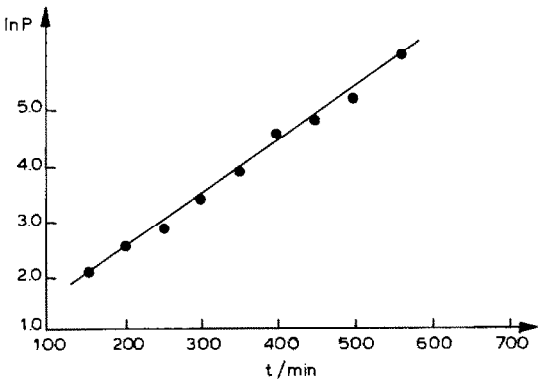

Fig. 2. Linear relation of ln P vs. t.

The thermokinetic equation of metabolism

From the data in Table 1, it is clear that the relationship ln P_t vs. t is satisfied in the linear equation $\ln P_t = 0.7913 + 0.008919t$, with correlation coefficient $r = 0.9954$ (see Fig. 2). More particularly, we can say that the P vs. t relationship is given by the thermokinetic equation $\mathrm{d}P/\mathrm{d}t = k_m P$, or $P_t = P_0\ \mathrm{e}^{k_m t}$, with order of growth metabolism $n = 1$. The metabolic rate constant is $k_m = 0.008919\ \mathrm{min}^{-1}$.

The thermal equation of metabolism

The P (μW) and C (cells ml^{-1}) data in Table 1 indicate that P and C are linearly related (see Fig. 3). The corresponding linear equation is

$$P = -91.4221 + 0.006561C \qquad r = 0.9939$$

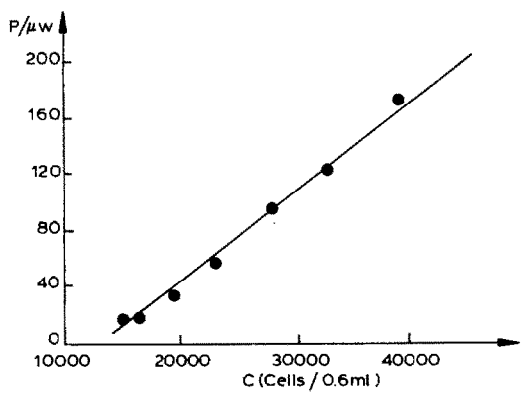

Fig. 3. Linear relation of P vs. C.

The $\bar{P}$ (nW per cell ml^{-1}) and ln C $\bar{P}$ taken from Table 1 fit the following linear equation:

$$\bar{P} = -34.5026 + 3.6734 \ln C \qquad r = 0.9927$$

This result indicates that $\bar{P}$ (power output by a single cell) depends on the population density.

Average generation time $\bar{G}$ *of* T. pyriformis

We can calculate the generation time by the following method. From the thermokinetic equation (eqn. (1)), $P_i = P_0 \, e^{k_m t_i}$, and the thermal equation (eqn. (2)), $P_i = KC_i + a$, we can obtain

$$KC_i + a = P_0 \, e^{k_m t_i}$$

For one generation, $C_{i+1} = 2C$ and $t_{i+1} - t_i = G_i$; thus

$$\frac{KC_{i+1} + a}{KC_i + a} = \frac{P_0 \, e^{k_m t_{i+1}}}{P_0 \, e^{k_m t_i}}$$

$$2 - \frac{a}{KC_i + a} = e^{k_m G_i} \qquad 2 - \frac{a}{P_i} = e^{k_m G_i}$$

$$G_i = \frac{\ln(2 - a/P_i)}{k_m} \tag{4}$$

where P_i is the power output at t_i, G_i is the corresponding generation time at t_i, and $a = 91.422$ μW (see eqn. (3)).

In order to calculate the average generation time G of the whole log phase time, the corresponding average power output P_a has to be known. From Fig. 1 and eqn. (1)

$$P_a = \frac{\int_0^t P_0 \, e^{k_m t} \, dt}{t} = \frac{1}{t} \int_0^t 2.2062 \, e^{0.008919t} \, dt$$

where t, the whole log phase time, corresponding to curve EF, equates to 410 min. Thus

$$P_a = \frac{1}{410} \times 2.2062 \times \frac{1}{0.008919} e^{0.008919 \times 410} = 23.37(\mu W)$$

$$G = \frac{\ln(2 - a/P_a)}{k_m} = \frac{\ln[2 - (-91.44/23.37)]}{0.008919} = 199.2(\text{min})$$

In this experiment the population density C was determined as a function of t and the data are given in Table 1. From the ln C vs. t data we can obtain the multiplication rate equation as follows:

$$\ln C_t = 8.8836 + 0.003369t \qquad r = 0.9983$$

or

$$C_t = C_0 \, e^{k_g t} \tag{5}$$

and the multiplication rate constant, $k_g = 0.003369$ min^{-1}.

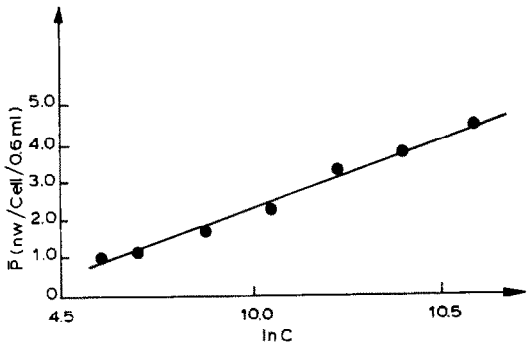

Fig. 4. Linear relation of $\bar{P}$ vs. ln C.

According to this method the generation time is

$$G = \ln 2/k_g = 0.6931/0.003369 = 205.7 \text{ (min)}$$

This result is close to $G = 199.2$ min, obtained by the microcalorimetric method, thus verifying the accuracy of the experiments.

Metabolic power of a single T. pyriformis *cell*

From Fig. 4 and the P data in Table 1, it is clear that the metabolic power of a single *T. pyriformis* cell is not constant but increases directly with ln C. In order to obtain a characteristic P, value we take $\bar{P}_{1/2}$ as the characteristic value of metabolic power of a single cell. Therefore, from Fig. 1 we can find a point H, which is at the halfway point on the power of growth peak, and $P = 86.4$ μW, so that we obtain $\bar{P}_{1/2} = 3.18$ nW per cell by using the interpolation method.

CONCLUSION

The experimental results demonstrate that microcalorimetry is a good method for metabolic studies. Using this method we can obtain perfect thermogram curves closely related to the metabolic processes. From these thermograms, thermokinetic and thermal equations for the metabolic processes can be established. These equations thus show some kinetic and energy metabolic relationships of the metabolic processes. Such important parameters as metabolic rate constant k_m, power output $\bar{P}_{1/2}$ and generation time $\bar{G}$ are very important in biology and thermochemistry.

REFERENCES

1 W.W. Forrest, in J.R. Norris and D.M. Robbins (Eds.), Biochemical Microcalorimetry, Academic Press, New York, 1969.

2 C.-L. Xie, H.-K. Tang, Z.-H. Shong and S.-S. Qu, Thermochim. Acta, 123 (1988) 33.
3 Z.-X. Zhang, Proc. 4th Symp. of the Chinese Protozoological Society, Wuhan, China, 10–14 November, 1987.
4 C.-L. Xie, D.-U. Sun, Z.-H. Shong and S.-S. Qu, Thermochim. Acta, 142 (1989) 211.
5 J. Suurkuusk and I. Wadso, Chem. Scr., 20 (1982) 155.