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THERMOCHEMICAL STUDIES OF CELL METABOLISM. PART 2. THERMAL EQUATION OF ENDOGENOUS METABOLISM

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ABSTRACT

An LKB-2277 Bioactivity Monitor was used to determine thermograms of the endogenous metabolism of *E. coli*. The experimental results indicate that the relation between cell concentration and power output can be characterized by the equations

$$\begin{cases} C = kP + a & (1) \\ dC/dP_0 = KC^0 & (2) \end{cases}$$

where P is the power output (μW), C is cell concentration (mg ml^{-1}), P_0 is the power output produced by the metabolism of a single cell ($P_0 = P/C$), and k, a and K are constants which depend on the culture conditions and physiogenic state of the cells.

The physical meaning of the group equation can be explained as follows: eqn. (1) indicates that the endogenous metabolic power and cell concentration conform to a linear relation; eqn. (2) is a zero order equation which indicates that P_0 is independent of cell concentration, i.e. it is not inhibited by cell density. This is very different from the metabolism of resting cells.

INTRODUCTION

A thermal equation for the metabolism of resting cells has been reported in a previous paper [1]. In the present partial work, we used an LKB-2277 Bioactivity Monitor to determine thermograms of the endogenous metabolism of *E. coli*. The experimental results indicate that the relation between cell concentration and power output can be characterized by the equations

$$\begin{cases} C = kP + a & (1) \\ dC/dP_0 = KC^0 & (2) \end{cases}$$

where P is the power output (μW), C is cell concentration (mg ml^{-1}), P_0 is the power output produced by the metabolism of a single cell ($P_0 = P/C$),

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and k, a and K are constants which depend on the culture conditions and physiogenic state of the cells.

For the endogenous metabolism of *E. coli* growing on pepton medium, the thermal equations can be written as

$$\begin{cases} C = 0.01085P + 0.00004708 & (1a) \\ P_0 = 92.0 & (2a) \end{cases}$$

The physical meaning of the group equation can be explained as follows: eqn. (1) indicates that the metabolic power and cell concentration conform to a linear relation; eqn. (2) is a zero order equation which indicates that P_0 is independent of cell concentration, i.e. it is not inhibited by cell density. This is very different from the metabolism of resting cells.

EXPERIMENTAL

Instruments

An LKB-2277 Bioactivity Monitor was used to determine the metabolic power of cells. The performance of this instrument and the details of its construction have been described in previous works [2–4].

Materials

Cells: *Escherichia coli* (ACCT).

Pepton medium: containing per 1000 ml NaCl, 5 g; K_2HPO_4, 2g; *p*-aminobenzoic acid, 0.02 g; pepton, 5 g; sodium citrate, 12 g; $MgSO_4$, 5 g; tryptone, 5 g; beef extract, 3 g; tryptose, 5 g; and NaOH (2 M), 2.5 ml.

Buffer: pH = 7, containing per 1000 ml 0.2 M KH_2PO_4, 66 ml; 0.2 M K_2HPO_4, 144.5 ml; NaCl, 0.5 g; $MgSO_4$, 0.12 g; $CaCl_2$ and $FeCl_2$, microadd.

All the above materials were provided by the Army Hospital of the Kwangchow Military District.

Procedure

Sample preparation

The *E. coli* strain was inoculated into the pepton medium (37 °C) and cultured by the cycle-flow method [2], with continuous monitoring of the thermogenesis curve. As the cells were going into the log phase, a series of bacterial samples (10 ml per sample) were taken simultaneously. The samples were separated by centrifuging (10^4 r.p.m.), and the residual medium then washed away with buffer solution. This separation process was re-

peated twice. Finally, the bacterial cells were suspended in buffer solution as samples of known concentration, and stored in the refrigerator (0 °C). Samples of this kind need to be used within 12 h, to avoid any change in the physiogenic state of the cells.

For each measurement of cell concentration, three bacterial samples (10 ml per sample) were removed from the culture medium and centrifuged separately. The residual medium was then washed away with distilled water, dried and weighed. The average dry weight of cells (mg ml^{-1}) could then be calculated.

Determination of endogenous metabolism

Endogenous metabolic thermograms of *E. coli* were determined by the experimental method (stop-flow method) described in a previous work [2]. The bacterial sample (of known concentration) was pumped into the flow cell. When the flow cell (volume 0.6 ml) was full, the pump was stopped but the monitor continued to record the thermograms. As the buffer solution does not contain nutrients, the bacteria are forced to metabolise nutrients inside the cells. The thermograms obtained by this method are called endogenous metabolic thermogenesis curves.

RESULTS

Endogenous metabolic thermogenesis curves of cell samples of different concentrations are shown in Fig. 1. From these figures we can see that each

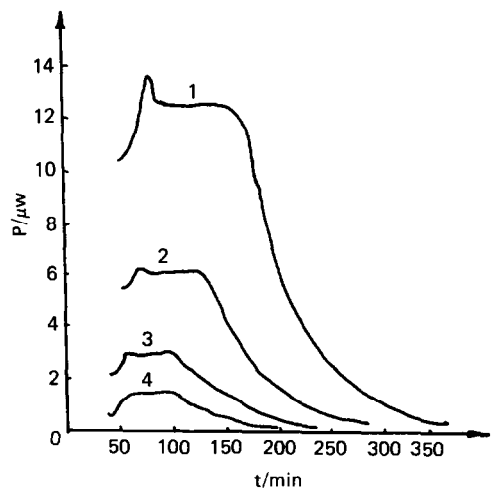

Fig. 1. Thermogenesis curves of the endogenous metabolism of *E. coli* (37 °C). 1, $C = 0.140$ mg ml^{-1}; 2, $C = 0.070$ mg ml^{-1}; 3, $C = 0.035$ mg ml^{-1}; 4, $C = 0.018$ mg ml^{-1}.

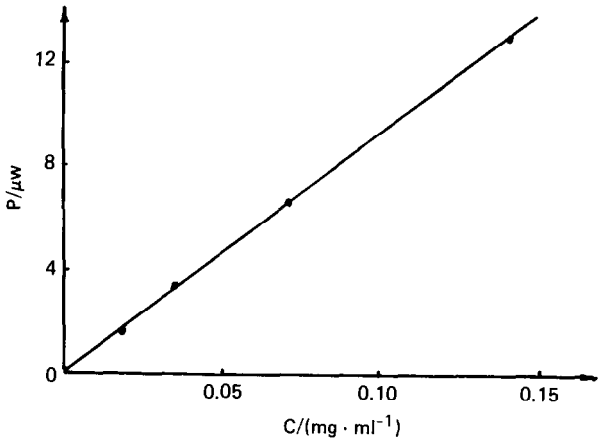

Fig. 2. $C-P$ relation for the endogenous metabolism of *E. coli*.

TABLE 1

$P-C$ data for endogenous metabolism of *E. coli* (37 °C)

Variable	Experiment no.			
	1	2	3	4
P (μW)	12.90	6.40	3.25	1.65
C (mg (0.6 ml)$^{-1}$) [a]	0.140	0.070	0.035	0.018
P_0 (μW mg^{-1} (0.6 ml)$^{-1}$)	92.1	91.4	92.8	91.7

[a] Flow cell volume $V = 0.6$ ml (realistic monitoring volume).

curve has a stationary phase, indicating steady endogenous metabolic power output at that period. Corresponding P vs. C data are given in Table 1.

The P vs. C data of Table 1 indicate that P and C are linearly related, as follows: $C = 0.01085P + 0.00004708$, with correlation coefficient $r = 0.9999$ (see Fig. 2).

Fitting the P_0 vs. C data from Table 1 to a linear equation (see Fig. 3), we obtain another equation, $dC/dP_0 = KC^0$, or $P =$ constant, a zero order equation. So the endogenous metabolic power output of a single cell is a constant value, in this case $P_0 = 92.0 \pm 0.50$ μW mg^{-1} (0.6 ml)$^{-1}$.

CONCLUSIONS

The power output of the endogenous metabolism of *E. coli* has been determined. The experimental results indicate that the relation between cell concentration and power output can be characterized by the equations

$$\begin{cases} C = 0.01085P + 0.00004708 \\ P_0 = 92.0 \end{cases} \quad \text{or} \quad \begin{cases} C = kP + a \\ dC/dP_0 = KC^0 \end{cases}$$

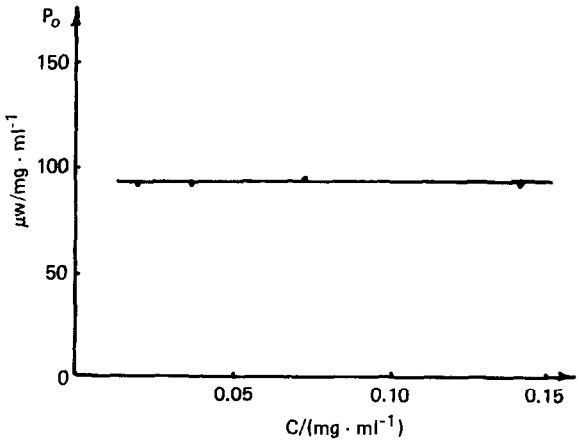

Fig. 3. $C-P_0$ relation for the endogenous metabolism of *E. coli*.

This group equation shows that the endogenous metabolic power and the cell concentration conform to a linear relation, but P_0 (single cell metabolic power) is independent of the concentration, i.e. it is not inhibited by cell density, there is no space effect. It is thus different from other kinds of metabolism.

This kind of equation for endogenous metabolism may have greater generality. As an example, we consider the endogenous metabolic thermograms of yeast determined by Nanomara and Fujita of Tokyo University, Japan [5]. The corresponding P (mW) vs. C (dry weight of cells in mg) data are represented in Fig. 4.

From Fig. 4 we can obtain the data given in Table 2 for the endogenous metabolism of yeast, and the process can be characterized by the following

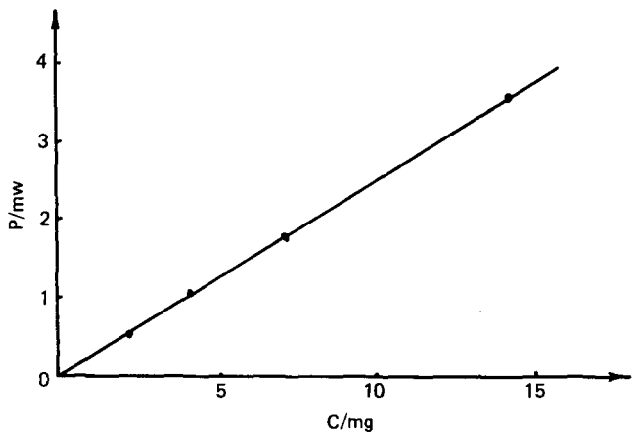

Fig. 4. $C-P$ relation for the endogenous metabolism of yeast.

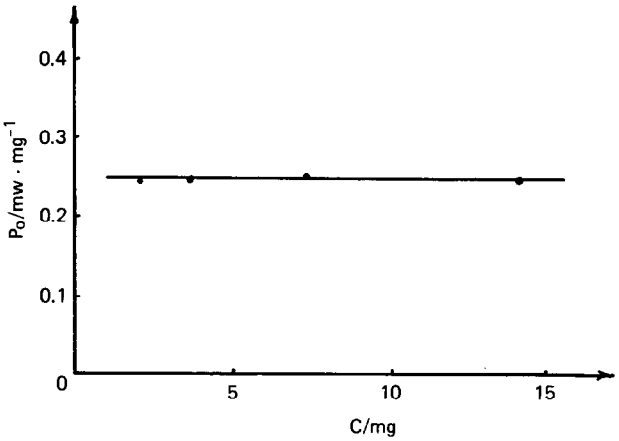

Fig. 5. $C-P_0$ relation for the endogenous metabolism of yeast.

TABLE 2

$P-C$ data for endogenous metabolism of yeast (30 °C)

Variable	Experiment no.			
	1	2	3	4
P (mW)	3.43	1.72	0.98	0.49
C (mg)	14	7	4	2
P_0 (mW mg^{-1})	0.245	0.245	0.245	0.245

equations:

$$\begin{cases} C = 4.0814P - 0.0046 & r = 0.9999 \\ P_0 = 0.245 \end{cases} \quad \text{or} \quad \begin{cases} C = k_1 P + a \\ \mathrm{d}C/\mathrm{d}P_0 = K_1 C^0 \end{cases}$$

It is clear that the endogenous metabolic equations for yeast have the same form as those for *E. coli*.

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