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A study of the optimum fungistatic action of a synthetic medicine using a microcalorimetric method

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Abstract

In this paper, we have determined bacterial growth thermograms using a thermal activity monitor. We have also determined bacterial growth thermograms of inhibitor and have studied the fungistatic action of a synthetic medicine (W2). We have calculated the rate constant at different concentrations and with the optimum allowable concentration of the synthetic medicine W2.

INTRODUCTION

In any living system, the various metabolic events occurring within the cells are all reactions producing heat. We can study the metabolic process of living cells by continuous measurement of the heat effect of the growing cells using a calorimeter. We have recorded the bacterial growth thermograms using a thermal activity monitor.

Thermograms were determined under inhibitory conditions of constant temperature, volume, nutrient matter and dissolved oxygen. We have established the experimental model of bacterial growth and determined the thermograms of *Staphylococcus aureus* and *Escherichia coli* with different concentrations of a synthetic medicine. We also calculated the growth rate constant and optimum allowable concentration of the synthetic medicine.

INSTRUMENT

A new typye of heat-flow microcalorimeter, the 2277 thermal activity monitor (ThermoMetric AB, Sweden) was used in this experiment in the flow-through mode. The sample was pumped through the flow cell by a 2132 microperspex peristaltic pump.

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EXPERIMENTAL METHOD

The complete cleaning and sterilization procedure for the flow tubing was as follows. Sterilized distilled water, 0.1 M HCl, and alcohol solution (75%) were pumped through the system for 30 min at a flow rate of 30 ml h^{-1}. Once the system had been cleaned and sterilized, the baseline was determined.

When a stable baseline had been obtained, the bacterial sample, medium and the synthetic medicine were pumped into the flow cell system and the monitor began to record the bacterial thermogram of continuous growth. When the recording pen returned to the baseline and became stabilized, the process of bacterial growth was complete.

MATERIALS

The bacteria *Staphylococcus aureus* and *Escherichia coli* were employed.

A soluble medium (pH 7.2–7.4) was used, containing NaCl (1 g), peptone (2 g) and beef extract (1 g) in each 200 ml.

Each 1 ml of soluble medium contained a different concentration of synthetic medicine (W2), the concentration being $A \times 0.05$ mg ml^{-1} where A is the volume of W2 in μl and the bacterial number is 10^7 cells.

DETERMINATION OF OPTIMUM GROWTH TEMPERATURE

We have determined the growth rate constants of *S. aureus* and *E. coli* at different temperatures. From these results, we can establish the non-linear equation $\mu = a + bT + CT^2$. When $\partial\mu/\partial T < 0$ and $\partial^2\mu/\partial T^2 = 0$, $T \approx 310$ K, which is the optimum growth temperature [1].

ESTABLISHMENT OF THE EXPERIMENTAL MODEL OF BACTERIAL GROWTH

For inhibitory conditions, bacterial numbers and time in the growth phase have the relationship [2]

$$dN(t)/dt = \mu N(t) - \beta N^2(t) \quad (1)$$

where μ is the growth rate constant, β the deceleration rate constant, and $N(t)$ the bacterial number at time t.

Thus, the power evolved in growth, if that of a single bacterium is P_0, is represented by

$$P(t) = P_0 N(t) \quad (2)$$

Therefore

$$dP(t)/dt = \mu P(t) - (\beta/P_0)P^2(t) \quad (3)$$

The integral equation is given by

$$P(t)^{-1} = a\, e^{-\mu t} + b \quad (4)$$

where $a = 1/P_0 - (\beta/\mu P_0)$ and $b = \beta/\mu P_0$.

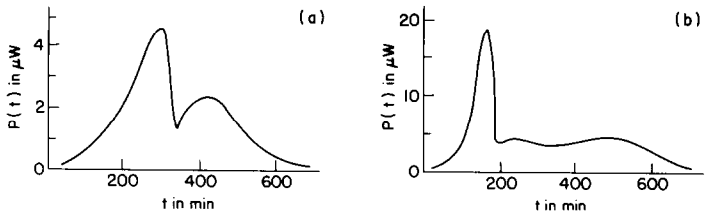

Fig. 1. Thermograms: (a) *S. aureus* with 2.5 mg ml^{-1} inhibitor; and (b) *E. coli* with 3 mg ml^{-1} inhibitor, both at 310 K.

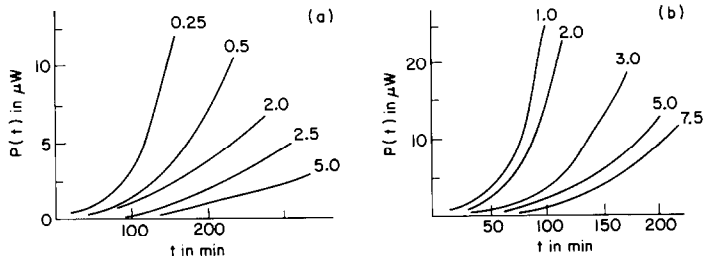

Fig. 2. Thermograms: (a) *S. aureus* and (b) *E. coli* at 310 K with different concentrations of inhibitor (W2 medicine) in mg ml^{-1}.

We determined the thermograms of *S. aureus* and *E. coli* (see Figs. 1 and 2). Using the data $P(t)$ and t obtained from the bacterial growth curve, fitted to the non-linear equation, we can obtain the growth rate constant μ at 310 K. The data obtained with inhibitor are shown in Tables 1 and 2.

TABLE 1

$P(t)$, $\hat{P}(t)$ and t values with inhibitor at 310 K

S. aureus 2.5 mg/ml			*E. coli* 3 mg/ml		
t/min	$P(t)/\mu$W	$\hat{P}(t)/\mu$W	t/min	$P(t)/\mu$W	$\hat{P}(t)/\mu$W
50	0.2	0.21	25	0.4	0.37
75	0.3	0.32	50	0.8	0.80
100	0.5	0.48	75	1.7	1.70
125	0.7	0.72	100	3.5	3.50
150	1.0	1.05	125	6.5	6.84
175	1.5	1.50	150	12.3	12.20
200	2.1	2.08	175	19.6	19.06
225	2.9	2.77			
250	3.6	3.53			
275	4.4	4.30			
300	4.9	5.00			

Key: $P(t)$ is the experimental data; $\hat{P}(t)$ is the data calculated from the model.

TABLE 2

Growth rate constants μ with different concentrations C of inhibitor (W2 medicine)

S. aureus		*E. coli*	
C/mg ml^{-1}	μ/min^{-1}	C/mg ml^{-1}	μ/min^{-1}
0.25	0.02684	1.0	0.03629
0.5	0.02459	2.0	0.03398
2.0	0.01751	3.0	0.03109
2.5	0.01739	5.0	0.02302
5.0	0.01332	7.5	0.02183

The corresponding non-linear equation of the experimental model at 310 K for *S. aureus* is

$$P(t)^{-1} = 11.0530\,e^{-0.01739t} + 0.1401 \quad \text{for} \quad t \leqslant 300$$

with $\mu = 0.01739$, at an inhibitor concentration of 2.5 mg ml^{-1}. For *E. coli*

$$P(t)^{-1} = 5.7862\,e^{-0.03109t} + 0.02737 \quad \text{for} \quad t \leqslant 175$$

with $\mu = 0.03109$, at an inhibitor concentration of 3 mg ml^{-1}.

In a similar way, we can also calculate the growth rate constant with different concentrations of inhibitor; the data are shown in Table 2.

CALCULATION OF THE OPTIMUM ALLOWABLE CONCENTRATION OF SYNTHETIC MEDICINE

We have determined the thermograms of bacterial growth and have calculated the rate constants μ of *S. aureus* and *E. coli* with different concentrations of medicine (inhibitor). From these results, we can establish the linear equation $\mu = A + BC$. For *S. aureus*

$$\mu_a = 0.03 - 2.7617 \times 10^{-3} C_a \qquad R = 0.9405$$

For *E. coli*

$$\mu_b = 0.04 - 2.4116 \times 10^{-3} C_b \qquad R = 0.9598$$

When $\mu_a = 0$, $C_a = 10.86$ mg ml^{-1}; and when $\mu_b = 0$, $C_b = 16.59$ mg ml^{-1}. C_a and C_b are the optimum allowable concentrations and R is the correlation coefficient.

CONCLUSIONS

These thermograms contain much information. We have established an experimental model of bacterial growth under inhibitory conditions in

order to calculate the growth rate constant with different concentrations of synthetic medicine and also the optimum allowable concentration.

These data are very useful in studies of fungistatic action on the growth of microorganisms.

REFERENCES

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