STUDIES ON THE ORIGIN OF BACTERIAL VIRUSES

V. THE EFFECT OF TEMPERATURE ON THE TERRAMYCIN-RESISTANT AND PHAGE-PRODUCING CELLS OF BACILLUS MEGATHERIUM CULTURES

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ABSTRACT

The growth rates, the mutation frequency rate constants of the terramycin-resistant cells, the burst size of the phage-producing cells, and the ratio of phage to cells all have a temperature coefficient of about 2 from 20 to 35° (μ = 9 × 10⁴ calories), with a maximum at 40°.

The mutation frequency rate constant (or time rate constant) of the phage-producing cells increases from 20 to 45° with a temperature coefficient of about 3 (μ = 2 to 3 × 10⁴ cal.).

The change in the values for the growth rate, mutation rate, and cell volume occurs in less than 1 hour, after the temperature is changed. The value for the burst size of phage-producing cells changes for 3 to 4 hours.

Prolonged growth of *megatherium* 899 at 48 to 50° results in the production of C + S phage, in place of T. Returning the culture to 25° results in the production of small T phage.

The growth rate, the incidence of terramycin-resistant cells and of phage-producing cells, their mutation rates, and the burst size of the phage-producing cells in *B. megatherium* cultures were determined at various temperatures. The results of these experiments are shown in Fig. 1.

The growth rate, the mutation frequency rate of the terramycin-resistant cells, the burst size of the phage-producing cells, and the ratio of phage to cells all have a temperature coefficient of about 2 per 10° (μ = about 1 × 10⁴ cal.) between 20 and 35° with a maximum at 40°. This is the usual type of curve for enzymes or biological processes (Johnson, Eyring, and Polissar, 1954). The mutation frequency rate constant for the phage-producing cells increases over the entire range with a temperature coefficient of about 3 per 10°, corresponding to a value for Arrhenius' constant μ of 2.3 × 10⁴ cal. (Fig. 2). The values for the 1957 series for both types of cells are higher than those observed in 1958, but
the temperature coefficient is the same in both series. This value is lower than that reported for other mutation rates (cf. Goldschmidt, 1955).

![Graph showing temperature vs. ratio of terramycin-resistant cells and phage to cells, burst size, growth rate, and mutation rates.](image)

**FIG. 1.** The ratio of terramycin-resistant cells and of phage to cells, the burst size of phage-producing cells, the growth rates, and the mutation rates of terramycin-resistant cells and phage-producing cells in cultures of *B. megatherium* growing at different temperatures.

The mutation frequency rate constant expresses the ratio of mutants to new cells, and has the dimensions of an equilibrium constant rather than those of a velocity constant. There is some uncertainty, therefore, in interpreting the results obtained when this value is used as a velocity constant.
The terramycin-resistant mutants appear as a result of cell division (Northrop, 1957 a) and the experiments may be taken to show that the proportion of terramycin-resistant mutants produced at any temperature is proportional to the growth rate at that temperature.

The phage-producing cells, however, appear as a result of a change in the cells which occurs without cell division (Northrop, 1957 b, 1958). The mutation time rate constant \( C \) is therefore the significant value. This constant has the dimensions of a true velocity constant; i.e., fraction per unit of time (Northrop and Kunitz, 1957, p. 127). The temperature coefficient of this constant is about 3 per 10°, corresponding to a value of 3.3 \times 10^4 \text{ cal. for Arrhenius’ constant (Fig. 2).}

The ratio of phage to cells at equilibrium shows a maximum at 40°, but this is the result of a maximum in burst size.

The equilibrium ratio of mutant cells to wild cells is given by the relation \( \frac{M}{W} = \frac{2\lambda A}{A - B} \) in which \( A \) is the growth rate of the wild cells and \( B \) is the growth rate of the mutant, and \( \lambda = \) the mutation frequency rate constant (Northrop and Kunitz, 1957).

In the case of phage-producing cells this becomes \( \frac{P}{W} = 2\lambda \), since the mutants do not grow and \( M = \frac{P}{l} \) (Northrop, 1958).
The values obtained in this way approximate the observed values (Table I). Both observed and calculated values vary quite widely in different experiments. The calculated value for terramycin-resistant mutants depends upon the

<table>
<thead>
<tr>
<th>Temperature</th>
<th>25°</th>
<th>40°</th>
<th>45°</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR X 10⁶  (\frac{W}{W})</td>
<td>Observed</td>
<td>7 to 10</td>
<td>6 to 8</td>
</tr>
<tr>
<td>2mA (A-B)</td>
<td>Calculated</td>
<td>6 to 10</td>
<td>30 to 50</td>
</tr>
<tr>
<td>P/W</td>
<td>Observed</td>
<td>1 to 2</td>
<td>3 to 7</td>
</tr>
<tr>
<td>(2\lambda_{d})</td>
<td>Calculated</td>
<td>0.05 to 0.2</td>
<td>0.6 to 2</td>
</tr>
</tbody>
</table>

Fig. 3. The effect of change of temperature on cell volume, growth rate, mutation rate, burst size, and ratio of phage to cells in cultures of *megatherium* 899 in yeast extract peptone.
Effect of Changes in Temperature on Cell Volume, Growth Rate, Mutation Rate, Burst Size, and Ratio of Phage to Cells

The results of an experiment in which 899 in logarithmic growth in yeast extract peptone at 25 ° was placed at 40 ° are shown in Fig. 3. The culture was maintained at constant cell concentration of 1 to 10 × 10⁷ cells per ml. by repeated dilution. The results at 40 ° are expressed in per cent of the corresponding values at 25 °. The growth rate increased twice in a few minutes. The cell volume and the mutation rate change in less than 1 hour, but the burst size and the ratio of phage to cells continue to increase for 4 hours.

Change in Phage Type at 48 to 50 °

At temperatures from 20 to 45 ° in yeast extract peptone medium, 899 produces almost entirely T phage with small numbers of C, S, and others (Murphy, 1954). If the organism is grown at 48 to 50 ° in the steady state apparatus, however, the C, S, and Br phages increase after 5 to 7 days, until they make up one-third to one-half the total phage titer. The change is very similar to that which occurs when 899 becomes adapted to ammonium sulfate culture medium (Northrop and Murphy, 1956). The colonies become wrinkled at the same time. Tests of separate colonies show that some form only T's, some T and C + S, and some no phage which can be detected by KM. These latter colonies are resistant to either C or T phage, however, so that they probably are also lysogenic. The culture continues to produce C + S phages for some time when grown at 25 ° in yeast extract peptone in the steady state apparatus, but eventually the number of C + S plaques decreases and they are replaced by small T plaques.

The culture continued to form C + S plaques after 6 months' transfer at 25 ° on 2 per cent peptone agar slants.

Experimental Procedure

The experimental procedure was the same as that described in previous papers (Northrop, 1957 a, 1957 c, 1958).

The figures are the means of 6 to 20 experiments. The average deviation of the mean is about ± 20 per cent. The mutation rate of the terramycin-resistant cells and of the phage-producing cells was determined at the same time, or nearly the same time. This is necessary because of changes in the culture.

BIBLIOGRAPHY


1 One culture of 899 growing in yeast extract peptone in the steady state apparatus at 25 ° changed in 2 days to a faster growing mutant which formed very large spreading colonies, similar to the SP strain obtained in ammonium sulfate culture medium (Northrop, 1957 a). This mutant produced about the same T phage as 899. It was resistant to terramycin.

Murphy, J. S., 1954, *J. Exp. Med.*, 100, 657.


