The effect of temperature variation on the multiplication rate of two strains of *Amoeba proteus*

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**Summary**

The effect of temperature variation on the multiplication rates of two strains of *Amoeba proteus* and the corresponding heterotransfer strains obtained by transplantation of nuclei between the 'parent' strains, has been studied. The two 'parent' strains multiply at a similar rate at 17° C but at different rates at 27° C and 11° C. The multiplication rates of the heterotransfers follow the cytoplasmic 'parent' at the low temperature and the nuclear 'parent' at the higher temperature.

**Introduction**

The technique of nuclear transfer in amoebae has been used for the last 13 years to obtain information on the relative importance of nucleus and cytoplasm in maintaining characteristic strain differences. Earlier results which have been published (Lorch and Danielli, 1950, 1953; Danielli, Lorch, Ord, and Wilson, 1955; Danielli, 1958, 1959), indicated a greater degree of cytoplasmic control than would be expected from purely Mendelian inheritance. Danielli and others (1955) advanced the hypothesis that the nucleus controls the nature of the macromolecules synthesized, while the cytoplasm controls the way in which these macromolecules are organized.

In the present study the multiplication rates of two strains of *Amoeba proteus* were compared at 11° C, 17° C, and 27° C. Over this range of temperatures, mitotic division is normal (Daniel and Chalkley, 1932; James, 1959).

**Materials and methods**

The two strains of *A. proteus* used in this work were *TJP* ('Bristol' *A. proteus*) and *ZP* ('Zealand' *A. proteus*). *TJP* was originally supplied by Sister Monica Taylor of the Notre Dame Training College, Glasgow, to Bristol University, date unknown, and from there sent to Copenhagen in 1952. This strain was sent to King's College in 1955 and a clone established by Dr. M. Ord. *ZP* was collected in North Zealand in 1952 by Dr. C. Chapman-Andreessen and sent to King's College in 1955.

Heterotransfers between these strains were obtained in 1957 using a de Fonbrune micromanipulator and methods described earlier (Comandon and de Fonbrune, 1938; Lorch and Danielli, 1950). As in earlier publications (Lorch and Danielli, 1950), the following notation has been used: *P* indicates *proteus* species, suffixes *n* and *c* denote nucleus and cytoplasm respectively and the strains are indicated by prefixes. Thus the heterotransfers used were

\[ T_{P_n} Z_{P} P_{Z_n} T_{P_c} \]

Cultures were grown in modified Chalkley's medium (Chalkley, 1930), as described by Lorch and Danielli (1953) and kept as clones in a constant temperature chamber at 17±1°C. The experiments were carried out in constant temperature chambers at 11±1° C, 17±1° C, and 27±1° C.

Amoebae were picked out of mass cultures which were in the logarithmic phase of growth, that is, in the 5th to 6th weeks after establishment. Forty amoebae of each strain were kept singly at each of the 3 chosen temperatures. Each amoeba was placed in a solid watch-glass containing 1·5 ml. Chalkley's solution and a known concentration of Colpidium sp. The food solution and watch-glasses were changed twice a week; each time both food and dishes were cooled or heated to the required temperature to avoid any temperature lag.

Since the multiplication rate is high at 27° C, all amoebae were observed
every 12 h and the numbers of amoebae in each watch-glass recorded. Thus all the experimental amoebae had the same amount of disturbance. The watch-glasses were always observed in the same order to standardize the slight time-lag, which must otherwise occur when 400 dishes are observed.

The 11°C and 17°C experiments were run for 12 days, the 27°C experiments for 5 days. These experiments were repeated 3 times.

**Results**

The results obtained were plotted on semi-log graph paper, the rate of multiplication on the log scale and time on the linear scale. Theoretically, results plotted in this way should give a straight line, since normal growth in amoebae is exponential (figs. 1, A to D; 2, A, B; 3, A, B).

It can be seen that the points lie roughly on straight lines; the lines drawn were calculated by the method of least squares. The calculated line did not go through the origin in every case; the reasons for this lag have not been
investigated but it is probably due to the abrupt change of temperature at the beginning of the experiment.

The effect of temperature change is best seen by comparing the gradients of these lines. The gradients may be expressed as \( \tan \theta \); values of \( \theta \) and the differences between values of \( \theta \) at the temperatures used are shown in tables 1 and 2.

It can be seen that at 27°C, the multiplication rate of \( T_4P \) increased to a greater extent than did the rate of \( ZP \). At 11°C, the multiplication rate of \( ZP \) decreased to a greater extent than \( T_4P \). That is, the multiplication rate of \( ZP \) was less affected by high temperature and more affected by low temperature when compared with the multiplication rate of \( T_4P \).

The results obtained from the heterotransfers showed that at 27°C both transfers followed the nuclear ‘parent’, the multiplication rate of \( T_4P_n ZP_c \) being increased more than the multiplication rate of \( ZP_n T_4P_c \). However, at

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**Fig. 3.** Graphs A and B showing the multiplication rates of two heterotransfer strains of *A. proteus* at 11°C and 17°C. •—• 11°C. ○—○ 17°C.
Both transfers followed the cytoplasmic 'parent', the multiplication rate of $z P_n z P_c$ decreasing more than $P_n z A P_c$.

**Table 1**

Multiplication rates of amoebae expressed as $\theta^*$ at $27^\circ C$ and $17^\circ C$ for 5 days

<table>
<thead>
<tr>
<th>Strain</th>
<th>$\theta$ at $27^\circ C$</th>
<th>$\theta$ at $17^\circ C$</th>
<th>Difference in $\theta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z P$</td>
<td>$51^\circ 47'$</td>
<td>$39^\circ 10'$</td>
<td>$12^\circ 37'$</td>
</tr>
<tr>
<td>$P_n z$</td>
<td>$55^\circ 19'$</td>
<td>$33^\circ 49'$</td>
<td>$21^\circ 30'$</td>
</tr>
<tr>
<td>$z P_n z P_c$</td>
<td>$53^\circ 28'$</td>
<td>$32^\circ 31'$</td>
<td>$20^\circ 57'$</td>
</tr>
<tr>
<td>$z P_n z A P_c$</td>
<td>$50^\circ 56'$</td>
<td>$37^\circ 4'$</td>
<td>$13^\circ 52'$</td>
</tr>
</tbody>
</table>

* See text.

**Table 2**

Multiplication rates of amoebae expressed as $\theta^*$ at $17^\circ C$ and $11^\circ C$ for 11 days

<table>
<thead>
<tr>
<th>Strain</th>
<th>$\theta$ at $17^\circ C$</th>
<th>$\theta$ at $11^\circ C$</th>
<th>Difference in $\theta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z P$</td>
<td>$32^\circ 15'$</td>
<td>$15^\circ 30'$</td>
<td>$16^\circ 45'$</td>
</tr>
<tr>
<td>$P_n z$</td>
<td>$31^\circ 4'$</td>
<td>$19^\circ 56'$</td>
<td>$11^\circ 14'$</td>
</tr>
<tr>
<td>$z P_n z P_c$</td>
<td>$30^\circ 24'$</td>
<td>$14^\circ 29'$</td>
<td>$15^\circ 55'$</td>
</tr>
<tr>
<td>$z P_n z A P_c$</td>
<td>$31^\circ 8'$</td>
<td>$19^\circ 55'$</td>
<td>$11^\circ 13'$</td>
</tr>
</tbody>
</table>

* See text.

**Discussion**

As the heterotransfer clones used in the experiments were established at least a year before the experiments were performed, the results obtained indicate stable nuclear or cytoplasmic influences on the clones.

If the multiplication rate of any clone were determined by a single step, then we should find

\[
\text{rate of growth} = \tan \theta = \text{const} \times e^{-QIRT},
\]

and $\log \tan \theta$ should be a linear function of $1/T$. Results plotted in this manner show that rate of growth is not a linear function of $1/T$ (fig. 4).

As the rate of multiplication depends upon the rates of phagocytosis, digestion, synthesis, &c., any one of which may become rate-determining under the appropriate conditions, it is not surprising that the relationship of multiplication to temperature is complex. It is probable that at high temperatures the rate of multiplication is determined by the rate of synthesis, whereas at low temperatures it is determined by the rate of phagocytosis. Previous observations, that phenomena at the chemical level are controlled by the nucleus, whereas complex physiological processes are controlled by the cytoplasm, are compatible with this view.
Daniel and Chalkley (1932) found that nuclear division was possible over a wider range of temperature than cytoplasmic division. They produced results demonstrating the existence of a cytoplasmic factor governing division, which was temperature-controlled. The results obtained in our experiments support this contention and show that this cytoplasmic control was apparent even under the influence of a ‘foreign’ nucleus.

![Graph of log tan \( \theta_1/T \) for two strains of A. proteus and the corresponding heterotransfers at the three temperatures used in the experiments.](image)

The reaction to temperature variation by increase or decrease in multiplication rate is, in amoebae, under the dual control of nucleus and cytoplasm; at high temperatures the nucleus is dominant, at low temperatures the cytoplasm becomes important.

References