MULTIPLICATION AND THERAPY OF TOXOPLASMA GONDII
IN TISSUE CULTURE

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ABSTRACT

MALONEY, EMILY D. (University of Florida College of Medicine, Gainesville), AND HERBERT E. KAUFMAN. Multiplication and therapy of Toxoplasma gondii in tissue culture. J. Bacteriol. 88: 319-321. 1964.—The effect of temperature on the rate of multiplication of the RH strain of Toxoplasma gondii and the results of treatment with pyrimethamine, an antifolate, at various temperatures were studied in tissue culture. At 37°C incubation, the organism was found to have a lag time of 10.5 hr and a mean generation time thereafter of 4.85 hr for binary multiplication during the exponential phase of growth. At 34°C, the lag time was 12 hr and generation time was 5.26 hr. When the organisms were incubated at 31°C, the lag time was 12.5 hr and generation time was 11.1 hr. Treatment of infected monkey kidney tissue cultures with 0.05 mg per 100 ml of pyrimethamine at 37°C for 4 days killed all organisms and permitted survival of the mice which were injected with the cultures. When cultures were incubated at 34°C, a dose of 0.6 mg per 100 ml was required to spare any mice, and with a 31°C incubation even this dose was only slightly effective when therapy was continued for 4 days. If the treatment was prolonged for 7 days before injection of the cultures into mice, however, no difference in therapy with different rates of multiplication was found. Since the rates of multiplication at 34 and 31°C are similar to the rates of multiplication of some naturally occurring strains, these data confirm the fact that prolonged therapy, as used by many, is rational.

Previous studies suggested that Toxoplasma gondii isolates from different sources (different strains) respond differently to antimetabolite drugs, and that this may be related to the rate of multiplication of the organisms (Kaufman et al., 1959). Toxoplasma is an obligate intracellular parasite, but is one of the few that is large enough (approximately 2 μ wide by 7 μ long) to observe within cells and to count with an ordinary light microscope. It is amenable to therapy with pyrimethamine and sulfonamides, both of which interfere with folic acid metabolism and block nucleotide synthesis in the organism. Organisms which multiply slowly have reduced requirements for nucleotides and these drugs should be less effective, but the importance of such an effect has not been defined.

Previous work indicated that slower multiplying strains of Toxoplasma are, in fact, more resistant to therapy with the antifolate agent pyrimethamine. Such studies are difficult with naturally occurring strains, however, because other properties also vary; for example, organisms from slowly multiplying strains are difficult to obtain in appreciable quantities from mouse peritoneal fluid. In this study, multiplication rate was regulated in a single strain by changing temperature, and the relationship between multiplication rate and pyrimethamine therapy was studied.

MATERIALS AND METHODS

Stationary monkey kidney tissue culture tubes were infected with 160,000 RH organisms. Growth curves of the organisms at the different temperatures were determined by staining the cultures and counting the organisms within cells after appropriate periods of incubation, as previously described (Kaufman and Maloney, 1961). Treatment was evaluated by incubating similar cultures with the organisms for 4 hr at 37°C to allow invasion of the cells, washing with fresh medium, and incubating with the appropriate concentrations of pyrimethamine at the desired temperatures for either 4 or 7 days. Survival of the organisms after drug treatment was determined by mouse inoculation of the treated tissue. The presence of live Toxoplasma organisms was ascertained by examining the peritoneal fluid or liver of all mice that died.

RESULTS

At 37°C, the organism multiplication had an apparent lag time of 10.5 hr and a mean generation
TABLE 1. Multiplication of the RH strain of Toxoplasma gondii at various temperatures*

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>18 C</th>
<th>28.5 C</th>
<th>31 C</th>
<th>34 C</th>
<th>37 C</th>
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<tr>
<td>1</td>
<td>1.39</td>
<td>1.39</td>
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<td>1.25 ± 0.11</td>
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<td>3.68</td>
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<td>19</td>
<td>3.05 ± 0.33</td>
<td>3.32 ± 0.01</td>
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<tr>
<td>20</td>
<td>2.99 ± 0.35</td>
<td>4.87 ± 0.50</td>
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<td>21</td>
<td>3.51 ± 0.53</td>
<td>8.09 ± 0.69</td>
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<tr>
<td>22</td>
<td>1.67 ± 0.04</td>
<td>3.98 ± 0.12</td>
<td>8.52 ± 0.41</td>
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</tr>
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<td>24</td>
<td>2.42 ± 0.50</td>
<td>5.75 ± 0.46</td>
<td>11.20 ± 1.28</td>
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<td>26</td>
<td>2.86 ± 0.26</td>
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<td>28</td>
<td>6.28 ± 0.64</td>
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<tr>
<td>30</td>
<td>3.89 ± 0.34</td>
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</tbody>
</table>

* Where a standard deviation is listed, a minimum of 400 invaded cells were counted. Otherwise, 200 cells were counted.

FIG. 1. Rate of multiplication of the RH strain of Toxoplasma at various temperatures.

FIG. 2. Rate of multiplication of the rapidly proliferating RH strain of Toxoplasma at controlled temperatures in rabbit kidney tissue culture can be made similar to that of the naturally occurring S7 and M7741 strains which multiply more slowly.

TABLE 2. Treatment of cultures with pyrimethamine for 4 days

<table>
<thead>
<tr>
<th>Amt of drug (mg/100 ml)</th>
<th>37 C</th>
<th>34 C</th>
<th>31 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0/18</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>0.01</td>
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<td>0/4</td>
<td>—</td>
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<tr>
<td>0.1</td>
<td>12/22</td>
<td>—</td>
<td>—</td>
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<tr>
<td>0.3</td>
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<td>3/22</td>
</tr>
<tr>
<td>0.6</td>
<td>—</td>
<td>10/28</td>
<td>3/20</td>
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</table>

Studies of resistance to antimetabolite therapy by slowly multiplying organisms indicate that, at 37 C, 4 days of therapy with only 0.05 mg per 100 ml of pyrimethamine killed most organisms and time (assuming binary multiplication) of 4.85 hr during the exponential phase of growth. At 34 C, the lag time was increased to 12 hr and the mean generation time was 5.26 hr. At 31 C, the lag time was 12.5 hr, and the generation time was 11.1 hr as compared with 4.85 hr at 37 C (Fig. 1 and Table 1).

Figure 2 illustrates that the growth characteristics of naturally occurring strains grown in monkey kidney cells at 37 C are similar to the rapidly multiplying RH strain with its rate of multiplication slowed by altering the incubating temperature. It is clear that altering the temperature permits the regulation of multiplication of the rapidly multiplying RH strain to a level where it is comparable with other slower growing, naturally occurring strains.
permitted the survival of mice which were injected with the treated cultures (Table 2). At 34 C, 0.6 mg per 100 ml, 12 times as much, was required to spare any mice, and at 31 C even this dose was only slightly effective.

The resistance to therapy of slowly multiplying organisms of this antimetabolite was so striking after 4 days of drug exposure that the effect of more prolonged therapy was studied. When therapy was extended to 7 days, there was no significant difference between the groups (Table 3).

### Acknowledgment

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### Literature Cited
