Curing of an R Factor from *Escherichia coli* by Trimethoprim

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R factor 1818, which we have shown previously to be eliminated by thymine starvation, was cured from three strains of *Escherichia coli* K-12 by overnight exposure to trimethoprim. Elimination was abolished in the presence of added thymine or thymidine, which suggests that curing is the result of the induction of thymineless conditions by trimethoprim. Starvation of the required amino acids proline and histidine had little effect on elimination, whereas methionine deprivation enhanced it. R factor curing was abolished by the presence of chloramphenicol, and it is concluded that protein synthesis is required for elimination to occur. It is suggested that elimination may result from the activity of a nuclease which is synthesized or induced during both direct thymine starvation and by trimethoprim treatment.

The mechanism of thymineless death (3) has yet to be adequately explained. Thymine deficiency promotes prophage induction in lysogenic strains (24, 27), which must contribute to the lethal effect, but strains which are apparently nonlysogenic also die during thymine starvation (12, 26). Mennigmann and Szybalski (28) first suggested that thymine deprivation produced single-strand breaks (or nicks) in bacterial deoxyribonucleic acid (DNA). Freifelder (15) later demonstrated that closed circular sex-factor DNA is indeed nicked during thymine starvation, and subsequently found an increase in endonuclease activity in thymine-starved bacteria (16). Chromosomal nicking occurs when some (36, 41), but not all (2, 37), bacterial strains are deprived of thymine, and it is therefore not clear whether this type of chromosomal DNA damage is responsible for the bactericidal effects of thymine starvation. However, Breitman, Maury, and Toal (6) recently reported that a substantial loss of DNA thymine occurs during thymine starvation of *Escherichia coli* and suggested that this loss is initiated from unrepairable single-strand breaks in the DNA.

Growth of thymine-requiring strains in suboptimal concentrations of thymine eliminates certain extrachromosomal elements (8) which are not susceptible to the classic acridine-curing techniques. Indeed, we have shown (33) that the incidence of R^- clones was increased up to 100-fold when thymineless strains of *E. coli* and Klebsiella aerogenes which contained R factor 1818 were starved of thymine. When logarithmically growing cultures of *E. coli* J6-2 pro^- his^- trp^- thy^- (R-1818) were deprived of all three required amino acids as well as thymine, 95% of the cells were still susceptible to thymineless death, but the plasmid curing effect was abolished (34). A similar result was obtained when organisms were starved of thymine in the presence of chloramphenicol (34). Both observations indicate that elimination is dependent on protein synthesis, and we have suggested that the inhibition of synthesis of a nuclease, which is more specific for the R factor genome than for the chromosome, could explain these observations.

With the exception of rifampin (4, 20, 25) and possibly nalidixic acid or chloroquine (7), the compounds which cure plasmids from their hosts are of little use as systemic chemotherapeutic agents. The antifolate drug trimethoprim is beginning to find extensive use in combination with sulfamethoxazole in the treatment of infections caused by gram-negative bacteria (17). Trimethoprim blocks the enzyme dihydrofolate reductase which catalyzes the reduction of dihydrofolate to tetrahydrofolate (40). The latter acts as the one carbon unit donor in thymidylate synthesis, being reoxidized to dihydrofolate in the process (32). Because tetrahydrofolate cannot be regenerated in cells treated with trimethoprim, the synthesis of thymidylate eventually ceases, and Cohen
has suggested that, under certain conditions, such antifolate drugs will induce thymineless death. We have consequently investigated whether trimethoprim can eliminate R factor 1818 and whether mechanisms similar to those induced by direct thymine starvation are in operation during trimethoprim treatment.

This paper reports that trimethoprim is effective in eliminating R-1818, and presents evidence that its mode of action in this respect is analogous to thymine deprivation.

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**MATERIALS AND METHODS**

**Bacterial strains.** The following derivatives of *E. coli* K-12 were used: 58.161/sp (met lac+; reference 19) = K-12; J5-3 (pro met lac+; reference 9) = J5-3; and J6-2 (pro his trp lac-; reference 9) = J6-2. A thymineless strain of K-12 which had previously been isolated by aminopterin selection (38) was used, and the thymine-requiring strains of J5-3 and J6-2 were isolated for use in this work by means of trimethoprim treatment (40). All thy- mutants required a high level (about 30 μg/ml) of thymine for optimal growth and were relatively resistant to thymineless death (26). No visible lysis or fall in viable count was observed after readdition of thymine to thymine-starved cultures. It is therefore unlikely that these strains harbor a prophage which is inducible by thymine deprivation (24).

**R factors.** R-1818 is an N-group R factor (13) and confers resistance to ampicillin, streptomycin, tetracyclines, and sulfonamides. R-TEM, which is fi+, mediates resistance to high concentrations of ampicillin and streptomycin. R-1818 and R-7268 were isolated from bacteria first characterized by E. S. Anderson of The Enteric Reference Laboratory, London, England, and have been renamed R46 and R1, respectively, by Meynell and Datta (29). R-TEM has been designated R6K by Kontomichalou, Mitani, and Clowes (23). Strains containing one or two R factors were constructed as described previously (33, 39).

**Media.** Nutrient broth no. 2 (code CM67) and MacConkey agar (code CM7) were supplied by Oxoid Ltd., London, England, and were prepared as recommended by the manufacturer. Minimal medium (DM) was that of Davis and Mingioli (14) supplemented with 0.28% glucose and, where necessary, L-proline (Pro), L-histidine (His), and L-tryptophan (Trp), all at 40 μg/ml, DL-methionine (Met) at 50 μg/ml, and thymine or thymidine at 60 μg/ml. Minimal agar was DM solidified with 1.5% Davis Japanese agar.

**Trimethoprim treatment.** Cultures were grown in suitably supplemented DM to late logarithmic or stationary phase, washed with unsupplemented DM salts solution, and diluted 1:100 or 1:50 into DM containing trimethoprim lactate (kindly supplied by S. R. M. Bushby) and other supplements as required. Samples from trimethoprim-treated cultures were diluted in nutrient broth and plated on MacConkey agar plus 60 μg of thymine/ml. Clones which grew after overnight incubation were replica-plated onto DM with or without suitable antibiotics and thyme to check for R factor content and thymine requirement. Antibiotics used for replica-plating were as follows: for R-1818, streptomycin (10 μg/ml) or tetracycline (10 μg/ml); for R-7268, chloramphenicol (10 μg/ml); and for R-TEM, ampicillin (400 μg/ml).

**Thymine starvation.** Thymine-requiring cultures were washed and diluted into suitably supplemented DM containing no added thymine. Samples were tested for R factor content as described for trimethoprim treatment.

**RESULTS**

**Thymine starvation.** Before investigating whether trimethoprim treatment eliminated R factor 1818 from thy- strains, it was necessary to check that the R factor could be cured from thy- derivatives of the bacteria by direct thymine starvation. We have shown previously (33, 34) that R-1818 is eliminated from thymineless mutants of K-12 or J6-2, and we found from similar experiments performed with J5-3 thy- (R-1818) that the proportion of R- clones increased from less than 0.2% in control cultures to approximately 10% in cultures which had been deprived of thymine for 120 min. This represented a greater than 50-fold increase in the proportion of R- cells, and we conclude that the R factor can be cured by thymine starvation from the thy- mutants of all three derivatives of *E. coli* K-12 used in this study.

**Trimethoprim treatment.** Loss in viability of the three strains when exposed to trimethoprim in DM medium was slow; greater than 50% J5-3 (R-1818) were still viable after 5 h in suitably supplemented DM containing concentrations of trimethoprim as high as 1,000 μg/ml. No elimination of the R factor could be demonstrated under these conditions. Cultures were therefore exposed to trimethoprim overnight before samples were plated, and the resulting clones were tested for R factor content. Table 1 shows that a substantial increase in the proportion of R- clones was produced when inocula from late logarithmic-phase cultures were treated with trimethoprim overnight. Similar results were obtained with inocula from stationary-phase cultures (see, for example, Table 2). Elimination rates exceeded those which could be attributed to the preferential survival of spontaneous R- segregants present in the initial inoculum.

We therefore conclude that, as with thymine starvation (33), trimethoprim treatment actively increased the proportion of R- cells.
There was no correlation between the level of survivors and the frequency of R factor elimination. Strain K-12 was the most susceptible to killing by trimethoprim treatment, whereas the highest curing rate was obtained in J6-2. Good curing was obtained from K-12 and J6-2 by use of trimethoprim at 50 μg/ml, but it was found that maximal elimination occurred from J5-3 when cultures were exposed to trimethoprim at 10 μg/ml. Increasing the concentration above 10 μg/ml actually decreased the frequency of R factor elimination from this strain.

**Effect on other R factors.** Previous results with thy⁻ strains (33) have shown that R factors TEM and 7268 were not eliminated by thymine starvation; it was also demonstrated that the presence of R-TEM prevented the elimination of R-1818 from K-12 thy⁻ (R-1818/R-TEM), whereas R-1818 was still cured from K-12 thy⁻ (R-1818/R-7268) by thymine starvation. Experiments were therefore performed to see whether similar results could be obtained by trimethoprim treatment of thy⁺ strains.

It was found (Table 2) that neither R-TEM nor R-7268 was eliminated by trimethoprim from stationary-phase cultures of J6-2, and that the presence of R-7268 had no effect on R-1818 curing. However, in contrast to the results with thymine starvation, R-1818 was still eliminated by trimethoprim from J6-2, which also contained R-TEM.

**Reversal of trimethoprim activity.** If loss of viability and elimination of R factor 1818 were the result of the induction of thymineless conditions, the addition of thymine or thymidine might be expected to reduce both effects. Table 3 shows that either thymine or thymidine did reverse the antimicrobial activity of trimethoprim and also its curing action on R-1818. The fact that none of the clones tested was thy⁻ rules out the possibility that the apparent protective effects produced by thymine or thymidine were due to multiplication of any thy⁻ mutants present in the initial inoculum.

Wild-type bacteria do not normally incorporate thymine (11, 21), whereas thymidine is readily incorporated for a short period until it is degraded by thymidine phosphorylase (35). The presence of an intracellular pool of deoxyribosyl groups can promote the incorporation of both thymine and thymidine (32). Trimethoprim, by preventing the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate, will produce a buildup of deoxyribosyl groups (32) and thus permit the uptake of both exogenous thymine and thymidine to reverse the effects of thymine deprivation.

**Effect of amino acid starvation.** When suitable auxotrophs are starved of thymine and amino acids, the extent of thymineless death is reduced (18) and R-1818 is no longer eliminated (34). The effects of amino acid deprivation on R factor elimination during trimethoprim treatment was therefore investigated. It can be seen in Table 4 that withdrawal of Pro or His from logarithmic cultures of J6-2 had little effect on R-1818 curing during trimethoprim treatment, whereas Trp starvation in the presence of trimethoprim reduced, but did not prevent, elimination of R-1818 from J6-2. These results contrast with those obtained previously with direct thymine starvation where amino acid depriva-

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**Table 1. Elimination of R factor 1818 from three strains of Escherichia coli K-12**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tri-methoprim (μg/ml)</th>
<th>Viable count per ml after 18 h</th>
<th>No. of clones sampled</th>
<th>No. of R- clones</th>
<th>Percent R- clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12 (R-1818)</td>
<td>0</td>
<td>1.6 × 10⁴</td>
<td>1,051</td>
<td>0</td>
<td>&lt;0.095</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.46</td>
<td>399</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td>J6-2 (R-1818)</td>
<td>0</td>
<td>1.3 × 10⁴</td>
<td>992</td>
<td>0</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.8</td>
<td>485</td>
<td>39</td>
<td>8.0</td>
</tr>
<tr>
<td>J5-3 (R-1818)</td>
<td>0</td>
<td>2.1 × 10⁴</td>
<td>556</td>
<td>0</td>
<td>&lt;0.18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27.3</td>
<td>677</td>
<td>25</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*Late exponential-phase cells grown in DM medium were washed in DM salts solution and diluted 1:50 into fully supplemented DM with or without trimethoprim at the concentrations shown. Cultures were incubated at 37°C for 18 h and were then diluted in broth and plated onto MacConkey agar plus thymine (60 μg/ml). Clones which grew after overnight incubation were replica-plated to check for thymine requirement and R factor content. No clone tested was found to be thymine-requiring.

*Expressed as a percentage of the initial count.

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**Table 2. Effect of trimethoprim on strains containing one or two R factors**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of clones sampled</th>
<th>No. of R- clones</th>
<th>Percent R- clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>J6-2 (R-1818)</td>
<td>616</td>
<td>77</td>
<td>12.5</td>
</tr>
<tr>
<td>J6-2 (R-7268)</td>
<td>778</td>
<td>0</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td>J6-2 (R-TEM)</td>
<td>369</td>
<td>0</td>
<td>&lt;0.27</td>
</tr>
<tr>
<td>J6-2 (R-1818/R-7268)</td>
<td>407</td>
<td>96</td>
<td>23.6</td>
</tr>
<tr>
<td>J6-2 (R-1818/R-TEM)</td>
<td>432</td>
<td>35</td>
<td>8.1</td>
</tr>
</tbody>
</table>

*E. coli J6-2 strains were grown overnight in DM, washed, and resuspended into fresh DM containing trimethoprim at 50 μg/ml. After 18 h, samples were plated and the resulting clones were tested for R factor content.

*The viability of all strains decreased by about 90% during trimethoprim treatment.

*Figures refer to elimination of R-1818 only; no elimination of R-7268 or R-TEM occurred.
tion did prevent R-1818 elimination (34). Met deprivation of K-12 during trimethoprim treatment increased, rather than decreased, the occurrence of R- clones (Table 4), and this effect, which was more pronounced when stationary-phase inocula were used (first three lines of Table 6), may reflect the requirement for tetrahydrofolate in Met synthesis (22).

It therefore seemed that Met starvation had a unique "synergistic" effect on the elimination of R-1818 by trimethoprim treatment. Further evidence for this was obtained by depriving J5-3 \( \text{pro}^- \text{met}^- \) (R-1818) of either Pro or Met during exposure to trimethoprim. It was found that the proportion of R- clones induced by trimethoprim treatment was the same whether Pro was present or absent, but when Met was withdrawn the elimination rate increased about fourfold (Table 4).

Although Pro starvation of J6-2 or J5-3, or His and Trp starvation of J6-2, had little effect on the viability of trimethoprim-treated cultures, there was some evidence that Met deprivation increased the lethal effect of trimethoprim on log-phase inocula of J5-3 (Table 4) or stationary-phase cells of K-12 (Table 6, lines 2 and 3). Since the increase in frequency of R factor elimination may have been a reflection of a decreased survivor level, the effects of withholding Met and thymine from a thymine-requiring mutant of J5-3 were investigated.

It was found that the surviving fraction was

\[ \text{Percent R- clones} \]

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**Table 3. Reversal by thymine and thymidine of trimethoprim-induced death and R factor elimination**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Conditions*</th>
<th>Viability per ml after 18 h*</th>
<th>No. of clones sampled</th>
<th>No. of R- clones</th>
<th>Percent R- clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12 (R-1818)</td>
<td>+Tm 50 + Thy</td>
<td>80 (0.46)</td>
<td>524</td>
<td>0</td>
<td>&lt;0.19 (3.8)</td>
</tr>
<tr>
<td></td>
<td>+Tm 50 + TdR</td>
<td>202</td>
<td>1,116</td>
<td>0</td>
<td>&lt;0.09</td>
</tr>
<tr>
<td>J6-2 (R-1818)</td>
<td>+Tm 50 + Thy</td>
<td>107 (5.8)</td>
<td>847</td>
<td>2</td>
<td>0.24 (8.0)</td>
</tr>
<tr>
<td></td>
<td>+Tm 50 + TdR</td>
<td>120</td>
<td>876</td>
<td>2</td>
<td>0.23</td>
</tr>
<tr>
<td>J5-3 (R-1818)</td>
<td>+Tm 10 + Thy</td>
<td>104 (27.3)</td>
<td>1,007</td>
<td>2</td>
<td>0.20 (3.6)</td>
</tr>
<tr>
<td></td>
<td>+Tm 10 + TdR</td>
<td>112</td>
<td>1,063</td>
<td>0</td>
<td>&lt;0.094</td>
</tr>
</tbody>
</table>

*Method as in Table 1. Figures in parentheses are values quoted from Table 1 for cultures containing no thymine or thymidine. No clone was found to be thymine-requiring.

*Concentration of trimethoprim (Tm) in micrograms per milliliter plus thymine (Thy) or thymidine (TdR).

*Expressed as a percentage of the initial count.

**Table 4. Effect of amino acid starvation on trimethoprim-induced elimination of R factor 1818 from three strains of Escherichia coli K-12**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Conditions*</th>
<th>Viable count per ml*</th>
<th>No. of clones sampled</th>
<th>No. of R- clones</th>
<th>Percent R- clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>J6-2 (R-1818)</td>
<td>+Pro +His +Trp +Tm 50</td>
<td>9.6</td>
<td>649</td>
<td>39</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>-Pro +His +Trp +Tm 50</td>
<td>8.9</td>
<td>774</td>
<td>34</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>-His +Trp +Pro +Tm 50</td>
<td>12.1</td>
<td>967</td>
<td>39</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>-Trp +Pro +His +Tm 50</td>
<td>10.6</td>
<td>772</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>-Pro -His -Trp +Tm 50</td>
<td>13.3</td>
<td>1,060</td>
<td>10</td>
<td>0.94</td>
</tr>
<tr>
<td>K-12 (R-1818)</td>
<td>+Met +Tm 50</td>
<td>0.46</td>
<td>399</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>-Met +Tm 50</td>
<td>0.46</td>
<td>311</td>
<td>27</td>
<td>8.7</td>
</tr>
<tr>
<td>J5-3 (R-1818)</td>
<td>+Pro +Met +Tm 10</td>
<td>27.3</td>
<td>697</td>
<td>25</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>-Pro +Met +Tm 10</td>
<td>29.0</td>
<td>721</td>
<td>25</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>-Met +Pro +Tm 10</td>
<td>10.0</td>
<td>309</td>
<td>41</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>-Pro -Met +Tm 10</td>
<td>26.0</td>
<td>628</td>
<td>33</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Late exponential-phase cultures were treated as described in Table 1. Spontaneous elimination rates were similar to those listed in Table 1. Amino acid deprivation in the absence of trimethoprim did not increase the frequency of R- clones above the spontaneous rate.

*Presence or absence of L-proline (Pro), L-histidine (His), L-tryptophan (Trp), and DL-methionine in addition to trimethoprim (Tm) at the concentration indicated in micrograms per milliliter.

*Expressed as a percentage of the initial count.
only slightly less when cultures of J5-3 pro- met- thy- were starved of thymine and Met than when they underwent thymineless death in the absence of Pro (Fig. 1). As expected (18, 34), extensive death occurred only when both required amino acids were present. Investigation of the R factor content of thymine-starved cultures showed that, in agreement with our previous findings (34), curing was produced only when cultures were starved of thymine in the presence of both required amino acids (Table 5). The increase in frequency of R factor elimination therefore appeared to be a peculiarity of methionine starvation which became evident during trimethoprim treatment, but not when thymine was withheld from thy- bacteria.

**Effect of chloramphenicol on trimethoprim-induced curing.** Since trimethoprim-induced curing still occurred during Pro or His deprivation, and as even greater elimination was produced by trimethoprim during Met starvation, our theory that protein synthesis is necessary for R-1818 elimination (34) appeared incorrect. However, Miovic and Pizer (31) have shown that some protein synthesis still takes place during trimethoprim treatment, and it is possible that protein turnover in the trimethoprim-inhibited cultures may have been sufficient to supply the amino acids required for the synthesis of protein(s) necessary for elimination of R-1818.

To investigate this point directly, protein synthesis was inhibited by including chloramphenicol in cultures of K-12 which were exposed to trimethoprim in the presence or absence of Met. The results (Table 6) show that in the presence of chloramphenicol the bactericidal effect of trimethoprim was reduced; moreover, the elimination of R-1818 was abolished both in the presence and absence of Met. Similar experimental results (not shown) showed that chloramphenicol also prevented elimination of R-1818 from Met-supplemented or Met-unsupplemented cultures of J5-3.

Consequently, it seems that some protein synthesis can occur in organisms which are both starved of an essential amino acid and treated with trimethoprim. Furthermore, it would appear that this synthesis is essential for the elimination of R-1818 (Table 6).

**DISCUSSION**

Trimethoprim has been shown to promote the elimination of R factor 1818. The fact that both thymine and thymidine abolish this effect (Table 3) suggests that R factor elimination results from the induction of thymineless conditions by trimethoprim. Further evidence for this is that neither thymine starvation nor trimethoprim treatment eliminates R factors 7289 or TEM (Table 2 and reference 35).

Starvation for the required amino acids Pro or His during trimethoprim treatment has little effect on the frequency of R factor elimination.
TABLE 6. Increase in trimethoprim-induced elimination of R factor 1818 from stationary-phase cells of Escherichia coli K-12 met+ (R-1818) produced by methionine starvation and its prevention by chloramphenicol

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Viable count after 18 h</th>
<th>No. of clones sampled</th>
<th>No. of R- clones</th>
<th>Percent R- clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Met</td>
<td>1.0 x 10^4</td>
<td>449</td>
<td>0</td>
<td>&lt;0.22</td>
</tr>
<tr>
<td>+Met +Tm 50</td>
<td>1.2</td>
<td>568</td>
<td>22</td>
<td>3.9</td>
</tr>
<tr>
<td>-Met +Tm 50</td>
<td>0.75</td>
<td>386</td>
<td>125</td>
<td>32.4</td>
</tr>
<tr>
<td>+Met +Tm 50</td>
<td>9.8</td>
<td>478</td>
<td>0</td>
<td>&lt;0.21</td>
</tr>
<tr>
<td>-Met +Tm 50</td>
<td>9.0</td>
<td>436</td>
<td>0</td>
<td>&lt;0.23</td>
</tr>
<tr>
<td>+Cm 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cultures were grown overnight in DM medium, washed in DM salts solution, and diluted 1:100 into DM medium with or without DL-methionine or chloramphenicol. After 18 h, samples were plated and the resulting clones were replica-plated to estimate R factor content.

*Presence or absence of DL-methionine (Met), trimethoprim (Tm), and chloramphenicol (Cm), with concentrations given in micrograms per milliliter.

*Expressed as a percentage of the initial count.

(The Table 4), whereas during Met deprivation elimination rates are actually increased (Tables 4 and 6). These results may appear to contradict our previous findings (34) that protein synthesis is necessary for the elimination of R-1818 from cultures undergoing thymineless death. However, since it was necessary to expose the cultures to trimethoprim for long periods before elimination could be demonstrated, it is probable that sufficient required amino acids became available, as a result of protein catabolism during the exposure period, for some protein synthesis to take place. Such a view is supported by the finding that the elimination of R-1818 was abolished by the presence of chloramphenicol during trimethoprim treatment (Table 6). We have suggested that R-1818 elimination during thymineless death is dependent on the synthesis of a nuclease which degrades the R factor; the requirements for protein synthesis during trimethoprim-induced curing could also indicate the necessity for synthesis of this, or a similar, nuclease.

The increase in frequency of R factor curing produced by Met starvation could be explained in terms of enhanced depletion of tetrahydrofolate. More subtly, however, Met starvation might also act by reducing the capacity of the cell to carry out DNA methylation. This, in turn, could increase the susceptibility of R factor DNA to nuclease attack in a manner analogous to host-cell restriction of unmodified DNA (1, 5). It has also been suggested that a restriction-type nuclease may even be responsible for some chromosomal degradation during thymine starvation (6).

ACKNOWLEDGMENTS

We thank Lorraine Pascoe for excellent technical assistance.

LITERATURE CITED

increased rate of loss of penicillinase plasmids from *Staphylococcus aureus* in the presence of rifampicin. J. Gen. Microbiol. 66:137–139.


