Mode of Action of Myxin on *Escherichia coli*  

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The effect of the new antibiotic, myxin, on the syntheses of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein in *Escherichia coli* (strains B and 15T-) was examined. Within 7 min of the addition of myxin at 5 μg/ml, the synthesis of new bacterial DNA was almost completely inhibited. This was followed by an extensive degradation of the pre-existing DNA to an acid-soluble form. All of the evidence indicated that the primary effect of the antibiotic was on cellular DNA. The synthesis of RNA was completely inhibited after 15 min of exposure to myxin (5 μg/ml), and the synthesis of protein was markedly reduced after 30 min. There was no measurable breakdown of either RNA or protein in the myxin-treated cells. A marked stimulation of 14C-uracil incorporation was found in the presence of myxin in 15T- cells only. This did not result from an increased rate of RNA synthesis but was due to an increase in the proportion of exogenous uracil, relative to endogenous uracil, incorporated into cellular RNA. This probably reflected a partial inhibition of the biosynthesis of uridine monophosphate from orotate. At 4.5 μg of myxin per ml and with 0.8 × 10^6 cells per ml, 50% of the antibiotic was reduced in 15 min from the biologically active oxidized form to the biologically inactive state. Under these conditions, a maximum of 0.6% (27 μg/μl) of the myxin was retained in the cells.

The newly discovered antibiotic, myxin (referred to previously as "3C"; recently named "myxin" in a patent application by F. D. Cook, E. A. Peterson, and D. C. Gillespie), has been reported to possess an unusually broad antimicrobial spectrum (20). The chemical structure of myxin has been established as 1-hydroxy-6-methoxyphenazine-5,10-dioxide (D. C. Gillespie, personal communication). Preliminary studies with actively growing *Agrobacterium tumefaciens* indicated that deoxyribonucleic acid (DNA) synthesis was inhibited at a myxin concentration of 1 μg/ml, without affecting the synthesis of either ribonucleic acid (RNA) or protein. A more detailed investigation into the effect of this antibiotic on DNA, RNA, and protein synthesis was undertaken with *Escherichia coli* as the test organism since, in this bacterium, these biosynthetic pathways have been well characterized. The results obtained are reported here.

**Materials and Methods**

*Bacterial cultures. E. coli* 15T- and 15T-A-U- (15TAU), kindly supplied by K. G. Lark, Kansas State University, and J. L. Suit, University of Texas, were grown in M9 synthetic medium with the required supplements (14). To ensure an actively multiplying culture, the cells were used in the following manner.

1 Contribution no. 623.

Glycerol (15%) was added to a log-phase culture in M9, and samples were stored frozen at −70°C. After fast thawing, a 1:100 dilution of these stock cells was made in M9 at 37°C. The cells multiplied, after a short lag period, with a generation time of 30 min, and reached a nephelometer reading of 45 after 3.5 hr. At this point, 8 ml of the culture was used in a total incubation volume of 10 ml to give about 0.8 × 10^6 cells/ml.

*Myxin.* The pure antibiotic, prepared by the method developed in this Institute (20), was the gift of E. A. Peterson. Radioactive myxin was made as described (S. M. Lesley and R. M. Behki, in press), with the modification that methyl-labeled 14C-methionine was also added to the culture medium. The labeled antibiotic was very stable when stored in acetone at 4°C.

*Radioactive materials. Glucose-U-14C,* thymine-2-14C, and L-leucine-U-14C were obtained from the Radiochemical Centre, Amersham, England; uracil-2-14C, thymine-U-13H, and orotate-6-14C were obtained from the New England Nuclear Corp., Boston, Mass.

*Synthesis of macromolecules.* DNA, RNA, and protein biosynthesis was followed by measuring the incorporation of radioactive thymine, uracil, and, to a lesser extent, into the cold trichloroacetic acid-insoluble fraction of the cells by the membrane filtration technique (2). The quantities of DNA and protein in the bacteria were determined colorimetrically by the methods of Burton (6) and Lowry et al. (17); RNA was measured by the orcinol method (18).

*Isolation and characterization of RNA.* Cells were lysed in the presence of lysozyme and deoxyribono-
nuclease by the method of Hayashi and Spiegelman (9) and the RNA was purified by phenol extraction (7).

The various species of bacterial RNA were separated by centrifugation of the purified RNA in a linear sucrose density gradient (15). Measurement of radioactivity. The amount of radioactivity retained on the filter membranes (Millipore Corp., Bedford, Mass.) was measured with a thin-window gas-flow counter. Fractions from sucrose density gradients, and other aqueous preparations as required, were counted in a liquid scintillation spectrometer. Appropriate corrections were made for self-absorption or quenching.

RESULTS

Preliminary experiments were done with log-phase 15T cells which were centrifuged at 4 C and then resuspended in fresh medium. Under these conditions, at 3 μg of myxin per ml at 37 C, DNA synthesis (14C-thymine incorporation) was 95% inhibited and the cells were quickly killed. However, such cells, in the absence of myxin, did not multiply for at least 135 min after resuspension (viable cell titration), although the DNA, RNA, protein, and optical density of the cells increased rapidly. This inhibition of cell multiplication indicated some temporary aberration in the overall metabolism of the resuspended bacteria, and it was, therefore, considered advisable to use a logarithmically growing culture without centrifugation.

Exposure time. It was established early in the study that the rate of 14C-thymine incorporation in cells exposed to myxin (5 μg/ml) was progressively inhibited during the first 7 min; subsequently, the fully inhibited rate was evident. The cells were, therefore, routinely exposed to myxin for 7 min prior to the addition of labeled precursors, except where otherwise noted.

Effect on DNA biosynthesis. Figure 1 shows the effect of different concentrations of myxin on 14C-thymine incorporation into the trichloroacetic acid-insoluble fraction of log-phase 15T cells. The extent of the inhibition of DNA synthesis was dependent on myxin concentration. With myxin at 5 μg/ml, the amount of DNA synthesized after 60 min was only 10% of that found in the control cells.

The possibility that myxin either blocked a thymine permease system or reacted directly with thymine so as to make it inaccessible to the cells was examined by the following experiments. The amount of intracellular DNA in actively multiplying cells of E. coli B was measured colorimetrically. These cells have no requirement for thymine. Inhibition of DNA synthesis was complete after 15 min of exposure to myxin (Table 1).

![Effect of myxin concentration on 14C-thymine incorporation into the DNA of 15T cells. Log-phase cells in M9 medium at 0.8 × 10^7/ml were exposed to myxin (concentration in micrograms per milliliter marked on the curves) for 7 min before the addition of 0.072 μmole of 14C-thymine per ml (zero-time). The amount of 14C incorporated into the trichloroacetic acid-insoluble fraction was measured with time and plotted as a percentage of the amount originally added to the culture.]

**TABLE 1. Effect of myxin (5 μg/ml) on DNA biosynthesis in log-phase Escherichia coli B**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Myxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA content, μg/ml of extract</td>
<td>9 μg/ml of extract</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>15</td>
<td>48</td>
<td>32</td>
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<td>30</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>45</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>60</td>
<td>98</td>
<td>14</td>
</tr>
</tbody>
</table>

* All assays were done on extracts of centrifuged and washed cells.

A 1-ml quantity of the extract was equivalent to 20 ml of cell culture.

* Zero-time represented the time of myxin addition.

1. In addition, about 50% of the DNA appeared to be lost from the cells during the next 45 min. Any interaction between thymine and myxin was tested by paper chromatography (butanol-acid-water, 4:1:5) of a mixture (in M9 medium) of 3H-thymine and 14C-myxin, at the same concentrations as in the previous experiment, after incubation at 37 C for 45 min. The chromatogram was cut into squares and the
amount of \(^3\)H and \(^{14}\)C in each segment was determined in a liquid scintillation spectrometer. The two labeled compounds were well separated on the chromatogram, and less than 5% of cross contamination was found.

It was clear from the results that the inhibition of DNA synthesis was a direct effect of myxin on the metabolism of the \(15\)T\(^-\) cells rather than an interference with the entry of thymine.

**DNA degradation.** The apparent loss of DNA in the presence of myxin (Table 1) could have been due either to its intracellular breakdown, similar to that reported to occur with mitomycin C (22), or could have resulted from cell lysis and loss of DNA into the medium. These possibilities were examined by use of \(15\)T\(^-\) cells in which over 90% of the DNA had been labeled during growth through more than four generations in the presence of \(^{14}\)C-thymine. Excess cold thymine was added and the cells were incubated both in the presence and absence of myxin (4.5 \(\mu\)g/ml). The loss of radioactivity from the acid-insoluble fraction of the cells with time is shown in Fig. 2. In 68 min, over 60% of the labeled DNA contained in the cells exposed to myxin had been degraded to an acid-soluble form. The acid-soluble radioactivity was not intracellular but was found in the cell-free culture supernatant fluid. In contrast, there was no loss of \(^{14}\)C from the control cells, which continued to incorporate some \(^{14}\)C-thymine from the medium.

In exactly the same manner, the RNA of \(15\)T\(^-\) cells was labeled with \(^{14}\)C-uracil and the protein was labeled with \(^{14}\)C-leucine. No degradation to an acid-soluble form of either RNA or protein was observed over a period of 60 min when such cells were incubated with myxin (5 \(\mu\)g/ml). Further evidence that cell lysis had not occurred in the presence of myxin was obtained when peptidase was added to samples of the \(^{14}\)C-leucine-labeled cells; no acid-soluble radioactivity was found to be released. This indicated that intracellular protein was not available for digestion by this enzyme, and the cell walls were, therefore, unlikely to have been damaged.

It is concluded on the basis of the above results that myxin inhibited the synthesis of new DNA and, in addition, caused an extensive degradation of the pre-existing bacterial cell DNA.

**Effect on \(^{14}\)C-uracil incorporation.** The effect of different concentrations of myxin on the incorporation of \(^{14}\)C-uracil into RNA in \(15\)T\(^-\) cells is shown in Fig. 3. At 5 \(\mu\)g/ml, the uptake of labeled uracil was completely inhibited by 45 min. At 1 \(\mu\)g of myxin per ml, a 100% stimulation of uracil incorporation was observed at 45 min relative to the control culture without myxin. This stimulatory effect was also evident at 3

![Fig. 2. Degradation of bacterial DNA in the presence of myxin. Log-phase \(15\)T\(^-\) cells were labeled by growth in the presence of \(^{14}\)C-thymine for more than four generations. Remaining \(^{14}\)C-thymine was diluted by an excess of unlabeled thymine, and the \(^{14}\)C in the acid-insoluble fraction of the cells was measured at intervals after the addition of myxin to 4.5 \(\mu\)g/ml. Control cells were similarly treated but with no addition of myxin.](image)

![Fig. 3. Effect of myxin concentration on \(^{14}\)C-uracil incorporation into the RNA of \(15\)T\(^-\) cells. The conditions were the same as in Fig. 1. The cells were exposed to myxin (concentration in micrograms per milliliter marked on curves) for 7 min before the addition of 6 \(\mu\)g of \(^{14}\)C-uracil per ml at zero-time. The amount of \(^{14}\)C incorporated into the acid-insoluble cell fraction was measured with time and plotted as a percentage of the amount originally added to the culture.](image)
μg/ml. At 5 μg/ml, this effect was observed only during the first 30 min.

That the presence of myxin caused an increase in the rate of RNA formation appeared unlikely, particularly since DNA synthesis had been shown to be inhibited. This effect was therefore examined in greater detail.

It was possible that the synthesis of only one intracellular species of RNA was stimulated, similar to the increased synthesis of soluble RNA found in E. coli after exposure to mitomycin C (21). To determine whether this had occurred, the RNA of log-phase 15′- cells was labeled by incubation with 14C-uracil (7 μg/ml) for 55 min with and without the addition of myxin (1.1 μg/ml). The amount of 14C-uracil incorporated into the myxin-treated cells was 60% greater than that taken up by the control cells (1,136,000 and 712,000 counts/min, respectively). The labeled RNA was extracted and purified from the cells in each culture, with a recovery, measured as 14C, of 85.5% from the control cells and 91% from the treated cells.

Each RNA preparation was separated into 4S, 16S, and 23S components by centrifugation of a 0.05-ml sample in a linear sucrose density gradient (15). The 14C (from 14C-uracil) was distributed over the three species of RNA in about the same relative proportions in the two RNA preparations (Fig. 4). There had not been a preferential synthesis of any one species of RNA in the cells exposed to myxin.

To determine whether the observed stimulation of 14C-uracil incorporation did represent increased RNA synthesis, the amount of purified RNA in each extract was measured colorimetrically; it was found to be similar, 505 μg/ml in the extract from the control cells and 490 μg/ml in the extract from the myxin-treated cells. However, the specific activity of the RNA from the myxin-treated cells was 76% greater than that from the control cells: 727 and 412 counts per min per μg, respectively. One possible explanation of the increased 14C-uracil incorporation was that myxin had caused a partial block of the endogenous supply of uridine monophosphate from orotate, with the result that the treated cells were forced to use a larger proportion of the exogenous 14C-uracil than was utilized by the normal cells.

This possibility was tested indirectly by using a uracil-requiring mutant (E. coli 15 TAU). Since these cells have no endogenous supply of uracil, they should show no stimulation of 14C-uracil uptake in the presence of myxin. Under the same cultural conditions as above and in the presence of 1.1 μg of myxin per ml, the incorporation of 14C-uracil was not stimulated; in fact, it was inhibited by 16% at 25 min (Table 2). The explanation for this inhibition was not apparent but may be related to the induction of a lyogenic phage, as was reported to occur in 15 TAU cells on treatment with nalidixic acid (8), which also inhibits DNA synthesis.

The stimulation by myxin of 14C-uracil incorporation was examined further by following the effect of myxin on the incorporation of 14C- orotate into the RNA of 15′- cells. It was found that, with 1.1 μg of myxin per ml of medium, the
incorporation of orotate was inhibited 25% after 60 min (Table 3). This small degree of inhibition could explain the increase previously found in the amount of uracil incorporated, if it is assumed that a sufficiently large proportion of the uracil in RNA was normally supplied from the orotate pathway in 15T- cells.

**Effect on RNA biosynthesis.** As shown above, it appeared that myxin, at a level of 5 μg/ml, completely inhibited the synthesis of RNA in 15T- cells after 45 min, as measured by 14C-uracil incorporation (Fig. 3). This finding was examined further by colorimetric determinations of total RNA with time in E. coli B under identical experimental conditions (Table 4). The chemical determination of RNA in these cells showed that, after 15 min of exposure to myxin, no significant synthesis of RNA occurred. The discrepancy in timing between the results of the two techniques is accounted for by the stimulated uptake of 14C-uracil as shown above. It should be noted that, unlike the results when DNA synthesis was examined in these cells (Table 1), there was no degradation of the pre-existing RNA.

**Effect on protein synthesis.** The results of 14C-leucine incorporation by 15T- cells (Fig. 5) indicated that the inhibition of protein synthesis was dependent on myxin concentration. At 5 μg of myxin per ml, protein synthesis was almost completely inhibited by 30 min. Subsequently, 14C-leucine incorporation continued at 5% of the rate observed in the control cells. The chemical determination of total protein synthesis in E. coli B at 5 μg of myxin per ml under the same conditions (Table 5) showed a similar general effect. The rate of protein synthesis at the time of myxin addition remained constant for 60 min, whereas in the control cells the rate increased with time, owing to cell multiplication.

**Bactericidal effect of myxin.** To correlate the biochemical results with the biological effect, the rate at which log-phase 15T- cells were rendered nonviable was determined with myxin at 4.5 μg/ml. The number of viable cells was measured, at various times after the addition of myxin, by spreading suitable dilutions (fresh M9 medium) on nutrient agar plates. The results (Fig. 6) showed a logarithmic decrease in the number of viable cells between 13 and 33 min, with only 0.1% of the cells surviving at 60 min.

These data indicate that, during the first 13 min, all of the previously described metabolic effects due to myxin were completely reversed (by dilution) in over 90% of the exposed cells; by 33 min, the damage was irreversible in nearly all of the cells and led eventually to the death of these cells.

**Uptake of 14C-labeled myxin.** It was of interest

### Table 3. Effect of myxin (1.1 μg/ml) on the incorporation of 14C-orotate by 15T- cells

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Myxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>20</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>42</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>63</td>
<td>4.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

### Table 4. Effect of myxin (5 μg/ml) on RNA biosynthesis in Escherichia coli B

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Myxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>15</td>
<td>125</td>
<td>120</td>
</tr>
<tr>
<td>30</td>
<td>185</td>
<td>120</td>
</tr>
<tr>
<td>45</td>
<td>225</td>
<td>125</td>
</tr>
<tr>
<td>60</td>
<td>340</td>
<td>125</td>
</tr>
</tbody>
</table>

* Assay conditions the same as in Table 1.

![Graph](http://jb.asm.org/)

**FIG. 5. Effect of myxin on the incorporation of 14C-leucine into the protein of 15T- cells. Cultural conditions the same as in Fig. 1. The cells were exposed to myxin (concentration in micrograms per milliliter marked on curves) for 7 min before the addition of 14C-leucine to 25 μg/ml at zero-time. The amount of 14C incorporated into the acid-insoluble cell fraction was measured with time. This was plotted as a percentage of the amount originally added.**
Table 5. Effect of myxin (5 μg/ml) on protein biosynthesis in Escherichia coli B

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Myxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.56</td>
<td>0.90</td>
</tr>
<tr>
<td>15</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>30</td>
<td>1.28</td>
<td>0.92</td>
</tr>
<tr>
<td>45</td>
<td>1.85</td>
<td>1.22</td>
</tr>
<tr>
<td>60</td>
<td>3.00</td>
<td>1.45</td>
</tr>
</tbody>
</table>

* Determined on the hot acid-insoluble residue obtained by Burton's method (6).

A 1-ml quantity of the extract was equivalent to 20 ml of cell culture.

Zero-time represents the time of myxin addition.

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Table 6. Uptake of 14C-myxin by Escherichia coli 1ST-

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>14C-myxin bound to cells, % of amount added</th>
<th>Uptake by cells μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.2</td>
<td>9.0</td>
</tr>
<tr>
<td>30</td>
<td>0.37</td>
<td>16.6</td>
</tr>
<tr>
<td>45</td>
<td>0.61</td>
<td>27.4</td>
</tr>
<tr>
<td>60</td>
<td>0.52</td>
<td>23.4</td>
</tr>
</tbody>
</table>

* Log-phase cells (5.0 ml, 0.8 × 10^6 cells per ml) in M9 medium were incubated at 37 C with 14C-myxin at 4.5 μg/ml. At times indicated, the cells were collected and washed in M9 by centrifugation; the cells were then dissolved in hyamine hydroxide and the 14C was counted in a liquid scintillation spectrometer.
with time. This color change appeared analogous to the chemical reduction of myxin and was, therefore, followed spectrophotometrically. In addition, the two forms of myxin were separated by paper chromatography, and the amount of each was determined.

\[ ^{14}\text{C}-\text{myxin} \] (4.5 \( \mu \text{g/ml} \)) was added to 15T\(^{-}\) cells, samples were taken at intervals, and the cells were removed by centrifugation. Myxin (oxidized plus reduced) was quantitatively extracted with chloroform from the supernatant fractions, and then concentrated; the two forms of myxin were separated by paper chromatography with methanol as the solvent (20). The chromatograms were scanned (Actigraph II, Nuclear Chicago Corp.) and the radioactivity in each peak was determined. The results (Fig. 7) show that 50\% of the myxin was in the reduced form at 15 min. The visible spectrum of the chloroform extract at 60 min is shown in Fig. 8. The spectrum of the original oxidized form as well as that of the chemically reduced form is also presented. By 120 min, essentially none of the myxin was present in the oxidized form and its spectrum was identical to that of the chemically reduced myxin.

Fully reduced myxin, prepared as above, was tested for its biological activity. At 5 \( \mu \text{g/ml} \), the reduced myxin had no effect on \( ^{14}\text{C}-\text{uracil} \) and \( ^{14}\text{C}-\text{thymine} \) incorporation into the RNA and DNA, respectively, of 15T\(^{-}\) cells (Table 7). The

\[ \text{Fig. 7. Reduction of myxin by 15T}\(^{-}\) cells. The bacteria (0.8 \( \times \) 10\(^{8}\) cells/ml) were incubated at 37 \( ^{\circ}\)C with \( ^{14}\text{C}-\text{myxin} \) (4.5 \( \mu \text{g/ml} \)), and the amount of \( ^{14}\text{C} \) in both the oxidized and reduced forms was determined in chloroform extracts of samples removed at various times. (○) Oxidized myxin; (○) reduced myxin.} \]

\[ \text{Fig. 8. Visible spectrum of oxidized and reduced myxin. The biologically reduced myxin (curve B) was that obtained from the 60-min sample in the experiment described in Fig. 7. The biologically active oxidized form of myxin (curve A) was the standard material, extracted with chloroform from solution (4.5 \( \mu \text{g/ml} \)) in M9 medium to give the same relative concentration as the solution of reduced myxin. Curve C represents the spectrum of the same concentration of myxin reduced with sodium thiosulfite before extraction from M9 medium with chloroform.} \]

\[ \text{TABLE 7. Effect of biologically reduced myxin (5 \( \mu \text{g/ml} \)) on the incorporation of \( ^{14}\text{C}-\text{thymine} \) and \( ^{14}\text{C}-\text{uracil} \) by 15T\(^{-}\) cells} \]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>( ^{14}\text{C}-\text{thymine} )</th>
<th>( ^{14}\text{C}-\text{uracil} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>25</td>
<td>11.0</td>
<td>10.5</td>
</tr>
<tr>
<td>40</td>
<td>21.0</td>
<td>20.1</td>
</tr>
<tr>
<td>60</td>
<td>34.4</td>
<td>34.0</td>
</tr>
</tbody>
</table>

The primary effect of myxin on E. coli appears to be the inhibition of DNA synthesis, followed immediately by the degradation of pre-existing
DNA. This activity of myxin is analogous to that reported for mitomycin C (22) and is similar to that found with nalidixic acid (8). However, unlike mitomycin C, with myxin the inhibition of RNA synthesis followed almost immediately after the establishment of DNA inhibition. The rapidity of this sequence could indicate that the DNA was changed to a form in which it was unavailable as a template for the synthesis of either DNA or RNA. This would assume that the synthesis of all normal cellular RNA is DNA-dependent in these cells, as previously shown in Staphylococcus aureus (13), Bacillus cereus (10), and B. subtilis (1). Such a change could occur either by multiple breaks of the DNA double helix as a preliminary stage in its degradation or by the extensive binding of myxin to DNA, with the result that the activity of both DNA polymerase and DNA-dependent RNA polymerase is prevented. This activity of myxin would be similar to that reported in studies of ethidium (23) and proflavine (10) on these two purified-enzyme systems. With both drugs, each of the enzymes is strongly inhibited and the inhibition is dependent on DNA concentration, leading to the conclusion that each drug is bound to DNA and not to either of the polymerase enzymes.

A small amount of myxin was bound to the susceptible cells used in the present work, but whether any of this was bound specifically to bacterial DNA is unknown at present. The reduction of myxin in contact with susceptible cells may have been associated with its biological activity, possibly by some interference with the electron transport system. However, the results of preliminary experiments with a strain of myxin-resistant bacteria, isolated from E. coli 15T⁻, have indicated that almost as much labeled myxin was bound to the resistant cells as to the susceptible parent strain. This was not unexpected, since it has been shown in experiments with ¹⁴C-porflromycin and E. coli K-12 that up to 80% of the bound label is attached to protein and a large part of the remainder is combined with ribosomal RNA (16). In addition to the uptake of myxin, it was found that the rate at which myxin was reduced was somewhat greater with the resistant cells than with the susceptible cells. There was no effect on the biosynthesis of DNA, RNA, or protein on or cell division of the resistant cells. It would, therefore, appear likely that the reduction of myxin in contact with susceptible cells was a secondary phenomenon unrelated to its biological effects.

The amount of ¹⁴C-myxin bound by the 15T⁻ cells was not sufficient for satisfactory intracellular-distribution studies. It is anticipated that labeled myxin of much higher specific activity can be made easily, and this would aid in determining the cellular binding sites of this antibiotic as has already been successfully done for several isotopically labeled antibiotics (19).

The absence of RNA degradation in E. coli exposed to myxin may reflect only the lack of sensitivity of the methods used. It is not unlikely that there was sufficient structural change in soluble or ribosomal RNA so that nucleases active on DNA were released, similar to the results found with cells treated with mitomycin C (11, 12).

The maximal inhibition of DNA synthesis was not established until about 7 min after myxin addition, and the degradation of DNA was not significant until about 3 min later. The lethal effect was largely reversible (by dilution in the viable-cell titrations) until about 13 min after exposure. These results could reflect the activity of a repair mechanism similar to that found in the excision of thymine dimers from bacterial DNA damaged by ultraviolet light in vivo (5). This possibility is open to test by the use of physical-chemical methods in a study of DNA isolated from bacteria exposed to myxin under various conditions.

Other experiments are planned in which cell-free enzyme systems concerned with the syntheses of DNA and RNA will be isolated from both susceptible and resistant cells, and the possible inhibitory effect of myxin will be tested in each system. DNA will be isolated from normal and myxin-treated cells and a comparison made between the two materials when serving as a template for the synthesis of either DNA or RNA. We would speculate that the results of such experiments may indicate that the activity of myxin is similar to that of edeine, which has been reported to inhibit DNA synthesis specifically (3) but without being bound to DNA (4) and which, therefore, is believed to act directly on the DNA-polymerizing enzyme.

Acknowledgments

We are indebted to J. Ross and K. Zahler for capable technical assistance.

Literature Cited