Effect of Myxin on Deoxyribonucleic Acid Synthesis in *Escherichia coli* Infected with T4 Bacteriophage¹

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Exposure of *Escherichia coli* cells to myxin results in the almost complete inhibition of new deoxyribonucleic acid (DNA) synthesis, extensive degradation of pre-existing intracellular DNA, and a rapid loss of viability in these cells (9). After exposure to myxin for 30 min (<1% survivors and >25% degradation of DNA), infection of these cells by T4 bacteriophage results in the renewal of DNA synthesis at a rate essentially equal to that found in T4-infected cells in the absence of myxin. This DNA was characterized as T4 DNA by hybridization and by hydroxyapatite chromatography. These results suggest that the primary site of action of myxin does not involve the biochemical pathways involved in either the energy metabolism or the biosynthesis of DNA precursors in the uninfected host cell. The yield of infectious T4 particles was reduced when myxin was present during multiplication. This effect may be partly accounted for by the finding that a significant fraction of the T4 DNA synthesized in the presence of myxin is apparently not properly enclosed by the bacteriophage protein coat since it is shown to be degraded by exogenous nuclease.

The primary action of myxin (1-hydroxy-6-methoxyphenazine-5,10-dioxide) at a concentration of 5 µg/ml has been shown to be the inhibition of deoxyribonucleic acid (DNA) synthesis in logarithmic cultures of *Escherichia coli* 15T⁻. This is closely followed by the inhibition of ribonucleic acid (RNA) and protein synthesis (9). The exact mechanism of action of this antibiotic is still unresolved.

There are several known differences between the biosynthetic pathways for the synthesis of T4 bacteriophage DNA and *E. coli* DNA (10); therefore, it appeared reasonable to investigate the effect of myxin in T4-infected 15T⁻ cells. The results reported here show that myxin does not inhibit the synthesis of DNA in 15T⁻ cells infected with T4 bacteriophage. These results would exclude certain metabolic pathways involved in DNA biosynthesis as the site of myxin action in uninfected *E. coli* cells.

MATERIALS AND METHODS

Bacterial cultures. *E. coli* 15T⁻, kindly supplied by K. G. Lark, was grown in M9 synthetic medium as described previously (9). The initial thymine concentration of the medium varied from 1.5 to 4.0 µg/ml depending on the amount of cell growth anticipated. The cells utilized about 0.36 µg of thymine per ml in growing to a density of 2×10^8 /ml.

Bacteriophage. T4 wild-type phage was obtained from R. C. French, and a stock preparation was made by standard procedures (1).

Myxin. The pure antibiotic myxin, prepared by the methods developed in this Institute (13), was the gift of E. A. Peterson. A stock solution of 1 mg of myxin (in acetone) per ml was stable in the cold. In preparation for use, the acetone was removed with nitrogen and the residue was dissolved in M9 medium.

Radioactive materials. Thymine-2-¹⁴C and thymine-6-³H were obtained from New England Nuclear Corp.

Synthesis of DNA. The host cells, growing logarithmically, were infected with T4 bacteriophage at a multiplicity of 7 when the bacterial population reached 1.8×10^8 /ml. The synthesis of DNA was followed at 37°C by measuring the accumulated incorporation of labeled thymine with time into the cold trichloroacetic acid-insoluble fraction of the cells, as described previously (9). Ninety-four per cent of the ¹⁴C-thymine incorporated into the acid-insoluble fraction of T4-infected cells was found in viable bacteriophage in the lysate under normal conditions.

Measurement of radioactivity. After cold trichloroacetic acid extraction of a suitable portion of the culture, the acid-insoluble residue was collected by filtration on a membrane filter (Millipore Corp., Bedford, Mass.) which was then air-dried and fastened to a planchet. The radioactive content was determined in a gas-flow thin-window counter (Nuclear-Chicago Corp.).

In the characterization of DNA by column chromatography and by hybridization, all of the samples

¹ Contribution no. 683.

were counted in Bray's scintillation fluid (3) by an Ansitron II liquid scintillation spectrometer.

Titration. When a suitable dilution of 15T⁻ cells growing in M9 medium is plated on nutrient agar, only about 50% of the cells give rise to colonies in comparison with the number of colonies formed on M9 agar. Our previously reported results, with nutrient agar in the titrations, showed that myxin (5 µg/ml) had no lethal effect on 15T⁻ cells growing in M9 medium during the initial 10 min of exposure. After 10 min, there was a rapid loss of cell viability (9). We now find that under the same experimental conditions, but with M9 agar replacing the nutrient agar in the titrations, the cells are rapidly killed from the time of myxin addition. The number of surviving cells present when plated on M9 agar after 5, 10, 15, 20, or 30 min of exposure to myxin is found to be 50, 28, 12, 6, and 0.4%, respectively, of the initial cell population at 0 min (1.8×10^8 cells/ml).

The preparation of rabbit T4 antiserum and the titration of infective centers or free bacteriophage particles was carried out by standard procedures (1).

Isolation of DNA. T4 was labeled with either ¹⁴C-thymine or ³H-thymine by infection of 15T⁻ cells growing in M9 medium containing the labeled precursor. The labeled T4 DNA was isolated from the bacteriophage, after purification by differential centrifugation and treatment with deoxyribonuclease and ribonuclease, by phenol extraction (11).

The labeled DNA was isolated from 15T⁻ cells (growth in M9 medium containing ¹⁴C- or ³H-thymine) and from T4-infected cells labeled as described above, essentially as described by Smith (15). The cells from 40 ml of culture, at a cell density of 1.8×10^8 /ml, were resuspended in 5 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris), pH 8, containing 0.01 M sodium ethylenediaminetetraacetate (EDTA). Lysozyme (1 mg; Mann Research Lab.) was added, and the suspension was incubated at 37 C for 5 min. Sodium dodecyl sulfate was then added to a final concentration of 0.6%. After incubation at 37 C for a further 5 min, the suspension had cleared; 0.7 ml of 10× SSC (1× SSC, 0.15 M NaCl and 0.015 M sodium citrate, pH 7) and 10 mg of Pronase (Calbiochem) were added, and the incubation at 37 C was continued for 30 min. The lysate was extracted with an equal volume of phenol (saturated with 0.1 M Tris, pH 7) and the aqueous layer was removed and dialyzed against 0.05 M phosphate buffer (pH 7) in the cold. The dialyzed preparation was treated for 15 min at 37 C with pancreatic ribonuclease (10 µg/ml; Miles Laboratories, Inc.) that had been previously heated at 80 C for 5 min. After another phenol extraction, the purified DNA was dialyzed exhaustively against 1× SSC in the cold. Recovery of labeled DNA was 85% in each experiment. The DNA content of each preparation was determined by the method of Ceriotti (4).

Characterization of DNA isolated from myxin-exposed cells infected with T4. The DNA was characterized by column chromatography on hydroxyapatite as described by Oishi (12) by using a column (1 by 3 cm) and a linear gradient of 0.15 M to 0.45 M sodium phosphate buffer (pH 7.0) to elute the DNA frac-

tions. Hybridization with membrane filters exactly as described by Denhardt (5) was also used to characterize samples of DNA.

Enzymes. Deoxyribonuclease (ribonuclease-free) was purchased from Worthington Biochemical Corp., pancreatic ribonuclease grade III from Miles Laboratories, Inc., and Pronase from Calbiochem.

RESULTS

Effect of myxin on DNA synthesis. The effect of increasing concentrations of myxin on the incorporation of ¹⁴C-thymine by T4-infected 15T⁻ cells is shown in Fig. 1. The cells, growing in the logarithmic phase, at 1.8×10^8 /ml were incubated with either 0, 5, 10, or 15 µg of myxin per ml for exactly 5 min at 37 C. At this point, T4 bacteriophage was added at a multiplicity of 7 along with ¹⁴C-thymine to give 0.02 µCi/ml in each culture. With 5 µg of myxin per ml, there was an apparent delay of about 10 min before the start of thymine incorporation. After this lag, the rate of thymine incorporation equaled that in the T4-infected cells without myxin. At higher concentrations of myxin, the rate of thymine incorporation was greatly reduced. Since the minimum concentration of myxin required to block DNA synthesis completely in uninfected 15T⁻ cells was known to be 5 µg/ml (9), all subsequent experiments were done at this concentration.

The 15T⁻ cells are killed at a rapid rate from the time of myxin addition at 5 µg/ml (*see above*), and it is known that the DNA in these cells is extensively degraded after 30 min of exposure to the antibiotic (9). Despite these effects of extended exposure to the antibiotic, the cells were still capable of supporting DNA synthesis when infected with T4, as measured by the incorporation of ¹⁴C-thymine (Fig. 2). In an experiment to demonstrate this point, the cells were exposed to myxin at 5 µg/ml (with simultaneous addition of ¹⁴C-thymine) for 0, 20, 30, 50, 60, or 90 min before infection with T4. The results show that, after 30 min of exposure to myxin, infection with T4 results in the renewal of DNA synthesis at a rate directly comparable to that in cells infected in the absence of myxin (Fig. 2, curve 4 and curve 1). Even after 50 min of exposure to myxin, there is an appreciable rate of DNA synthesis shortly after T4 infection (Fig. 2, curve 5); however, after 60 min of exposure, the rate was seriously impaired and, after 90 min of exposure to myxin before T4 infection, no DNA synthesis was evident. The incorporation of ¹⁴C-thymine by unexposed uninfected host cells at 30 min was 980 counts per min per ml (not shown in the figure). The effect on ¹⁴C-thymine incorporation by myxin in these cells is shown by curve 8 (Fig.

FIG. 1. Effect of myxin concentration on ^{14}C -thymine incorporation into DNA of 15T^- cells infected with T4 bacteriophage. Log-phase cells in M9 medium at 37°C were incubated with myxin for exactly 5 min. Labeled thymine ($0.02 \mu\text{Ci/ml}$) and T4 at a multiplicity of 7 were then added (zero time in the graph), and the amount of ^{14}C -thymine incorporated into cold trichloroacetic acid-insoluble material was measured with time. The concentration of myxin in micrograms per milliliter is marked on the curves.

2) and represents $> 90\%$ inhibition of DNA synthesis.

The effect of the addition of myxin, at various times after T4 infection, on the incorporation of ^{14}C -thymine into DNA is shown in Fig. 3. Cells were infected with T4 at zero time, and myxin was added at the times shown by the arrows in the figure. Although no significant effect of myxin addition on the rate of DNA synthesis was observed, it is shown later that the physical state of

FIG. 2. Recovery of DNA synthesis as a result of bacteriophage infection in cells exposed to myxin for various times and its relationship with infective centers. Cells were exposed to myxin ($5 \mu\text{g/ml}$), and ^{14}C -thymine was added (zero time in the graph). Bacteriophage was added at 0, 20, 30, 50, 60, or 90 min (curves 2, 3, 4, 5, 6, and 7, respectively), and the incorporation of radioactivity into the cold trichloroacetic acid-insoluble fraction was measured in each infected culture with time. The incorporation of ^{14}C -thymine by infected cells in the absence of myxin is shown in curve 1, and the effect of myxin in uninfected host cells is shown by curve 8. The dotted line in the figure represents infective centers titrated through rabbit antiserum within 10 min of T4 addition in each case. (100% = all viable cells infected with T4.)

the portion of this DNA made immediately after the addition of myxin is different from that made in the absence of myxin.

Effect of myxin on bacteriophage infectivity and yield. In this experiment, the recovery of DNA biosynthesis as measured by the incorporation of ^{14}C -thymine was correlated with the ability of the 15T^- cells to support T4 infection at various times after exposure of the host cells to myxin. Bacterio-

FIG. 3. Effect on the synthesis of T4 DNA of myxin addition at various times after bacteriophage infection. The bacteriophage and ^{14}C -thymine were added to 15T $^{-}$ cells at zero time. Myxin at a final concentration of 5 $\mu\text{g/ml}$ was added at 0, 10, 20, and 40 min (arrows in the figure), and the incorporation of radioactivity into the cold trichloroacetic acid-insoluble fraction was measured with time. Curve A, infected control without myxin; curve B, myxin addition at 0 min; curve C, myxin addition at 10 min; curve D, myxin addition at 20 min; curve E, myxin addition at 40 min.

phage at a multiplicity of 7 was added to myxin-exposed cells and 10 min later the number of infective centers present was determined by titration through T4 antiserum to neutralize all unadsorbed virus particles. The results (Fig. 2, dotted line) show that up to 25 min after exposure to 5 μg of myxin per ml all of the cells were capable of supporting at least some T4 synthesis. By 40 min, this number was reduced by 50% and approached zero after exposure to myxin for 90 min.

The yield of bacteriophage was measured in an experiment similar to the one described above except that a constant period of 60 min was allowed for multiplication after the addition of the T4 bacteriophage. At that time, a suitable dilution was made to prevent loss of intracellular phage by adsorption to cellular debris and KCN was added to interrupt cellular metabolism with consequent cell lysis. The yield of bacteriophage from these cells exposed to myxin for various times before

T4 infection is given in Table 1. An almost constant yield of about 20 to 25%, as compared to the control without myxin, was obtained with as much as 10 min of myxin exposure. The yield of T4 fell rapidly thereafter, and, after 90 min of exposure to myxin before T4 infection, the yield fell to 0.8% of that in the control.

Characterization of DNA synthesized in myxin-exposed cells after T4 infection. To ensure that all of the DNA synthesized in myxin-exposed cells after T4 infection was in fact T4 DNA, the isolated DNA was characterized by two techniques. A culture of 15T $^{-}$ cells was exposed to myxin (5 $\mu\text{g/ml}$), and suitable amounts of ^{14}C -thymine and T4 were added 5 min later. After 60 min at 37 C, the ^{14}C -labeled DNA was isolated and analyzed by column chromatography on hydroxypatite along with a sample of ^3H -labeled DNA isolated from uninfected 15T $^{-}$ cells grown in M9 medium containing ^3H -thymine. Each DNA

TABLE 1. *Effect of myxin on T4 replication*^a

Time of infection after myxin addition (min)	Bacteriophage yield 60 min later	
	Infectious particles/ml	Per cent of control without myxin
0	4.3×10^9	27
5	3.0×10^9	19
10	3.5×10^9	22
20	1.4×10^9	9
40	6.4×10^8	4
60	4.8×10^8	3
90	1.3×10^8	0.8
Control (no myxin addition)	1.6×10^{10}	100

^a Cells at 1.5×10^8 /ml were exposed to myxin at 5 μ g/ml. T4 at a multiplicity of 7 was added at the indicated times, and the total number of T4 particles produced was determined by titration by using M9 for both layers. Bacteriophage replication was stopped at 60 min, and the cells were lysed by a 1:100 dilution into cold 0.01 M KCN in M9 medium.

species was eluted from the column as a separate well-defined fraction with > 80% recovery of each labeled DNA. No radioactivity was found in any other tube. The ³H-labeled 15T⁻ DNA was found in tubes 8, 9, and 10, whereas the ¹⁴C-labeled DNA was contained in tubes 11, 12, and 13. With the same experimental conditions, ¹⁴C-DNA was applied to a fresh column of hydroxyapatite along with a sample of authentic ³H-labeled T4 DNA. All of the radioactivity was eluted in tubes 11, 12, and 13. These results are similar to those reported by Oishi (11) for the separation of *E. coli* DNA from T4 bacteriophage DNA by the same method.

The above ¹⁴C-labeled DNA was also examined by a test for hybridization with T4 DNA or 15T⁻ DNA by the membrane filter technique of Denhardt (5). In addition, ¹⁴C-labeled DNA isolated from T4-infected cells under slightly different conditions was also tested. As will be shown below, the DNA synthesized during the 20 min immediately after myxin addition to T4-infected cells is different from that made later, in that it is susceptible to the action of nuclease *in vitro*. In this instance, the cells were infected with T4, and 20 min later both myxin and ¹⁴C-thymine were added. After incubation for a further 20 min, the cells were collected by centrifugation and their content of DNA was isolated.

The results of the hybridization test show that at least 80% of the DNA synthesized after T4 infection of 15T⁻ cells exposed to myxin is able to hybridize with known T4 DNA (Table 2). Prac-

TABLE 2. *Hybridization characteristics of DNA isolated from T4-infected 15T⁻ cells cultured in the presence of myxin*

DNA on filter ^a	Input DNA (counts/min)	¹⁴ C-labeled input DNA ^b	Per cent hybridized ^c
T4	430	T4	60.5
T4	1,950	15T ⁻	1.2
T4	685	T4-15T ⁻ (20) ^d	50.4
T4	840	T4-15T ⁻ (60) ^e	50.2
15T ⁻	1,950	15T ⁻	5.8
	430	T4	0.7
	685	T4-15T ⁻ (20)	1.0
	840	T4-15T ⁻ (60)	1.0

^a DNA on filter. An 8- μ g amount $\pm$ 0.1 μ g of ³H-labeled T4 or 15T⁻ DNA was found to be fixed to each membrane filter as determined by measurement of radioactivity.

^b Input DNA. Exactly 0.5 μ g in 0.1 ml of each input DNA (sonically treated and heat denatured) was added to the membranes in 1.3 ml of preincubation medium.

^c Per cent hybridization: (μ g of input DNA bound to DNA on membrane filter/ μ g of input DNA) $\times$ 100. Only a trace of ³H-labeled DNA was released from the filters during 16 hr of incubation at 65 C. Suitable corrections were made for adsorption to blank membrane filters in each case.

^d T4-15T⁻ (20). 15T⁻ cells were first infected with T4 followed by the addition of myxin and ¹⁴C-thymine at 20 min, and 20 min later the DNA was isolated (90% recovery of acid-insoluble ¹⁴C).

^e T4-15T⁻ (60). 15T⁻ cells were exposed to myxin for 5 min and then ¹⁴C-thymine and T4 were added. DNA was isolated at 60 min (90% recovery).

tically none of the DNA synthesized under these conditions was able to form a hybrid with authentic 15T⁻ DNA. Taken together with the results of the column chromatography on hydroxyapatite, these data indicate that only T4 DNA is synthesized in the presence of myxin.

T4 DNA synthesized in the presence of myxin.

In this experiment, the susceptibility to deoxyribonuclease of the T4 DNA synthesized by the infected cells after the addition of myxin was examined. The experimental design was similar to that in Fig. 3, with one difference. After infection of the cells with T4, in the presence and absence of myxin, there was a 12-min incubation period to allow sufficient time for DNA synthesis to begin in both cultures. ¹⁴C-labeled thymine (0.3 μ g/ml) was then added, followed 5 min later by unlabeled thymine (40 μ g/ml) to reduce effectively further incorporation of labeled thymine. At the time points shown in Table 3, duplicate samples were removed from each culture. One sample was

TABLE 3. *Susceptibility to deoxyribonuclease of DNA in T4-infected 15T⁻ cells^a*

Sample time (min)	¹⁴ C-labeled DNA (counts per per min per ml)		Deoxyribonuclease-resistant ¹⁴ C-DNA (%)	
	- Myxin	+ Myxin	- Myxin	+ Myxin
30	4,050	4,010	73	49
45	4,220	4,110	81	53
60	4,460	4,440	94	60
75	4,800	4,340	90	68

^a 15T⁻ cells were infected with T4 in the presence and absence of myxin at 5 µg/ml. After incubation at 37 C for 12 min, ¹⁴C-thymine (0.03 µg/ml) was added, followed by unlabeled thymine (40 µg/ml) exactly 5 min later. Duplicate samples were removed from each culture at the times indicated. One sample was extracted with cold 10% trichloroacetic acid for ¹⁴C-thymine incorporation, and cell lysis was induced in the other by the addition of KCN (to 0.01 M) and chilling for 30 min. The lysed sample was digested with deoxyribonuclease, after the addition of MgSO₄ (0.04 M), at 37 C for 20 min; the amount of cold trichloroacetic acid-insoluble radioactivity remaining was measured.

extracted with 10% trichloroacetic acid in the cold for the estimation of acid-insoluble radioactivity. Sufficient KCN was added to the other (to 0.01 M) to stop further biosynthesis and, with chilling, to induce cell lysis. After the addition of MgSO₄ (to 0.04 M) and deoxyribonuclease (5 µg/ml), each lysate was incubated at 37 C for 20 min, chilled, and extracted with cold 10% trichloroacetic acid to measure the residual acid-insoluble radioactivity.

It is seen (Table 3) that in cultures without myxin the amount of deoxyribonuclease-resistant T4 DNA increased from 70% at 30 min to about 90% at 75 min after infection. In the culture with myxin present, this deoxyribonuclease-resistant T4 DNA increased from 50% to about 70% over the same time period. These results show that, of the T4 DNA synthesized in the presence of myxin, a larger proportion is susceptible to deoxyribonuclease than is the case with the T4 DNA made in the absence of myxin.

The above results become more meaningful when the results of the following control experiments are considered. Here the effect of the nuclease on both T4 DNA and intact T4 bacteriophage added to the KCN lysates was examined. Purified ³H-labeled T4 DNA and purified ³H-labeled T4 were each added to separate portions from the above T4-infected cultures after lysis with KCN. The labeled T4 DNA was found to be almost completely degraded to an acid-soluble

form (97%), whereas the labeled bacteriophage was essentially unaffected (5%) by the 20-min digestion with deoxyribonuclease. Separate samples (at 30 min) of the KCN lysates with added ³H-labeled T4 DNA were incubated without the addition of nuclease. Measurement of the acid-insoluble residue showed that in the lysate from the T4 infected culture with myxin present the ³H-DNA was degraded by 40%; in that from the culture without myxin, it was degraded by only 20%.

DISCUSSION

It is known that the addition of myxin (5 µg/ml) to uninfected 15T⁻ cells results in essentially complete inhibition of DNA synthesis within 7 min, followed shortly afterwards by the inhibition of RNA and protein synthesis, and accompanied, after 10 min of exposure, by degradation of intracellular DNA to an acid-soluble form (9). At the same concentration of myxin, 15T⁻ cells are rapidly killed from the time the antibiotic is added (50% survivors at 5 min).

The results presented here show that these effects of myxin are largely nullified when the cells are infected with T4 bacteriophage. The rate of DNA synthesis found in the normal T4-infected cells is not reduced to any significant extent when the infection occurs in the presence of myxin (Fig. 1). There is a slight, but reproducible, delay before the onset of DNA synthesis after infection when myxin is present. The reason for this is unknown, but it does show, along with the fact that the yield of infectious mature T4 particles is reduced in myxin-exposed cells, that there is no significant hindrance to the rapid entry of myxin into the T4-infected cell. Nearly all of the DNA synthesized by the infected cells during 60 min of incubation in the presence of myxin is shown to be T4 DNA, and not host cell DNA, by hybridization tests and by column chromatography on hydroxypatite. A significant proportion of this T4 DNA is shown to be degraded by deoxyribonuclease *in vitro*; it is, therefore, not protected to the same extent in these cells by being enclosed in mature T4 bacteriophage particles as it is in cells not exposed to myxin. One effect of myxin, which would account for the decreased yield of infectious T4 particles, may be an interference with the insertion of the T4 DNA into the protein coat to form the completed virus particle. There is no direct evidence that the synthesis of T4 coat protein is normal in the myxin-exposed cell, but the rate of synthesis of protein and RNA (measured by the incorporation into the acid-insoluble cell fraction of ¹⁴C-labeled leucine and uridine, respectively) is closely the same in the T4-infected cell in

either the presence or absence of myxin (*data not shown*).

It is of interest to compare our results with previously reported effects of nalidixic acid and mitomycin C on phage development. In the presence of nalidixic acid, the multiplication of the DNA coliphages T2, T5, ϕ X174, and ϕ R was inhibited and no infectious progeny particles were produced (16). However, with the same drug, it was found that nearly normal replication of the SP01 bacteriophage occurred in the host cell, *Bacillus subtilis* (5). It should prove interesting to make a direct comparison of the effect of myxin and of nalidixic acid on these bacteriophage systems.

At mitomycin C concentrations sufficient to inhibit DNA synthesis in uninfected cells, T2-infected *E. coli* B did actively synthesize DNA, but the infected cells did not produce any infective bacteriophage particles (14). This differential effect of mitomycin C on DNA synthesis in normal and in T2-infected cells is similar to the results reported above with myxin. However, the T2 DNA produced in the presence of mitomycin C was resistant to the action of deoxyribonuclease after the action of alkali, indicating that the DNA had probably been protected by the bacteriophage coat and, since they were uninfected particles, it was possible that the DNA was biologically inactive.

As mentioned above, previous results (9) show extensive inhibition of host cell metabolism 30 min after the addition of myxin, whereas the present results show that at 30 min each cell is capable of nearly normal T4 DNA synthesis and the production of some infectious progeny particles (Fig. 2). It is unlikely, therefore, that the primary effect of myxin in the *uninfected* host cell involves interference with either energy metabolism or the biosynthesis of any DNA precursor. The possible exception would be the synthesis of deoxycytidylic acid which is replaced by hydroxymethyldeoxycytidylic acid in the infected cells and is synthesized in response to information supplied by the T4 genome (6). Since infection of *E. coli* with T4 results in the production of a new T4-specific DNA polymerase (2, 16) and this polymerase is not inhibited by myxin, the possibility exists that myxin does inhibit the polymerase present in the uninfected host cell. In this regard, our results (*unpublished data*) show that polymerase isolated from *E. coli* by the procedure of Kornberg (8) was not inhibited when tested in

vitro with myxin. One possibility to be investigated is that myxin interferes with DNA biosynthesis by establishing a block in the action of DNA ligase (18).

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