Differential Effects of DNA Gyrase Inhibitors on the Genetic Transformation of *Neisseria gonorrhoeae*

CHRISTINE PARHAM,† ELOISE CUNNINGHAM, AND E. MCGINNIS*

Department of Microbiology, Meharry Medical College, Nashville, Tennessee 37208

Received 27 May 1988/Accepted 13 September 1988

Inhibitors of DNA gyrase in *Escherichia coli* exerted differential effects on the genetic transformation of *Neisseria gonorrhoeae*. When competent cells of the gonococcus were exposed to novobiocin before the uptake of transforming antibiotic resistance DNA, there was a 50 to 60% reduction in the number of transformants compared with the number of control untreated cells. Norfloxacin, a more potent inhibitor of DNA gyrase and an analog of nalidixic acid, nearly abolished the production of transformants by recipient cells. On the contrary, exposure of competent cells to nalidixic acid had no effect on transformant yield. The target of these inhibitors appears to be at the level of recombination. Possible mechanisms are discussed.

The chromosome of *Escherichia coli* can be isolated as a highly compacted structure which is organized into negatively supercoiled domains (29, 31). Negative supercoiling of DNA facilitates unwinding of the duplex and therefore promotes those processes which require DNA denaturation, such as replication, transcription, and recombination (5, 7). The introduction of negative supercoils in a DNA molecule as well as relaxation of such superwists are carried out by DNA gyrase, a type II topoisomerase, originally described in *E. coli* (9). Gyrase is composed of two subunits, GyrA and GyrB, which are encoded on nonadjacent genes in *E. coli* (8, 10). The GyrA subunit, which is inhibited by nalidixic acid and oxolinic acid, is responsible for the breakage and reunion activity of the enzyme (8, 30); the GyrB subunit is an ATPase which is inhibited by two structurally related antibiotics, novobiocin and coumermycin A1 (10, 19). Increasingly more potent derivatives of nalidixic acid are pipemidic acid, ciprofloxacin, norfloxacin, and rosoxacin, all of which have a common target, the GyrA subunit (22). DNA gyrase inhibitors have been shown to block the supercoiling activity of DNA gyrase in vivo and in vitro, and a specific correlation has been made between the coumermycin-induced loss of chromosomal superhelicity and inhibition of growth and DNA synthesis (6, 19, 20).

Genetic transformation in *Neisseria gonorrhoeae* occurs in several distinct stages: binding of DNA, uptake of DNA, formation of a preintegration complex, and incorporation of the complex into the recipient genome. Competence in *N. gonorrhoeae*, or the ability to take up DNA, is constitutive in the piliated, virulent colony types 1 and 2 and persists throughout the growth cycle. Colony types 3 and 4 are noncompetent and avirulent (1, 3, 17). The integration of donor DNA during transformation is achieved by a recombination event between a single-stranded donor DNA fragment and the recipient genome. The significance of negative supercoiling or DNA gyrase in recombination was realized upon the observation that closed circular lambda DNA without supercoils was an ineffective substrate for integrative recombination (16). Moreover, the reduction in number of transformants produced by *Streptococcus sanguis* after exposure to coumermycin was correlated with a loss of titratable supercoils in the chromosome (18).

In this study, we report differential effects of inhibitors of DNA gyrase on the genetic transformation of the gonococcus by various antibiotic resistance markers. The data support the notion that novobiocin- and norfloxacin-sensitive targets may be involved in the genetic transformation of *N. gonorrhoeae*, probably at the level of recombination. We presume these targets to be similar to the GyrA and GyrB proteins of *E. coli*. Possible mechanisms are discussed.

MATERIALS AND METHODS

**Chemicals.** Novobiocin, nalidixic acid, and rifampin were purchased from Sigma Chemical Company, St. Louis, Mo. Fresh solutions of nalidixic acid, novobiocin, and norfloxacin were prepared in 0.1 N NaOH at 10 mg/ml before the start of each experiment. Norfloxacin was kindly provided by Hans H. Gadebusch, of Merck Institute for Therapeutic Research; fusidic acid was provided by W. O. Godtfredsen, of Leo Pharmaceutical Products, Ballerup, Denmark; streptomycin sulfate was obtained from Pfizer Inc., New York, N.Y.; and [2-1H]Adenine was purchased from Dupont, NEN Research Products, Boston, Mass.

**Organisms.** A proline-requiring laboratory strain of *N. gonorrhoeae* (strain 2686) was used as the recipient in all transformation assays. Donor DNA was extracted from strains JN25, JN33, and JN35, which contain Str', Fus', and Rif' markers, respectively, and are spontaneous isolates of strain 2686. Wild-type *N. gonorrhoeae* cannot form colonies in the presence of norfloxacin, novobiocin, or nalidixic acid at concentrations of more than 6 ng/ml, 0.2 μg/ml, and 0.4 μg/ml, respectively. To select for drug-resistant mutants, 0.1-ml portions of a suspension of strain 2686 at a density of 10⁵ CFU/ml were spread onto fresh GC medium base (Difco Laboratories, Detroit, Mich.) plates containing various concentrations of the drugs and incubated for 48 h at 37°C. Several colonies appeared which were able to grow in the presence of novobiocin and nalidixic acid at concentrations of 5.0 and 20 μg/ml, respectively. One of the novobiocin-resistant isolates was designated MN1431. Strains 2686, JN25, JN33, and JN35 were kindly provided by Fred Jones. All strains were stored in gonococcal broth containing 20% glycerol at -70°C.

**Growth conditions and media.** Cells were grown overnight
on GC medium base agar plates containing 1.0% defined supplements (12). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air in a Forma Scientific-ultrex incubator. Gonococcal broth, which also contained 1.0% defined supplements and 420 μg of sodium bicarbonate per ml, was used for growth of cells in liquid culture. Flasks were incubated in a water bath shaker (model G76; New Brunswick Scientific Co., Inc., Edison, N.J.), and growth was measured by Klett-Summerton colorimetry at 540 nm.

**Radiolabeling of DNA.** Cells from overnight gonococcal agar plates were suspended in a diphasic growth medium (consisting of an upper GC broth phase and a lower GC medium base agar phase) at a density of 10⁸ CFU/ml. After a 2-h lag period, 14C]adenine (specific activity, 15 to 20 Ci/mmol) was added and the cells were incubated for one doubling (28). DNA was extracted as outlined below.

**Transferring DNA Preparation.** The partially purified DNA used in this study was prepared by a modification of the method of Sparling (26), which consisted of sodium dodecyl sulfate lysis of cells followed by NaCl deproteinization and ethanol precipitation. In transformation experiments carried out with unlabeled DNA, concentration was determined by the diphenylamine assay of Burton (2). Radiolabeled DNA, however, was placed in 2.0 ml of SSC (0.15 NaCl plus 0.015 sodium citrate) and incubated in the presence of 100 μg of pancreatic RNase per ml at 37°C for 1 h. At the end of the incubation, 2.5 volumes of cold 95% ethanol were added and the precipitated DNA was placed in a final volume of 1.0 ml of SSC. After extensive dialysis against sodium citrate buffer, the DNA concentration was determined at 260 nm.

**Transformation Procedure.** The transformation procedure was a modification of the method of Biswas et al. (1). Cells of colony type 2 were scraped from 14- to 16-h gonococcal broth agar plates and resuspended in GC broth containing defined supplements 1 and 2. Cells (10⁸) receiving drug treatment were incubated in the presence of the drug at 37°C for 30 min. At the end of the incubation period, 5.0 ml of gonococcal broth was added to the cells, which were vortexed and spun at 4,500 rpm for 5 min. Any additional washings or centrifugations resulted in cell killing. The cell pellet was resuspended in 1.0 ml of fresh broth and diluted 10-fold. Control cells were incubated without drug and treated similarly. To tubes containing 1 to 5 μg of DNA per ml and 2 × 10⁻⁴ M CaCl₂, 2 × 10⁻³ to 5 × 10⁻³ drug-treated and untreated cells were added to allow DNA uptake. To terminate the reaction, 50 μg of pancreatic DNase per ml was added to destroy unbound DNA. After a 10-min incubation period, 0.1 ml of serial dilutions were plated in triplicate onto antibiotic-free GC medium base agar. After 4 to 5 h of incubation at 37°C, plates received three overlays: 200 μg of streptomycin per ml to select for the Str' marker, 0.1 μg of rifampin per ml to select for the Rif' marker, and 0.3 μg of fusidic acid per ml to select for the Fus' marker. CFU and number of transformants were determined at 48 h.

To determine whether the effect of novobiocin on transformation was at the level of DNA binding or post-DNA uptake, cells were allowed to take up radiolabeled DNA for 15 min and for 30 min at 37°C. After treatment with DNase to destroy unbound DNA, 10% trichloroacetic acid (TCA) was added to samples at 0°C for 30 min. Samples were washed onto membrane filters (type GC; Millipore Corp., Bedford, Mass.) with 5% TCA and 95% ethanol and air dried. DNase-resistant TCA-insoluble radioactivity was determined in a liquid scintillation spectrometer (Packard Instrument Co., Inc., Rockville, Md.).

**Alkaline sucrose gradients.** By using a modification of the method of McGrath and Williams (14), a spheroplast suspension of prelabeled cells (10⁷) was added to a layer of 0.5 N NaOH and 0.2% sodium dodecyl sulfate on top of 5.0-ml gradients of 5 to 20% sucrose containing 0.7 M NaCl, 0.3 N NaOH, 0.01 M EDTA, and a 60% sucrose shelf. After 10 min, tubes were centrifuged in an SW50.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.) in a Beckman L5-50 ultracentrifuge at 30,000 rpm for 120 min at 20°C. 3H-labeled lambda DNA (double-strand mass of 3.3 × 10⁶ daltons; single-strand mass of 1.65 × 10⁶ daltons) was added to each gradient as a molecular weight marker. Six-drop fractions were collected on no. 1 chromatography paper strips (Whatman, Inc., Clifton, N.J.) and processed by the method of Carrier and Setlow (4). Radioactivity was determined in a Packard scintillation spectrometer.

**S1 nuclease digestion.** S1 nuclease from Aspergillus oryzae, type III, was obtained from Sigma. Reaction mixtures (1 ml) contained 20 to 50 μg of prelabeled DNA in 30 mM sodium acetate buffer (pH 4.5)-1 mM ZnSO₄-0.2 M NaCl. Excess amounts of the enzyme were added to the mixture at 0°C and transferred to a 50°C water bath for 1 h. Samples received 100 μl of 1.0% bovine serum albumin as a carrier and were placed on ice in the presence of 10% TCA for 3 h. The percent TCA-precipitable counts remaining was determined after a wash of the sample in 5% TCA and 95% ethanol.

**RESULTS**

**Effect of novobiocin on transformation.** When competent cells of *N. gonorrhoeae* (colony type 2) were exposed to novobiocin before the uptake of transforming DNA, transformation was inhibited (Table 1). Maximum inhibition occurred at a drug concentration of 25 μg/ml, and there was no significant difference in viable cell counts determined at the time of plating for control and drug-treated cells. Incubation of streptomycin-sensitive cells in the presence of novobiocin for 30 min before the uptake of Str' marker DNA resulted in a 60% reduction in transformant yield. The magnitude of inhibition was similar for both limited and full DNA uptake. Transformation by Fus' and Rif' marker DNA was inhibited by approximately 50%. Whether or not additional inhibition of transformation was possible by using a post-DNA uptake drug treatment protocol is not known, since repeated washings of the cells to remove residual traces of drug resulted in a log loss of cells. The data suggest that the production of these transformants may involve a novobiocin-sensitive target.

**Effect of novobiocin on transformation in post-DNA uptake.** DNA extracted from log-phase streptomycin-resistant cells (JN25) was used as the donor DNA in transformation experiments. After uptake of DNA into a DNase-resistant form, 10-fold serial dilutions of the reaction mixture were spread onto GC plates to determine transformants and CFU, and another portion was used to determine TCA-insoluble radioactivity after DNA uptake as outlined in Materials and Methods. The data in Table 2 show similarity in DNase-resistant TCA-insoluble counts between the drug-treated and control samples. However, there was an approximately 60% reduction in number of transformants produced by cells exposed to novobiocin compared with the number produced by untreated control cells. The data suggest that novobiocin probably has no effect on DNA binding and uptake but...
exerts its effect at a stage of transformation which is subsequent to the uptake of DNA into a DNase-resistant form.

**Effect of a nov$^\circ$ mutation on transformation.** A nov$^\circ$ mutant of *N. gonorrhoeae* produced a two- to threefold-higher transformant yield than did the parental strain (2686) (Table 1). The increase in number of transformants was observed for both Str$^\circ$ and Fus$^\circ$ marker DNA but not for Rif$^\circ$ marker DNA. The molecular basis for this observation is not completely understood and may be related to intrinsic properties of these markers. Furthermore, when the mutant was incubated in the presence of novobiocin, there was no effect on the ability of the cells to produce transformants. The data suggest the possible involvement of a novobiocin-sensitive target at a stage post-DNA uptake in the production of streptomycin-, fusidic acid-, and rifampin-resistant transformants.

**Effect of nalidixic acid and norfloxac in on transformation.** When strain 2686 was exposed to increasing concentrations of nalidixic acid, there was little or no effect on the transformant yield after uptake of streptomycin resistance DNA (data not shown). In contrast, exposure of cells to norfloxacin, a fluorinated analog of nalidixic acid, resulted in nearly a complete inhibition of transformation by streptomycin resistance transforming DNA (Table 3). There was more than a 95% reduction in transformant yield when cells were exposed to the drug for 30 min at a concentration of 1 µg/ml. Variations in CFU between treated and control cells were less than 20%. The MIC of norfloxacin is 70-fold less than the MIC of nalidixic acid for *N. gonorrhoeae*, which correlates with the apparent differences in potency as DNA gyrase inhibitors.

**Effect of novobiocin on chromosomal DNA.** On the basis of the premise that DNA gyrase is needed to maintain the chromosome in a superhelical state, one would expect that an inhibition of the enzyme would affect the degree of superhelicity of both chromosomal and plasmid DNA. When the DNA of competent cells of *N. gonorrhoeae* was subjected to neutral sucrose gradient analysis, however, folded chromosomes sedimented in a manner similar to that of relaxed chromosomes without negative supercoils. Moreover, there was no apparent effect of novobiocin on the sedimentation rate of these chromosomes (data not shown). In an effort to understand the basis of the already reduced sedimentation rate of supercoiled DNA from the competent colony type 2 cells, an analysis was made of single-stranded DNA from these cells. The data in Fig. 1 indicate the

### Table 1. Effect of novobiocin treatment before DNA uptake in transformation of competent *N. gonorrhoeae* recipients by unlabeled antibiotic resistance marker DNA

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Phenotype</th>
<th>DNA uptake (min)</th>
<th>Transformants/ml (10$^6$) with given marker DNA</th>
<th>% Inhibition$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Str$^\circ$ Fus$^\circ$ Rif$^\circ$</td>
<td>Str$^\circ$ Fus$^\circ$ Rif$^\circ$</td>
</tr>
<tr>
<td>2686</td>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$ Rif$^\circ$ Pro$^-$</td>
<td>15</td>
<td>0.213/0.536</td>
<td>0.214/0.466</td>
</tr>
<tr>
<td>MN4131</td>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$ Rif$^\circ$ Pro$^-$</td>
<td>15</td>
<td>1.27/1.23</td>
<td>1.47/1.44</td>
</tr>
<tr>
<td>2686</td>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$ Rif$^\circ$ Pro$^-$</td>
<td>30</td>
<td>1.09/2.73</td>
<td>0.258/0.565</td>
</tr>
<tr>
<td>MN4131</td>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$ Rif$^\circ$ Pro$^-$</td>
<td>30</td>
<td>7.74/7.21</td>
<td>1.57/1.26</td>
</tr>
</tbody>
</table>

$^a$ Recipient cells (10$^7$) were used in the transformation assay after initial exposure to 25 µg of novobiocin per ml. There was no significant difference between viable cell counts of control and treated cells determined at the time of plating.

$^b$ Donor DNA was present at a concentration of 1 to 5 µg/ml during DNA uptake.

$^c$ Expressed as mean ratio of treated cells/control cells from three determinations.

$^d$ Calculated as [(mean number of transformants of drug-treated cells/mean number of transformants of control cells) × 100] – 100.

### Table 2. Effect of novobiocin treatment before DNA uptake in transformation of competent *N. gonorrhoeae* recipients by [3H]adenine-labeled Str$^\circ$ marker DNA

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>DNA uptake (min)$^b$</th>
<th>Novobiocin treatment$^b$</th>
<th>TCA-insoluble cpm/ml$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1 2686</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$</td>
<td>15</td>
<td>-</td>
<td>952</td>
</tr>
<tr>
<td>Rif$^\circ$ Pro$^-$</td>
<td>15</td>
<td>+</td>
<td>933</td>
</tr>
<tr>
<td>MN4131</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$</td>
<td>15</td>
<td>-</td>
<td>921</td>
</tr>
<tr>
<td>Rif$^\circ$ Pro$^-$</td>
<td>15</td>
<td>+</td>
<td>870</td>
</tr>
<tr>
<td>Expt 2 2686</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$</td>
<td>30</td>
<td>-</td>
<td>1.190</td>
</tr>
<tr>
<td>Rif$^\circ$ Pro$^-$</td>
<td>30</td>
<td>+</td>
<td>1.100</td>
</tr>
<tr>
<td>MN4131</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov$^\circ$ Str$^\circ$ Fus$^\circ$</td>
<td>30</td>
<td>-</td>
<td>1.164</td>
</tr>
<tr>
<td>Rif$^\circ$ Pro$^-$</td>
<td>30</td>
<td>+</td>
<td>1.200</td>
</tr>
</tbody>
</table>

$^a$ Recipient cells (10$^7$) were used in the transformation assay after exposure to novobiocin.

$^b$ Specific activity of donor DNA was 10$^6$ cpm/µg (strain JN25, Pro$^-$ Str$^\circ$) and was present at a concentration of 1 to 5 µg/ml during DNA uptake.

$^c$ Exposure to novobiocin (25 µg/ml) for 30 min at 37°C.

$^d$ Determined after uptake of DNA into a DNase-resistant form by the addition of 10% TCA to samples at 0°C for 30 min. Numbers represent average values from duplicate experiments.

### Table 3. Effect of norfloxac in treatment before DNA uptake in transformation of strain 2686 by Str$^\circ$ marker DNA

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>DNA uptake (min)$^a$</th>
<th>Norfloxacin treatment$^b$</th>
<th>Transformants/ml (10$^6$)</th>
<th>% Inhibition$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>-</td>
<td>4.550</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>+</td>
<td>0.200</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>+</td>
<td>0.078</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>+</td>
<td>0.082</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>-</td>
<td>0.920</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>+</td>
<td>0.040</td>
<td>96</td>
</tr>
</tbody>
</table>

$^a$ Donor DNA was present at a concentration of 1 to 5 µg/ml during DNA uptake.

$^b$ Norfloxacin treatment (1 µg/ml) was for 30 min before DNA uptake. The amount of cell killing produced by norfloxacin was less than 20%.

$^c$ Calculated as [(number of transformants of drug treated cells/number of transformants of control cells) × 100] – 100.
presence of more single-strand breaks in the DNA of competent cells than in the DNA of noncompetent colonial variants, types 3 and T4. Alkaline sucrose gradient analysis of single-stranded DNA from the various colony types revealed the presence of a single slow-sedimenting peak of DNA from colony type 2 cells which cosedimented with lambda DNA. A similar analysis of DNA from colony types 3 and 4 confirmed the presence of a predominant fast-sedimenting peak, which suggested that there are fewer discontinuities in these molecules. Furthermore, analysis of the DNA with S1 nuclease confirmed the presence of single-stranded regions in the DNA of colony type 2 cells. Approximately 20% of the radioactivity from prelabeled DNA of colony type 2 cells was released by the enzyme, compared with the release of 3 to 6% of the radioactivity from the DNA of noncompetent colonial variants (Table 4).

**DISCUSSION**

We have provided evidence that inhibitors of DNA gyrase in *E. coli* exert differential effects on the genetic transformation of *N. gonorrhoeae* by antibiotic resistance marker DNA. When competent novobiocin-sensitive cells were exposed to novobiocin before uptake of streptomycin, fusidic acid, and rifampin resistance markers, there was a 50 to 60% reduction in transformant yield. Interestingly, a novobiocin-resistant mutant produced a transformant yield that was twofold greater than that of the novobiocin-sensitive parental strain. However, exposure of the mutant to novobiocin before the uptake of transforming antibiotic resistance marker DNA had no effect on transformant yield. Furthermore, an assessment of TCA-insoluble radioactivity after uptake of radiolabeled DNA into a DNase-resistant form suggested that the target of the novobiocin-induced inhibition of transformation was subsequent to DNA uptake. In addition, exposure of nalidixic acid-sensitive cells to nalidixic acid before DNA uptake had no effect on transformant yield. Interestingly, norfloxacin, one of the most potent inhibitors of DNA gyrase and an analog of nalidixic acid, nearly abolished the production of transformants in the gonococcus. There was an approximate 95% reduction in the number of transformants after uptake of streptomycin resistance marker DNA.

The apparent drug-induced effects on the genetic transformation of *N. gonorrhoeae* may involve several phenomena: (i) effects on RecA synthesis and therefore alterations in RecA-mediated activities during homologous recombination, (ii) a DNA gyrase-mediated recombinational pathway, and (iii) effects on chromosomal superhelicity. Inhibitors of the A subunit of DNA gyrase in *E. coli* such as nalidixic acid and oxolinic acid are known to induce the RecA system (23, 24). Furthermore, inhibitors of enzyme activity cause an increase in the synthesis of DNA gyrase (15). Coumermycin and novobiocin both caused a 10-fold increase in the relative rate of synthesis of GyrA and GyrB, whereas nalidixic acid caused only a two- to threefold increase in synthesis. This induction of the GyrA and GyrB proteins by coumermycin was also accompanied by an induction of several other proteins, including RecA. However, there was a lack of stimulus on RecA by novobiocin but instead an inhibition (15). These data lend support to the notion that the novobiocin-induced inhibition of transformation in the gonococcus may relate to an inhibition of RecA synthesis and a subsequent decline in D-loop formation and branch migration during homologous recombination (25). Moreover, the increased efficiency of antibiotic resistance markers in transforming a novobiocin-resistant mutant may be related to increased synthesis of the gyrase A or B protein, or both, in this mutant. A possible model which summarily explains these observations would consist of a DNA gyrase–RecA-mediated pathway which effects strand exchange between homologous segments of the chromosome. The prevalence of single-stranded regions in the DNA of colony type 2 cells is intriguing and reminiscent of similar regions in competent *Haemophilus influenzae* (13) which may represent pairing sites for incoming donor DNA molecules during recombination. Interestingly, it was reported that [3H]norfloxacin preferentially binds single-stranded regions of DNA rather than the A subunit of DNA gyrase (22). Therefore, with these observations in mind, the inhibitory effect of norfloxacin on transformation may result from a primary interaction of the drug with the single-stranded regions of DNA in...
competent cells. Further, drug-binding sites may be the binding sites for DNA gyrase or a similar enzyme involved in recombination. The precise mechanism by which DNA gyrase or a similar enzyme may be involved in the genetic transformation of the gonococcus is still unclear. However, an attractive speculation is the idea that a norfloxacin-sensitive target bound to single-stranded regions in the DNA of the gonococcus may help mediate strand exchange during recombination in this organism. Moreover, such regions may result from the gene rearrangements and gene switching which have been reported to occur in the pilin-coding region of the gonococcal genome (27), a proposed hot spot for recombination (21). Interestingly, the proteins that are used to achieve gene rearrangements and gene switching may be similar to proteins that are used by the gonococcus during genetic transformation. Such proteins may be similar to the E. coli A and B subunits of DNA gyrase and therefore targets for inhibitors of the enzyme. An alternate explanation of these data, however, may involve drug-induced alterations in the superhelical density of the gonococcal chromosome (11).

ACKNOWLEDGMENTS
This research was supported by Public Health Service Minority Biomedical Support Grant RR08037 from the Division of Research Resources, National Institutes of Health, and by Minority Research Initiation Research Grant PRM-8115076 from the National Science Foundation.

We thank Carol McClendon for assistance in preparation of the manuscript.

LITERATURE CITED