Effect of Nalidixic Acid and Hydroxyurea on Division Ability of Escherichia coli fil+ and lon− Strains

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Short periods of incubation in medium containing nalidixic acid or hydroxyurea, followed by a return to normal growth conditions, induced filament formation in Escherichia coli B (fil+) and AB1899NM (lon−) but not in B/r (fil+) and AB1157 (lon+). These drugs reversibly stopped deoxyribonucleic acid (DNA) synthesis with little or no effect on ribonucleic acid (RNA) synthesis or mass increase. The initial imbalance caused by incubation in these drugs was the same for B and B/r as was macromolecular synthesis following a return to normal growth conditions. DNA degradation caused by nalidixic acid was measured and found to be the same for B and B/r. Hydroxyurea caused no DNA degradation in these two strains. Survival curves as determined under various conditions by colony formation suggested that the property of filament formation was responsible for the extraseptivity of fil+ and lon− strains to either nalidixic acid or hydroxyurea. E. coli B was more sensitive to either drug than was B/r or B−. Pantoyl lactone or liquid holding treatment aided division and colony formation of nalidixic acid-treated B but had no effect on B/r. Likewise, the filament-former AB1899NM was more sensitive to nalidixic acid than was the non-filament-former AB1157. The sensitivity of B/r and B− to nalidixic acid was nearly the same except at longer times in nalidixic acid, when B− appeared more resistant. Even though nalidixic acid, hydroxyurea, and ultraviolet light may produce quite different molecular alterations in E. coli, they all cause a metabolic imbalance resulting in a lowered ratio of DNA to RNA and protein. We propose that it is this imbalance per se rather than any specific primary chemical or photobiological alteration which leads to filament formation by some genetically susceptible bacterial strains such as lon− and fil+.

Certain strains of Escherichia coli, referred to as fil+ or lon−, grow into long multinucleate filamentous cells after exposure to low doses of ultraviolet light (UV) (1, 6, 21). This property of filament formation is genetically determined and affects the UV sensitivity of these strains as measured by colony formation (12, 20). The UV-induced lesions leading to filament formation are photoreactivable (6, 8, 14), suggesting that they are pyrimidine dimers in the deoxyribonucleic acid (DNA). Since these pyrimidine dimers act as temporary blocks to DNA synthesis in E. coli strain B (fil+) (19), a short period of DNA synthesis inhibition, without concomitant inhibition of ribonucleic acid (RNA) or protein synthesis, occurs after UV irradiation. It is possibly this short period of "unbalanced growth" induced by UV that leads to filament formation in this and other fil+ and lon− strains. If so, other procedures known to specifically and reversibly halt DNA synthesis in E. coli should also induce filament formation in these strains. We have examined the effect on filament formation of stopping DNA synthesis for a period without stopping RNA or protein synthesis in these strains by using nalidixic acid and hydroxyurea. Nalidixic acid and hydroxyurea specifically inhibit DNA synthesis in E. coli (9, 17). This inhibition is reversible, and DNA synthesis resumes if the drug is removed from the bacterial cultures. We have also investigated (i) the effect of these drugs on DNA degradation and colony-forming ability in both filament-forming and non-filament-forming strains and (ii) the effect of pantoyl lactone and liquid-holding treatment on colony survival after nalidixic acid treatment. These experiments were done to help clarify the events which lead to filament formation in fil+ and lon− strains and to obtain further support for existing evidence relating their extraseptivity, as determined by colony formation, to the property of filament formation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this investigation were E. coli B,
originally obtained from the Yale University Biophysics Department; E. coli B/r, obtained from E. Witkin; E. coli B+, obtained from R. F. Hill; the E. coli K-12 strain AB1157 (uvr+*, lon+) obtained from R. P. Boyce; and the E. coli K-12 strain AB1899NM (uvr+*, lon+) obtained from H. L. Adler.

The E. coli B strains were grown in liquid C-1 minimal medium (16) and the K-12 strains were grown in C-1 supplemented with 10 µg/ml of thiamine and 50 µg/ml of each of the five required amino acids—arginine, histidine, leucine, proline, and threonine. Liquid cultures were incubated at 37 C with aeration. Log-phase cells were used for all experiments.

Chemicals. Nalidixic acid was obtained from W. A. Goss of the Sterling-Winthrop Research Institute, Rensselaer, N.Y. A stock nalidixic acid solution containing 5 mg/ml was prepared by dissolving 100 mg of nalidixic acid in 1 ml of 1 N sodium hydroxide and diluting to a volume of 20 ml with sterile distilled water.

Hydroxyurea (Nutritional Biochemicals Corp., Cleveland, Ohio) was prepared fresh each day just before use. A quantity was dissolved in sterile distilled water and the solution was sterilized by filtration with a membrane filter (0.45-µ pore size; Millipore Corp., Bedford, Mass.).

Measurement of DNA synthesis and DNA degradation. DNA synthesis in the presence of nalidixic acid or hydroxyurea was determined by measuring uptake of tritiated thymidine by use of a procedure described previously (13). Adenosine (200 µg/ml) and tritiated thymidine (2 µg/ml; specific activity, 16.7 c/mmole; New England Nuclear Corp., Boston, Mass.) were added to control and drug-treated cultures incubated at 37 C. The initial cell concentration was about 2 × 10^9 per ml. Samples of cells taken at various times were extracted for 30 min with ice-cold 5% trichloroacetic acid and the insoluble fraction was collected on membrane filters (0.45-µ pore size; Millipore Corp.), dispersed in scintillation fluid (3), and counted in a liquid scintillation counter. Removal of nalidixic acid or hydroxyurea was accomplished by collecting cells on membrane filters, washing with several volumes of warm C-0 medium (C-1 without glucose), and resuspending in fresh C-1 medium.

DNA degradation in nalidixic acid or hydroxyurea-treated cultures was determined by observing the trichloroacetic acid-insoluble fraction of prelabeled cells. Cells were labeled prior to the addition of drug for at least four generations in C-1 supplemented with 200 µg of adenosine per ml and tritiated thymidine, collected on a membrane filter, washed thoroughly with warm C-0 medium, and resuspended in fresh C-1 medium containing 200 µg of adenosine and 2 µg of cold thymidine per ml. These cells were incubated for 30 min at 37 C before nalidixic acid or hydroxyurea was added. After the addition of drug, samples were taken at various times, and the trichloroacetic acid-insoluble fraction was collected and counted in a scintillation counter. The concentration of cells was about 10^9 to 2 × 10^9 per ml when growth in ²H-thymidine was started and about 6 × 10^9 to 9 × 10^9 per ml after resuspension in cold medium and at the time of drug addition.

Measurement of RNA synthesis. RNA synthesis was followed in nalidixic acid- or hydroxyurea-treated cultures by observing the incorporation of ¹⁴C-uracil (7 µg/ml; specific activity, 30 mc/mmole; New England Nuclear Corp., Boston, Mass.) into the trichloroacetic acid-insoluble fraction by use of techniques similar to those described for measurement of DNA synthesis, with the exception that adenosine was not used. Samples taken at various times were extracted for 30 min with ice-cold 5% trichloroacetic acid, and the insoluble portion was collected on a membrane filter. The filter was glued to a stainless-steel planchet, allowed to dry, and counted by use of a gas-flow counter. Cell concentrations were the same as described for measurements of DNA synthesis.

Observation of filament formation. The effect of nalidixic acid or hydroxyurea on division ability was observed by incubating cells with either drug for various lengths of time. Cells were collected on a membrane filter after drug treatment, washed thoroughly with warm C-0 medium, resuspended in C-1 medium, and incubated at 37 C with aeration. After two mass-doubling times, as determined by turbidity increase, a drop of the culture was examined under the microscope and the relative number of filamentous cells was determined. Filaments scored at this time were four to eight times longer than normal cells and could easily be distinguished from them. Increase in turbidity of cultures was observed with a Bausch & Lomb Spectronic-20 colorimeter at 625 mµ.

Determination of survival curves. Survival, as measured by colony formation, of nalidixic acid- or hydroxyurea-treated cultures was determined by incubating cultures with either drug at 37 C and taking samples at various times. Dilutions were done in room-temperature C-0 medium, and samples of dilutions were immediately plated on 2-day-old C-1 agar plates. Incubation of plates at 37 C was started 5 min after plates were spread and was continued for 48 hr. The effect of pantoyl lactone (DL Pantoyl Lactone, Nutritional Biochemicals Corp., Cleveland, Ohio) on survival was observed by plating on 12-hr-old C-1 agar plates supplemented with 0.2 M pantoyl lactone. The effect of liquid-holding treatment on survival was determined by holding drug-treated samples in C-0 medium at 37 C for various times before plating on C-1 agar plates.

Results

DNA synthesis. The effect of nalidixic acid and hydroxyurea on DNA synthesis in E. coli B and B/r is shown in Fig. 1. Log-phase cells were labeled for 100 min with tritiated thymidine in C-1 containing adenosine. At that time, the cultures were split, and either nalidixic acid or hydroxyurea was added to one part. After a period of incubation at 37 C, part of the drug-treated cultures was removed; cells were washed free from drug and incubated again at the same cell concentration in C-1 containing tritiated thymidine and adenosine. The results show that nalidixic acid stopped DNA synthesis in both E. coli B (Fig. 1a) and B/r (Fig. 1b). DNA synthesis resumed after nalidixic acid was removed,
and the rate of synthesis for both strains paralleled that of the controls not treated with nalidixic acid. Hydroxyurea also inhibited DNA synthesis in E. coli B (Fig. 1c) and B/r (Fig. 1d). Removal of hydroxyurea allowed DNA synthesis to resume immediately in both strains, and, as with nalidixic acid, rate of synthesis paralleled that of the untreated controls. The response of DNA synthesis in both E. coli B and B/r to either nalidixic acid or hydroxyurea and to their removal was the same. After a time in hydroxyurea, the incorporation of tritiated thymidine into the trichloroacetic acid-insoluble fraction began even if the hydroxyurea was not removed. At this concentration of hydroxyurea, the block to DNA synthesis was not permanent.

**RNA synthesis.** The effect of nalidixic acid and hydroxyurea on RNA synthesis in E. coli B as measured by incorporation of $^{14}$C-uracil is shown in Fig. 2. Cultures grown in the presence of $^{14}$C-uracil for 100 min were split, and either nalidixic acid (5 μg/ml) or hydroxyurea (5.6 mg/ml) was added to one part. Samples were taken at various times, and the trichloroacetic acid-insoluble fractions were collected and counted. The results of Fig. 2a show that nalidixic acid had no effect initially on RNA synthesis. For the first 70 min in nalidixic acid, the uptake of $^{14}$C-uracil was the same as that of the control culture. A decrease in the rate of uptake not observed in control cultures was observed after more than 70 min in nalidixic acid. Hydroxyurea did not completely inhibit RNA synthesis but did cause a decrease in the rate of synthesis, as is shown in Fig. 2b. If hydroxyurea was removed, the rate of RNA synthesis increased immediately and proceeded at the same rate as that of an untreated control.

**Mass increase.** Concentrations of nalidixic acid and hydroxyurea large enough to inhibit DNA synthesis in E. coli B and B/r do not initially inhibit mass increase as indicated by measurement of turbidity increase with a Bausch & Lomb Spectronic-20 colorimeter at 625 μm. Figure 3a shows that cultures of E. coli B, B/r, and B+; growing in C-1 containing 5 μg of nalidixic acid per ml increased in turbidity three to four times. For at least the first 50 min, the rate of turbidity increase was the same as that of a culture grown in C-1 without drug. The turbidity increased at a slower rate after more than 50
min in nalidixic acid and eventually stopped. Figure 3b shows the results obtained when hydroxyurea (5.6 mg/ml) was used. The turbidity increase of B and B/r cultures was not affected until incubation times in the presence of hydroxyurea exceeded 50 min. At times greater than this, the turbidity increased at a slower rate than that of a control and continued at this slower rate for at least 200 min.

The rate of mass increase after a period of incubation in the presence of either drug was also examined, and the results obtained for E. coli B and B/r are shown in Fig. 4. Cells incubated at 37°C in C-1 containing either nalidixic acid (5 μg/ml) or hydroxyurea (5.6 mg/ml) were collected, washed, resuspended in fresh C-1 medium, and incubated at 37°C with aeration. Turbidity measurements showed that the rate of mass increase of cells pretreated with drug was the same as that of untreated controls, regardless of whether nalidixic acid (Fig. 4a and 4b) or hydroxyurea (Fig. 4c and 4d) was used. A 60-min incubation period in the presence of either drug caused a slight lag in mass increase when cells were subsequently incubated in fresh C-1, but after this slight lag mass increased at a rate equal to that of untreated controls. The results obtained were the same for E. coli B and B/r.

DNA degradation. The DNA of E. coli 15 TAU is degraded when cells are incubated in the presence of nalidixic acid (4). The stability of the DNA in nalidixic acid-treated cultures of E. coli B and B/r was determined (Fig. 5a and 5b). Cells labeled with tritiated thymidine were incubated for 30 min in C-1 containing cold thymidine and then nalidixic acid at a concentration of 20 μg/ml was added. The results show that there was a loss of trichloroacetic acid-insoluble label in nalidixic acid-treated cultures and that the amount of label lost was approximately the same.
for B (Fig. 5a) and B/r (Fig. 5b). Incubation for 120 min caused approximately 14% of the original label in E. coli B and 11% in B/r to become acid-soluble. Longer incubation times caused little or no further degradation. Similar results were obtained when experiments were done without the 30-min chase period following the tritiated thymidine labeling period. The amount of degradation observed here is considerably less than that observed previously for E. coli 15 TAU (4).

Hydroxyurea-treated cultures of E. coli B and B/r were also examined for DNA degradation by the same technique (Fig. 5c and 5d). Tritiated thymidine-labeled cells were incubated in C-1 medium containing 5.6 mg of hydroxyurea per ml. No loss of label was observed for either strain. The concentration of cells at the time hydroxyurea was added was the same as that used in all previous experiments, about 6 \times 10^7 per ml. These results are similar to that previously observed for E. coli C600 (17).

Effect of nalidixic acid and hydroxyurea on filament formation in E. coli B and B/r. Incubation of either E. coli B or B/r in the presence of nalidixic acid or hydroxyurea stops DNA synthesis (Fig. 1) but does not initially inhibit RNA synthesis (Fig. 2) or mass increase (Fig. 3). Further, when either drug is removed, DNA synthesis begins immediately (Fig. 1) and RNA synthesis and mass increase continue (Fig. 2 and 4). Therefore, the effect of short intervals of inhibition of DNA synthesis, induced by pulses of either of these drugs, on filament formation in several E. coli strains was examined. The results obtained for E. coli B, B/r, and Bp-1 with nalidixic acid are shown in Fig. 6. Cultures of these strains treated with nalidixic acid (5 \mu g/ml) for various times were washed free from drug by filtration on a membrane filter, resuspended in C-1, and incubated at 37 C. After 100 min of turbidity increase (two mass-doubling times), the cultures were examined for filaments. The data are presented as percentage of filaments four to eight times normal length observed in the culture at this time. The results show that short periods of exposure to nalidixic acid, with its subsequent removal, induced filament formation following further growth for E. coli B but not for B/r or Bp-1. Exposure for 60 min to nalidixic acid, followed by a period of growth in fresh C-1 that allowed the mass to double twice, caused about 88% of the cells of a B culture to appear as filaments, whereas only 14% of the B/r cells and 19% of the Bp-1 cells appeared as filaments. Longer incubation in nalidixic acid did not cause the percentage of filaments to increase appreciably in an E. coli B culture when examined after a period of subsequent growth in C-1, but did cause it to increase in B/r and Bp-1 cultures. However, after growth in fresh C-1 for periods longer than 100 min following nalidixic acid treatment, very
long filamentous cells observable in *E. coli* B cultures were not observed in B/r cultures. Some long filaments persisted in B/r cultured.

Incubation for short times in medium containing hydroxyurea also induced filament formation in *E. coli* B but not in B/r. Cells incubated for various times in C-1 containing 5.6 mg of hydroxyurea per ml at 37°C were collected on a membrane filter, washed free from drug, suspended in fresh C-1, and incubated at 37°C. After 100 min of mass increase, cultures were examined for filaments. As shown in Fig. 7, a 60-min incubation period in hydroxyurea caused an *E. coli* B culture to contain approximately 95% filaments after two mass-doubling times in C-1 whereas similar treatment caused a B/r culture to contain only 7% filaments. As was noted for nalidixic acid, long filaments observed in hydroxyurea-treated *E. coli* B cultures after periods in fresh C-1 greater than 100 min were not observed in similarly treated B/r cultures.

**Effect of nalidixic acid on filament formation in *E. coli* AB1157 and AB1899NM.** The effect of nalidixic acid on filament formation was studied further by use of some *E. coli* K-12 strains having

![Graph 1: Nalidixic Acid](image)

**Fig. 5.** Stability of DNA in cultures of *Escherichia coli* B and B/r incubated in (a, b) nalidixic acid (20 μg/ml) or (c, d) hydroxyurea (5.6 mg/ml). Open symbols, controls; closed symbols, incubated in drug-containing medium. The initial trichloroacetic acid-insoluble counts for (a) were 4,000 (○) and 1,000 (△) counts/min per 10 uliters, respectively; for (b), 2,000 (○) and 1,000 (△); for (c), 2,000; for (d), 1,200.

UV-survival and filament-forming characteristics parallel to *E. coli* B and B/r. The strains used were *E. coli* AB1157, a lon<sup>−</sup>, uvr<sup>+</sup> strain similar to B/r, and *E. coli* AB1899NM, a lon<sup>−</sup>, uvr<sup>−</sup> strain similar to B. Experiments similar to those described for B and B/r were done. The concentration of nalidixic acid used was 20 μg/ml, and the K-12 strains were grown in C-1 supplemented with thiamine and required amino acids. Cultures were examined for filaments after 120 min of mass increase in fresh medium (two mass-doubling times). The results shown in Fig. 8 are qualitatively similar to those obtained for B and B/r. A short period of exposure to nalidixic acid induced filament formation in AB1899NM while having only a slight filament-forming effect in AB1157.

**Effect of nalidixic acid on nonproliferating cultures of *E. coli* AB1899NM.** The effect of nalidixic acid on filament formation in nonproliferat-
ing cultures was also examined. Log-phase cells of E. coli AB1899NM were collected on a membrane filter, washed with C-0, and resuspended in C-1 containing thiamine but no amino acids. After 30 min of incubation at 37°C, nalidixic acid at a final concentration of 20 μg/ml was added to the culture. At various times, cells were washed free of nalidixic acid, resuspended in fresh complete medium (C-1, thiamine, and amino acids), incubated at 37°C, and examined for filaments after 120 min of mass increase. As shown in Fig. 8, nalidixic acid had no effect on filament formation in AB1899NM under these conditions. A culture incubated for 80 min in medium lacking required amino acids and containing nalidixic acid had the same percentage of filamentous cells after growth for two mass-doubling times in complete medium as did a log-phase untreated culture growing in the same complete medium.

**Colony survival after nalidixic acid treatment.**

The effect of nalidixic acid on colony survival of several E. coli strains possessing wide variations in UV sensitivity, as measured by colony-forming ability, was examined. Samples of log-phase cultures treated with nalidixic acid were diluted and plated on C-1 agar plates which were subsequently incubated at 37°C. Plates were counted after 48 hr of incubation. Results with two different nalidixic acid concentrations are shown in Fig. 9 for E. coli strains B, B/r, and B_{-1}. Figure 9a shows the results obtained when cultures containing cells at an initial concentration of 10^8/ml were incubated with 5 μg of nalidixic acid per ml. A loss of viability was observed in all three strains, occurring more rapidly in B than in B/r or B_{-1}. The survival curve for each strain exhibited an initial shoulder, followed by a portion decreasing exponentially. The slope of the exponential portion decreased at lower survival levels. At this concentration, an increase in colony count of about 5% occurred for both B/r and B_{-1} during the first 25 min. The initial shoulder observed in the survival curve for B_{-1} was not as great as that observed for B/r, but at later times the curves crossed and B_{-1} appeared more resistant than B/r. The results obtained with 20 μg of nalidixic acid per ml are shown in Fig. 9b. As observed for the lower concentration,
E. coli B was more sensitive than either B/r or B_{s+1}. Each curve exhibited an initial shoulder followed by an exponentially decreasing portion and a decrease in slope at lower survival levels. No "break" or decrease in slope at lower survival levels was observed for B/r. For all strains, loss of viability began at an earlier time at the higher drug concentration, although the inactivation rates were similar. After longer periods of incubation, B_{s+1} was more resistant than B/r, a result similar to that observed at the lower drug concentration. Nalidixic acid was previously reported to be bactericidal for E. coli 15 TAU under conditions similar to those used here (7).

The effect of nalidixic acid on colony survival of the E. coli K-12 strains AB1157 and AB1899NM was also examined (Fig. 10). Log-phase cultures of these strains incubated in C-1 supplemented with thiamine and the required amino acids were treated with 50 μg of nalidixic acid per ml. Samples taken at various times were diluted and plated on 2-day-old C-1 agar plates containing thiamine and required amino acids which were subsequently incubated at 37°C. The results are qualitatively similar to those obtained for E. coli B and B/r. The general shape of the curves was similar and the filament-former AB1899NM was more sensitive to nalidixic acid than was the non-filament-former AB1157.

**Pantoxy lactone effect.** The effect of pantoxy lactone on the colony survival of nalidixic acid-treated E. coli B and B/r is shown in Fig. 11. Pantoxy lactone is a division-promoting agent (2, 10). Its effect on colony survival can give information about the effect of filament formation on colony-forming ability. Samples of cultures incubated with 20 μg of nalidixic acid per ml were diluted and plated on 12-hr-old C-1 agar plates supplemented with 0.15 m pantoxy lactone. Plates were then incubated for 72 hr at 37°C. The results in Fig. 11 show that under these conditions the survival of E. coli B was the same as that of E. coli B/r. Pantoxy lactone caused the colony-forming ability of nalidixic acid-treated E. coli B to increase but had no effect on the colony survival of B/r.

We have also observed that pantoxy lactone aids division in nalidixic acid-treated cells of E. coli B. A culture of B exposed to 5 μg of nalidixic acid per ml for 60 min had 90% filaments after two mass-doubling times in fresh C-1 medium and less than 30% filaments after two mass-doubling times in C-1 containing 0.1 m pantoxy lactone.

**Liquid holding effect.** The effect of liquid holding in non-nutrient medium on colony survival after exposure to nalidixic acid was examined for E. coli B, B/r, and B_{s+1}. These experiments were done to obtain further data for a comparison between the action of UV and nalidixic acid. Cells were treated with 5 μg of nalidixic acid per ml for
a period that allowed approximately 1% of the cells to form colonies. They were then washed free of nalidixic acid by filtration on a membrane filter, resuspended in an equal volume of C-0 medium and incubated at 37 C. This procedure required about 3 min. Samples were diluted and plated on 2-day-old C-1 agar plates after various times of liquid holding, and plates were placed in an incubator at 37 C. *E. coli* B/r showed no liquid-holding recovery (LHR), as measured by colony formation from nalidixic acid treatment, whereas *E. coli* B exhibited an increase by at least a factor of 10 (Fig. 12). B<sub>r</sub>-1 showed a small LHR effect and increased in survival by a factor of approximately 2. Holding untreated cells in non-nutrient medium for an equivalent time had no effect on their colony-forming ability.

Liquid holding after nalidixic acid treatment also aids division in *E. coli* B. It was observed that an *E. coli* B culture treated for 90 min with 5 μg of nalidixic acid per ml had 90% filaments after 100 min of growth in fresh medium and 27% filaments if held 150 min in non-nutrient medium before incubation in C-1.

An unsuccessful attempt was made to photoreactivate the nalidixic acid-induced damage in *E. coli* B. A culture of *E. coli* B treated with 5 μg of nalidixic acid per ml for 90 min (1.5% survival of colony-forming ability) was exposed to doses varying from 0 to 3.2 × 10<sup>6</sup> ergs/mm<sup>2</sup> of 406-mu light. A slight increase in colony survival occurred. However, the increase in colony count corresponded directly to the LHR effect when the time required to give the dose of 406-mu light was considered. It was therefore concluded that nalidixic acid-induced damage is not photoreactivable.

**Colony survival after hydroxyurea treatment.** The effect of hydroxyurea on colony survival of *E. coli* B, B/r, and B<sub>r</sub>-1 is shown in Fig. 13. Hydroxyurea at a final concentration of 5.6 mg/ml was added to log-phase cultures growing in C-1 medium. The turbidity of the cultures was the same at the time the hydroxyurea was added. The control plates indicated that the cell concentrations used were approximately the same for all strains, about 3 × 10<sup>8</sup>/ml. Hydroxyurea caused no loss of viability in cultures of *E. coli* B/r during the interval of incubation used here. Loss of colony-forming ability was observed for both *E. coli* B and B<sub>r</sub>-1, occurring to a lesser extent for B<sub>r</sub>-1. The total incubation time used here (250 min) caused the colony count to decrease to 70% of the original count for B<sub>r</sub>-1 and to 23% for B.

**DISCUSSION**

Nalidixic acid and hydroxyurea reversibly inhibit DNA synthesis in several *E. coli* strains but affect RNA synthesis, protein synthesis, and mass increase to a lesser extent. Periods of unbalanced growth resulting from brief treatments with either drug, followed by a return to normal growth conditions, lead to a greater degree of filament formation in those *E. coli* strains classed as "filament formers" by virtue of their UV response (fil<sup>+</sup>,

\[ \text{Percent Survival - Colony Forming Ability} \]

\[ \text{Time in Nalidixic Acid (Min.)} \]

\[ \text{Liquid Holding Time (Min.)} \]

\[ \text{Liquid Holding} \]

\[ \text{reversibly inhibit DNA synthesis in several *E. coli* strains but affect RNA synthesis, protein synthesis, and mass increase to a lesser extent. Periods of unbalanced growth resulting from brief treatments with either drug, followed by a return to normal growth conditions, lead to a greater degree of filament formation in those *E. coli* strains classed as "filament formers" by virtue of their UV response (fil<sup>+</sup>,}
tion). If unbalanced growth in nalidixic acid is prevented by also blocking protein synthesis by withholding required amino acids, as was done here for the filament former E. coli AB1899NM, then filaments are not formed after a subsequent period of growth under normal conditions. The imbalance in macromolecular synthesis created by nalidixic acid and hydroxyurea appears to be the same in the filament-formers as compared to the non-filament-formers. It is the response to this imbalance which differs in these two groups of bacteria. Recent data of Cummings and Mondale (5) suggest also that unbalanced growth caused by a period of thymine starvation induces filaments in a thymine-requiring strain of E. coli B.

The nalidixic acid-induced growth imbalance is accompanied by DNA degradation in some bacteria (4). Such degradation might occur to a different degree in filament-formers as compared to non-filament-formers. This was checked and no such difference was found. Slight degradation of DNA was observed during the nalidixic acid-induced growth imbalance, occurring to about the same degree in E. coli B and B/r. Hydroxyurea at the concentration and exposure times used here produced no degradation in either strain. It is therefore unlikely that the filament formation induced by these drugs is caused by DNA degradation.

Among other requirements, cell division by E. coli is necessary for colony formation. After a perturbation, colony-forming ability reflects the filament-forming characteristics of a particular strain, at least in part, provided other cellular and metabolic responses are equal. A cell with the greater tendency to form a filament after a certain treatment has a lesser chance of forming a colony. Thus, a tendency of E. coli B to form filaments after UV treatment makes its colony-forming ability appear more sensitive to UV when compared to E. coli B/r, a non-filament-former. Correspondingly, nalidixic acid and hydroxyurea, which induce more filaments in E. coli B than B/r, are more effective in reducing the colony-forming ability of B as compared to B/r. This also holds for E. coli AB1899NM and AB1157 which, respectively, do and do not form filaments after short exposure to nalidixic acid or hydroxyurea. Pantoyl lactone, a chemical agent which aids the division of UV-induced filaments (13, 20) and increases their colony-forming potential, both reduces filament formation and increases colony-forming ability for nalidixic acid-treated cultures of E. coli B, while having no effect on nalidixic acid-treated cultures of E. coli B/r. Liquid holding, which reduces filament formation (14) and increases colony survival (11, 15) of UV-irradiated E. coli B, has a similar effect on nalidixic acid-treated E. coli B but not B/r. These results are consistent with other data which show that bacterial strains giving more filament formation exhibit lower colony survival when perturbed and exhibit various reactivation phenomena that are not observed for non-filament-formers, which are otherwise genetically the same.

E. coli B sub-1, a strain which is UV-sensitive because it cannot efficiently repair dimer-initiated blocks to DNA synthesis (18), is about as resistant to nalidixic acid as B/r with respect to both filament formation and colony formation. This result tempts us to class it as a non-filament-former. However, since our experiments are limited to only a few E. coli strains, and the molecular mechanisms of division inhibition and subsequent filament formation after short nalidixic acid or hydroxyurea pulses are not understood, it is not yet clear whether these agents can be used as faithful indicators of the filament-forming characteristic. It should also be pointed out that, although the number of filaments in nalidixic acid-treated B sub-1 cultures was considerably less after a period of normal growth conditions than that observed in similarly treated B cultures, a few very long filaments (20 to 30 times normal size) that were similar in appearance to the B filaments persisted in the B sub-1 cultures. B/r filaments never exceeded lengths greater than 10 times normal. It is interesting that E. coli B sub-1 shows higher colony survival than either B or B/r after long nalidixic acid treatment. We have also observed that UV-sensitive K-12 mutants are more resistant to nalidixic acid than the UV-resistant parent. If this were a general property of UV-sensitive mutants, then this might lead to a method for isolating such mutants.
Since nalidixic acid damage is not photoreactivable and $B_{r,1}$ is not more sensitive to it than $B$ or $B/r$, it is clear that production of pyrimidine dimers is not the major mode of action of this drug. The way in which these drugs inhibit division and colony formation is not known.

We would like to propose that it is the brief period of unbalanced growth induced by nalidixic acid or hydroxyurea pulses, ultraviolet light, or other treatments which leads to filament formation in some $E. coli$ strains which are not able to cope with such disturbances. Other $E. coli$ strains (non-filament-formers) can cope with this imbalance and are quick to recover their ability to divide. It should be noted that the delays in DNA synthesis necessary to produce filaments of $E. coli$ B by UV are somewhat less than those produced by nalidixic acid or hydroxyurea when the same fraction of filaments is observed. Thus, even though a lag in DNA synthesis produced by nalidixic acid and hydroxyurea causes filaments in $E. coli$ B, as does low UV exposure, the apparent lags do not agree well quantitatively. This may not be surprising, since the initial "lesions" leading to imbalance are different for these agents. Further, initial DNA synthesis observed after UV irradiation may be partly repair synthesis, and sequential synthesis may actually be delayed longer than it appears to be. However, the possibility remains that any agent slowing down DNA synthesis more than RNA and protein synthesis may tend to produce filaments in some strains of bacteria which cannot recover easily from such an imbalance.

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