

Induction of Excessive Deoxyribonucleic Acid Synthesis in *Escherichia coli* by Nalidixic Acid

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Prior treatment of *Escherichia coli* with nalidixic acid in nutritionally complete medium altered the subsequent pattern of deoxyribonucleic acid (DNA) synthesis normally observed in nutritionally deficient medium. Transfer of *E. coli* 15 TAU to an amino acid- and pyrimidine-deficient medium usually resulted in a 40 to 50% increase in DNA content. Previous treatment with nalidixic acid caused a 200 to 300% increase in DNA content under these conditions. The extent of this DNA synthesis depended on the duration of prior exposure to nalidixic acid. The maximal rate of synthesis was obtained after a 40- to 60-min exposure to nalidixic acid and was two to three times that of the control. The induction of this excessive DNA synthesis was prevented by chloramphenicol or phenethyl alcohol, but the synthesis of this DNA was only partially sensitive to these agents. With *E. coli* TAU-bar, the rate of DNA synthesis, after removal of nalidixic acid, was similar to that of *E. coli* 15 TAU, but the maximal amount of DNA synthesized was 180 to 185% of that initially present. Cesium chloride density gradient analysis demonstrated that DNA synthesis after removal of nalidixic acid occurs by a semiconservative mode of replication. The density distribution of this DNA was similar to that obtained after thymine starvation. These results suggest that nalidixic acid treatment may induce additional sites for DNA synthesis in *E. coli*.

It has been demonstrated that the chromosome of *Escherichia coli* is replicated sequentially by means of a single replicating fork (3, 19, 20). When *E. coli* 15 TAU is transferred from a nutritionally complete medium to a medium containing thymine but no arginine or uracil, deoxyribonucleic acid (DNA) synthesis continues for approximately 60 min, then ceases. The amount of DNA synthesized under these conditions is 40 to 50% of that initially present and represents completion of the DNA replication cycle (18). Physical evidence indicates that completion of this cycle occurs at a unique point on the chromosome and that initiation of DNA synthesis from this point is prevented in the absence of exogenous amino acids (13, 16, 17). Present evidence indicates that thymine starvation of *E. coli* 15T⁻ induces an extra cycle of DNA replication from this same region (21). Likewise, the increased rate of DNA synthesis observed after thymine or thymine-deoxyriboside starvation of *Lactobacillus acidophilus* R-26 has been attributed to the induction of an extra cycle of DNA replication (24).

To date, the accelerated DNA synthesis oc-

curing in amino acid-deficient medium has been observed only after thymine or deoxyriboside starvation. Preliminary studies with *Alcaligenes faecalis* LB suggested that DNA synthesis was not accelerated after inhibition of DNA synthesis by deoxyadenosine (14). No increased rate of DNA synthesis was observed in *E. coli* 15T⁻ after inhibition of DNA synthesis by cytosine arabinoside (13). On the basis of these and their previous observations, Lark and co-workers have postulated a regulatory role for thymine, or a thymine-deoxyriboside, in the initiation of DNA synthesis (13, 24).

Previous studies in our laboratory have demonstrated that the rate of DNA synthesis, in *E. coli* 15 TAU, is accelerated after removal of nalidixic acid (4, 5, 8). This increased rate of DNA synthesis also occurs after thymine starvation of *E. coli* 15T⁻ (1). This observation prompted the present comparison between the effects of nalidixic acid and thymine starvation on the regulation of DNA synthesis in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* 15 TAU was cultured in a glucose-salts synthetic medium at 37 C with aeration as previously described (8).

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E. coli TAU-bar (kindly supplied by P. C. Hanawalt of Stanford University) was cultured in the same glucose-salts medium supplemented with thymine (2 $\mu\text{g}/\text{ml}$), uracil (10 $\mu\text{g}/\text{ml}$), and the amino acids, arginine (100 $\mu\text{g}/\text{ml}$), proline, tryptophan, and methionine (45 $\mu\text{g}/\text{ml}$). The cell concentrations were maintained between 10^8 and 3×10^8 cells/ml by periodic dilution with warm medium. Under these conditions, the generation time of *E. coli* 15 TAU was approximately 60 min and that of *E. coli* TAU-bar, approximately 45 min.

Medium changes were performed by membrane filtration (0.45 μ , Millipore Corp., Bedford, Mass.), washing, and resuspension in fresh warm basal medium. The entire process of filtration and resuspension took 2 min. Supplements were added after resuspension to give the desired medium.

In experiments with *E. coli* 15 TAU, (+T, -AU) will refer to a nutritionally deficient medium containing thymine but no arginine or uracil. A nutritionally complete medium, containing all three growth requirements, will be designated (+T, +AU). Similarly, for *E. coli* TAU-bar, (+T, -supplements) will refer to a medium containing thymine but lacking all other required supplements. Likewise, (+T, +supplements) will designate a nutritionally complete medium.

DNA synthesis. DNA synthesis was monitored by determining the incorporation of ^3H - or ^{14}C -labeled thymine and ^{14}C -labeled bromouracil into the cold trichloroacetic acid-insoluble material (8). The amount of radioactivity incorporated was measured with a liquid scintillation spectrometer (Packard Instrument Corp., Chicago, Ill.).

Density gradient centrifugation. Samples (15 to 20 ml) for CsCl density gradient analysis were harvested by centrifugation (8,000 rev/min, 5 min), washed with tris(hydroxymethyl)aminomethane (Tris) buffer (0.1 M, pH 8) and resuspended in 1.5 to 2 ml of Tris-EDTA buffer (0.1 M Tris, 0.02 M ethylenediaminetetraacetate, pH 8.2). Lysozyme (0.05 ml of a 4 mg/ml solution) was added, and the samples were incubated for 1 to 2 hr at 37 C. Cell lysis was enhanced by intermittent freezing and thawing in dry ice and acetone or liquid nitrogen. Deproteinization was effected by addition of an equal volume of chloroform-octanol (9:1) followed by 10 min of shaking on a vortex mixer (9). The aqueous phase was diluted to 3 ml with 0.1 M Tris (pH 8.0), and the density was adjusted to 1.710 ± 0.005 g/cm 3 by addition of solid cesium chloride (optical grade, Harshaw Chemical Co., Cleveland, Ohio). Density was calculated from the refractive index by use of an Abbe Refractometer (Bausch & Lomb Inc., Rochester, N.Y.). Samples for analysis were centrifuged at 33,500 rev/min for 60 hr in an SW 39 rotor of a Spinco Model L-2 ultracentrifuge (Spinco Division, Beckman Instruments Inc., Palo Alto, Calif.). Drops were collected from a pin-hole in the bottom of the tube directly into 5 ml of ice-cold 5% trichloroacetic acid. The samples were filtered by membrane filtration (0.45 μ , Millipore Corp., Bedford, Mass.) and washed once with cold 1% trichloroacetic acid; the filter pads were added to liquid scintillation fluid in vials. Radioactivity was measured as described above. In cases where double isotopes (^3H and ^{14}C)

were employed, the channel ratios of the liquid scintillation spectrometer were adjusted for simultaneous measurement of ^{14}C and ^3H .

Chemicals. Thymine-2- ^{14}C (45.5 mc/mole) and thymine-methyl- ^3H (6.7 c/mole) were purchased from the New England Nuclear Corp., Boston, Mass. Bromouracil-5- ^{14}C (31 mc/mole) was obtained from Calbiochem, Los Angeles, Calif. Chloramphenicol was obtained from Parke Davis & Co., Detroit, Mich. Phenethyl alcohol, obtained from the Eastman Kodak Co., Rochester, N.Y., was distilled once before use.

RESULTS

Induction of excessive DNA synthesis by nalidixic acid and thymine starvation. When exponentially growing cultures of *E. coli* 15 TAU were treated with nalidixic acid (20 $\mu\text{g}/\text{ml}$) or starved of thymine for 60 min, there was no detectable DNA synthesis (Fig. 1). In the control culture, the amount of ^{14}C -thymine incorporated into acid-insoluble material (DNA) increased 105% during this period. Upon transfer to the (+T, -AU) medium, the amount of DNA synthesized in cultures previously treated with nalidixic acid or starved of thymine increased 200 and 180%,

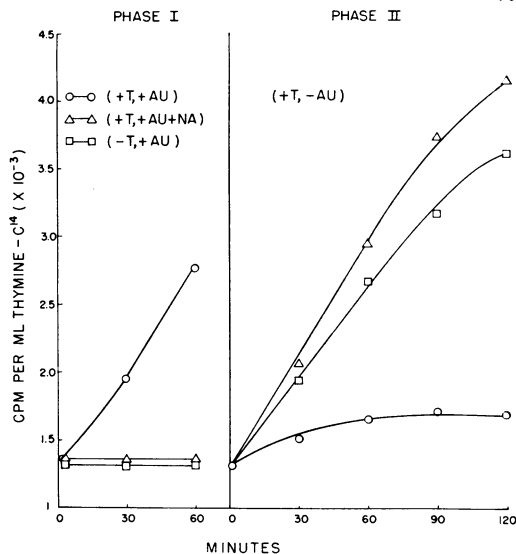


FIG. 1. Induction of excessive DNA synthesis in *Escherichia coli* 15 TAU by thymine starvation or exposure to nalidixic acid (NA). A culture of *E. coli* 15 TAU was prelabeled with ^{14}C -thymine (0.01 $\mu\text{C}/\text{ml}$) for six generations and divided into three equal portions. One (control) was transferred directly to (+T, -AU) medium; the second was treated with nalidixic acid (20 $\mu\text{g}/\text{ml}$) and the third was starved of thymine (Phase I). After 60 min, the treated cultures were filtered, washed, and transferred to (+T, -AU) medium containing ^{14}C -thymine at the same specific activity (Phase II). Samples were removed to ice-cold 10% trichloroacetic acid for determination of the radioactivity in acid-insolubles.

respectively. In contrast, the DNA content of the control culture increased 42%.

Extent of the DNA synthesis induced by nalidixic acid. It has been demonstrated that when *E. coli* 15T⁻ is starved of thymine, both the rate and extent of DNA synthesis in the absence of amino acids are dependent upon the duration of thymine starvation (21). An analogous relationship exists with respect to nalidixic acid treatment.

An exponential culture of *E. coli* 15 TAU was treated with nalidixic acid (20 µg/ml) in complete (+T, +AU) medium. After various periods of incubation, portions of the culture were transferred to (+T, -AU) medium. The DNA content of the cultures previously exposed to nalidixic acid for 30, 60, and 80 min increased 105, 180, and 230%, respectively, during the subsequent 120 min incubation in (+T, -AU) medium

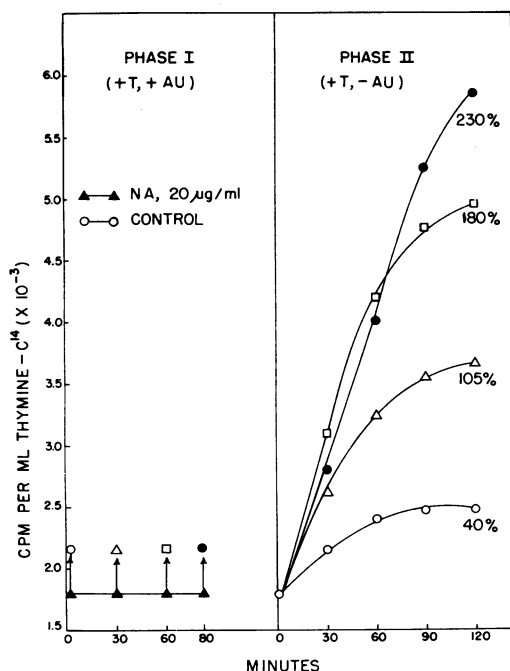


FIG. 2. Dependence of the rate and extent of excessive DNA synthesis in *Escherichia coli* 15 TAU on the duration of exposure to nalidixic acid (NA). An exponentially growing culture of *E. coli* 15 TAU, pre-labeled with ¹⁴C-thymine (0.01 µc/ml) for several generations, was exposed to nalidixic acid for 80 min (Phase I). At 0, (○), 30 (△), 60 (□), and 80 (●) min, 20-ml portions were removed, filtered, washed, and transferred to drug-free (+T, -AU) medium containing ¹⁴C-thymine at the same specific activity (Phase II). Samples were removed to ice-cold 10% trichloroacetic acid for determination of radioactivity in the acid-insolubles. The arrows indicate the time of transfer to Phase II.

(Fig. 2). Although the greatest amount of DNA was synthesized after prior exposure to nalidixic acid for 80 min (230%), the rate of DNA synthesis was comparable to that obtained after a 60-min exposure to the drug. During the same period of incubation, the DNA content of the control culture increased 40%.

Maximal amount of DNA synthesized after exposure to nalidixic acid. In a preliminary communication, we reported that, under conditions of amino acid starvation (+T, -AU), a culture of *E. coli* 15 TAU, previously treated with nalidixic acid for 75 min, synthesized up to 10 times as much DNA as an untreated control culture (Bacteriol. Proc., p. 82, 1966). In view of the ability of *E. coli* 15 TAU to synthesize as much as 2% of its uracil requirement and 5% of its arginine requirement (11), the observed DNA increase under these conditions may have resulted from "leakiness" and initiation of new cycles of DNA replication in deficient (+T, -AU) medium. To explore this possibility, we examined the extent of excessive DNA synthesis in the presence of chloramphenicol and phenethyl alcohol. Chloramphenicol has been shown to decrease the rate of DNA synthesis after initiation (13). Phenethyl alcohol prevents the initiation of DNA synthesis (16, 25).

The amount of DNA synthesized by *E. coli* 15 TAU in (+T, -AU) medium, after removal of nalidixic acid, was reduced in the presence of chloramphenicol (Table 1). However, some residual DNA synthesis was observed even after 150 min of incubation in the (+T, -AU) medium containing chloramphenicol. Chloramphenicol had no detectable effect on completion of a round of DNA synthesis (Table 1, zero-time transfer).

Inhibition of DNA synthesis in *E. coli* 15 TAU,

TABLE 1. Effect of chloramphenicol on the DNA synthesis in *Escherichia coli* 15 TAU induced by nalidixic acid^a

Time of treatment with nalidixic acid (20 µg/ml)	DNA, % increase in (+T, -AU) medium	
	- chloramphenicol	+ chloramphenicol
0	50	43
30	100	80
60	220	184
80	300	230

^a After nalidixic acid treatment, cultures were filtered, washed, transferred to (+T, -AU) medium with and without chloramphenicol (30 µg/ml), and incubated for 150 min. DNA synthesis was monitored by the incorporation of ¹⁴C-thymine into acid-insoluble material.

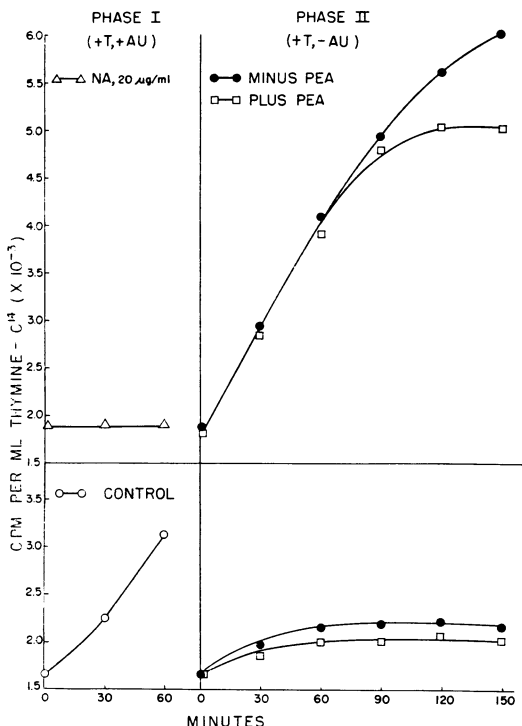


FIG. 3. Effect of phenethyl alcohol (PEA) on the extent of DNA synthesis in *Escherichia coli* 15 TAU after exposure to nalidixic acid (NA). An exponential culture of *E. coli* 15 TAU was labeled for several generations with ¹⁴C-thymine (0.01 μg/ml). At a cell density of 1.5×10^8 to 2×10^8 cells/ml, the culture was divided into two portions in the presence and absence of nalidixic acid (Phase I). After 60 min of incubation, the cultures were filtered, washed, and transferred to drug-free (+T, -AU) medium of the same specific activity with and without 0.20% phenethyl alcohol (Phase II). Radioactivity was determined as previously described.

by nalidixic acid, in nutritionally complete medium resulted in a 180% increase in DNA content after transfer of the culture to (+T, -AU) medium containing phenethyl alcohol. In contrast, a 235% increase in DNA was observed in the absence of phenethyl alcohol (Fig. 3). In the presence of phenethyl alcohol, DNA synthesis ceased by 90 min, whereas synthesis continued in the absence of phenethyl alcohol. The DNA contents of the control cultures increased 35 and 46%, respectively, in the presence and absence of phenethyl alcohol, respectively.

Results similar to those observed with chloramphenicol and phenethyl alcohol were obtained when a mutant (*E. coli* TAU-bar) showing a more stringent uracil requirement than *E. coli* 15 TAU was employed. Treatment of *E. coli* TAU-

bar with nalidixic acid for different periods of time in nutritionally complete medium resulted in a 180 to 190% increase in DNA content after drug removal and transfer to (+T, -supplements) medium (Fig. 4). The maximal rate of DNA synthesis was three times that of the control and synthesis had ceased by 90 to 100 min. The maximal amount of DNA synthesized was three to four times that of the untreated control.

Requirement for protein and ribonucleic acid (RNA) synthesis during induction of excessive DNA synthesis by nalidixic acid. The induction of excessive DNA synthesis by nalidixic acid requires protein or RNA synthesis, or both. Furthermore, the effect induced by nalidixic acid occurs during the period of exposure to the drug and not after transfer to drug-free (+T, -AU) medium.

This was demonstrated in an initial experiment in which protein and RNA synthesis were restricted during nalidixic acid treatment by omission of arginine and uracil. When a culture of *E.*

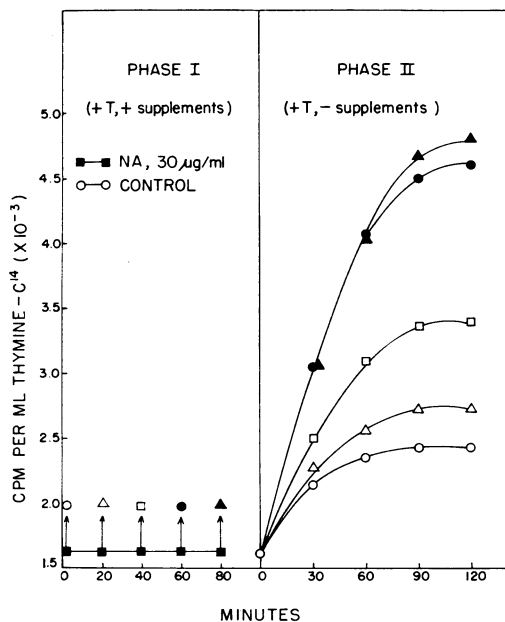


FIG. 4. Induction of excessive DNA synthesis in *Escherichia coli* TAU-bar by exposure to nalidixic acid (NA). An exponential culture of *E. coli* TAU-bar, pre-labeled with ¹⁴C-thymine (0.01 μg/ml) for several generations was exposed to nalidixic acid for 80 min (Phase I). At 0 (○), 20 (△), 40 (□), 60 (●), and 80 (▲) min, 20 ml samples were filtered, washed, and transferred to (+T, -AU) medium (Phase II) containing ¹⁴C-thymine (0.01 μg/ml). Samples were removed to ice-cold 10% trichloroacetic acid for determination of acid-insoluble radioactivity. The arrows indicate the time of transfer to Phase II.

coli 15 TAU, in (+T, -AU) medium, was treated with nalidixic acid, DNA synthesis ceased. On subsequent transfer to drug-free (+T, -AU) medium, the DNA content increased 36 to 38% (Table 2). In contrast, inhibition of DNA synthesis in nutritionally complete (+T, +AU) medium resulted in a 186% increase in DNA content on transfer of the culture to drug-free (+T, -AU) medium. The DNA content of the control culture increased 38% during incubation in (+T, -AU) medium. If a period of thymine starvation (-T, -AU) was substituted for the nalidixic acid treatment, the DNA content increased 33% during the subsequent incubation in (+T, -AU) medium.

Excessive DNA synthesis was prevented when inhibitors were employed to block protein or RNA synthesis, during nalidixic acid treatment, in nutritionally complete (+T, +AU) medium. Concomitant treatment of *E. coli* 15 TAU with nalidixic acid and chloramphenicol or phenethyl alcohol prevented excessive DNA synthesis on transfer to drug-free (+T, -AU) medium (Table 3). Under these conditions, the increase in DNA content in (+T, -AU) medium was of the same order of magnitude as that observed in the untreated control culture. Similarly, the induction of excessive DNA synthesis by thymine starvation was blocked by phenethyl alcohol.

TABLE 2. Effect of nutritional conditions on the induction of excessive DNA synthesis in *Escherichia coli* 15 TAU by nalidixic acid^a

Nutritional condition (Phase I)	DNA, % increase	
	Phase I ^b	Phase II ^c
+T, +AU	110	43
+T, +AU, +NA	0	186
+T, -AU	38	0
+T, -AU, +NA	0	36
-T, -AU	0	33

^a An exponential culture of *E. coli* 15 TAU, prelabeled with ¹⁴C-thymine (0.01 μg/ml), was divided into five portions. One was treated with nalidixic acid (NA, 20 μg/ml) for 40 min and subsequently transferred to (+T, -AU) medium for 120 min. The remaining portions were filtered, washed, and transferred to (+T, -AU) medium with and without nalidixic acid (20 μg/ml) or to (-T, -AU) medium. After 40 min, the cultures were filtered, washed, and transferred to (+T, -AU) medium for 120 min. Radioactivity was monitored as previously described.

^b Phase I refers to the nutritional condition and the per cent increase in DNA during this phase.

^c Phase II refers to the (+T, -AU) medium and the per cent increase in DNA during incubation in this medium.

TABLE 3. Effects of chloramphenicol and phenethyl alcohol on the induction of excessive DNA synthesis in *Escherichia coli* 15 TAU by nalidixic acid^a

Condition (Phase I)	DNA, % increase	
	Phase I	Phase II
+T +AU	95	45
+T +AU +NA	0	192
+T +AU +CAP	70	23
+T +AU +NA +CAP	0	42
+T +AU +PEA	36	0
+T +AU +NA +PEA	0	44
-T +AU	0	175
-T +AU +PEA	0	43

^a Exponential cultures of *E. coli* 15 TAU, pre-labeled with ¹⁴C-thymine (0.01 μg/ml), were treated for 60 min under the various conditions described (Phase I) and subsequently transferred to drug-free (+T, -AU) medium (Phase II) having the same specific radioactivity. Inhibitor concentrations were: nalidixic acid (NA), 20 μg/ml; chloramphenicol (CAP), 30 μg/ml; and phenethyl alcohol (PEA), 2 mg/ml.

Effect of growth rate on the extent of DNA synthesis after removal of nalidixic acid. Lark reported that the pattern of DNA replication is related to the growth rate in *E. coli* (12). Radioautographic data indicate that, when *E. coli* 15 T⁻ is grown in a synthetic medium containing succinate as carbon source, only one of the two chromosomes per cell is replicating at any given instant. In contrast, cells growing in a medium containing glucose appear to replicate both chromosomes simultaneously (15). If cells growing in succinate are transferred to an amino acid-deficient medium, the net increase in DNA is approximately one-half that obtained when cells are previously grown in glucose. If the succinate-grown cells are previously starved of thymine, the DNA content increases approximately 2.8-fold over the control on subsequent incubation in amino acid-deficient medium (15). From these data, it has been suggested that the amount of DNA synthesized in deficient medium is a measure of the number of chromosomes undergoing replication, and that thymine starvation initiates replication prematurely only on those chromosomes in the process of replication (15).

A similar result was obtained when *E. coli* 15 TAU, growing in succinate, was treated with nalidixic acid for 80 min and subsequently transferred to (+T, -AU) medium containing glucose. Under these conditions, the DNA content of the treated culture increased 77%, whereas that of the control culture increased 27% (Fig. 5)

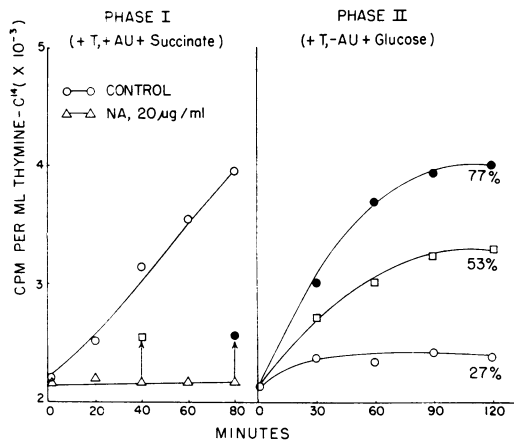


FIG. 5. Effect of growth rate on the extent of nalidixic acid (NA)-induced DNA synthesis in *Escherichia coli* 15 TAU. An exponential culture (generation time = 110 min) of *E. coli* 15 TAU, growing in succinate (0.5%) was labeled for three generations with ¹⁴C-thymine (0.01 µg/ml). At a cell density of 1.5×10^8 to 2×10^8 cells/ml, the culture was divided into two portions with and without nalidixic acid (Phase I). At 40 (□) and 80 (●) min, samples were removed and transferred to drug-free (+T, -AU) medium containing glucose and ¹⁴C-thymine at the same specific activity (Phase II). The arrows indicate the time of transfer to Phase II.

This represented a 2.85-fold increase over the control in the amount of DNA synthesized by the treated culture. [It can be noted that under these conditions the amount of DNA synthesized in (+T, -AU) medium is independent of the carbon source in this medium.] No DNA synthesis was detectable during treatment with nalidixic acid, whereas the DNA content of the control culture increased 75%.

Density gradient distribution of the DNA synthesized after nalidixic acid treatment and thymine starvation. To gain some insight into the replication pattern of the DNA synthesized after nalidixic acid treatment, we employed CsCl density gradient centrifugation. In these studies, ¹⁴C-labeled bromouracil (BU) was used as a density label.

A culture of *E. coli* 15 TAU, growing in glucose, was treated with nalidixic acid or starved of thymine. After inhibition of DNA synthesis for 60 min, the cultures were transferred to (+¹⁴C-BU, -AU) medium for 110 min, at which time samples were removed for CsCl density gradient centrifugation. The DNA density distributions in CsCl of the nalidixic acid- and thymine-starved cells were qualitatively similar (Fig. 6). In both cases, three DNA bands were evident in the gradient: light, ³H-containing DNA; hybrid

DNA, containing both ³H and ¹⁴C; and heavy DNA (BU) containing only ¹⁴C. In contrast, DNA from the control culture gave only light (³H) and hybrid (³H + ¹⁴C) material.

DISCUSSION

The data presented demonstrate some basic similarities between the effects of thymine starvation and nalidixic acid treatment on the regulation of DNA synthesis in *E. coli* 15 TAU. As reported for thymine starvation (12, 14, 24), the accelerated DNA synthesis induced by nalidixic acid depends on concomitant synthesis of protein or RNA, or both, during the period when DNA synthesis is blocked. After both treatments, the amount of DNA synthesized is reduced approximately one-half if succinate is substituted for glucose during the treatment period. This suggests that the excessive DNA synthesis induced by nalidixic acid results from its action on those chromosomes actively replicating. Furthermore, it provides an explanation for the failure of nalidixic acid to affect nongrowing cultures of *E. coli* (7).

After removal of nalidixic acid, the amount of DNA synthesized in (+T, -AU) medium is reduced in the presence of chloramphenicol or phenethyl alcohol. Although reduced, some DNA synthesis still occurs in the presence of chloramphenicol. In the presence of phenethyl alcohol, DNA synthesis ceases by 90 min, and the extent of DNA synthesis is limited to 175 to 180% of that initially present. This maximum is also observed when *E. coli* TAU-bar is substituted for *E. coli* 15 TAU. These results indicate that at least some of the excessive DNA synthesized by *E. coli* 15 TAU, in (+T, -AU) medium, after treatment with nalidixic acid, is due to initiation of new cycles of DNA synthesis. Under conditions in which initiation is inhibited (phenethyl alcohol), or the rate of DNA synthesis after initiation is reduced (chloramphenicol), the maximal amount of DNA synthesized is 175 to 180% of that initially present.

Protein or RNA synthesis, or both, are required for the induction of excessive DNA synthesis by nalidixic acid. The sensitivity to phenethyl alcohol may result from its effect on RNA synthesis (22), since the initiation of DNA synthesis in (+T, -AU) medium, after nalidixic acid removal, is not prevented by phenethyl alcohol. Alternatively, the mechanism of initiation of this DNA synthesis may differ from that of the normal initiation of DNA synthesis from the chromosomal origin in *E. coli* 15 TAU.

Under conditions of restricted protein and RNA synthesis (+T, -AU, +nalidixic acid),

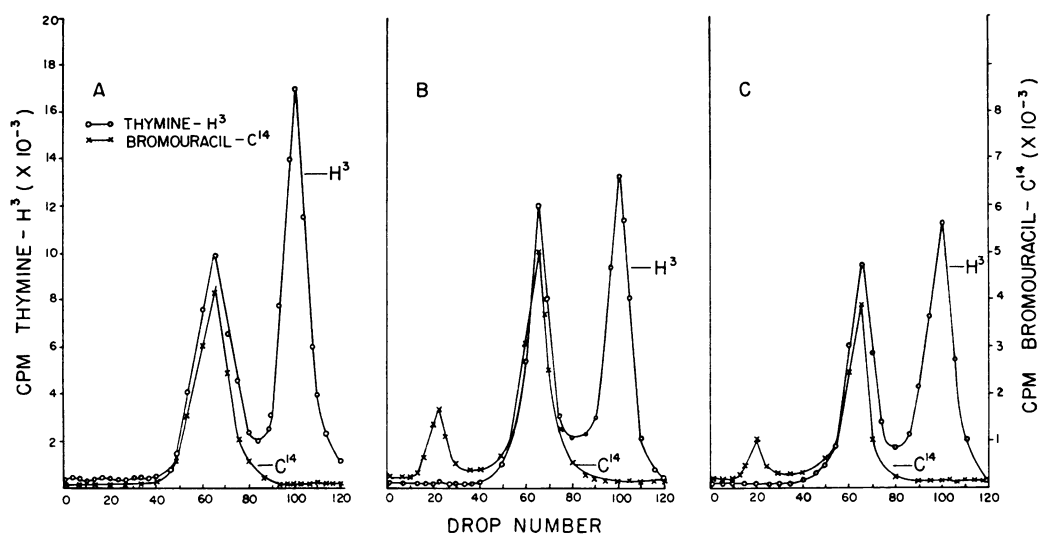


FIG. 6. Cesium chloride density distribution of DNA synthesized after exposure to nalidixic acid or thymine starvation. A culture of *Escherichia coli* 15 TAU was uniformly labeled with ^3H -thymine ($3 \mu\text{g/ml}$) for three generations and divided into three equal portions. The control (A) was transferred directly to (+T, -AU) medium containing unlabeled thymine ($2 \mu\text{g/ml}$) for 6 min and then to (+BU, -AU) medium containing unlabeled bromouracil ($2 \mu\text{g/ml}$) for 10 min. After 10 min in unlabeled bromouracil, the culture was transferred to (+BU, -AU) medium containing ^{14}C -bromouracil ($0.33 \mu\text{g/ml}$, $2 \mu\text{g/ml}$) for 110 min. The same procedure was employed for the treated cultures except that cells were exposed to nalidixic acid (B) or starved of thymine (C) for 60 min before the initial transfer to (+T, -AU) medium. The calculated densities of heavy, hybrid, and light DNA were 1.795 g/cm^3 , 1.757 g/cm^3 , and 1.715 g/cm^3 , respectively.

DNA synthesis is blocked, but the induction of excessive DNA synthesis is prevented. Upon drug removal, the DNA replication cycle is completed. This suggests that nalidixic acid may affect DNA at or near the replicating fork and that, upon drug removal, DNA synthesis is reinitiated from this original point of replication.

The CsCl density distribution of the DNA synthesized by *E. coli* 15 TAU, after removal of nalidixic acid, is qualitatively similar to that obtained after thymine starvation. The simultaneous presence of heavy, hybrid and light DNA may reflect: (i) heterogeneity in the chromosome population; (ii) premature initiation of new cycles of DNA replication; or (iii) replication of portions of some chromosomes twice before others have replicated once. The present data do not permit the resolution of these alternatives, and the use of bromouracil may preclude accurate quantitation. In this respect, it has been reported that some thymineless strains of *E. coli* demonstrate anomalous DNA replication when bromouracil is employed as a density label (9, 10). Also, it has been observed that the DNA content of *E. coli* 15 TAU increased only 56% in a (+BU, -AU) medium after thymine starvation (2).

On the basis of the experimental data, we suggest that nalidixic acid alters the normal DNA

replication pattern in *E. coli* 15 TAU and *E. coli* TAU-bar. This alteration appears to involve the induction of new sites for DNA synthesis and requires protein or RNA synthesis, or both. The rapid rate and extent of DNA synthesis in (+T, -AU) medium, after drug removal, strongly suggests that DNA synthesis proceeds from new sites on the chromosome.

The present experiments do not eliminate conclusively the possibility that the DNA synthesis induced by nalidixic acid is associated with episomal or phage nucleic acid. Recent reports have established the presence of defective phage particles in both *E. coli* 15 TAU and *E. coli* TAU-bar (6, 23). Further studies are in progress to examine the chromosomal or nonchromosomal nature of the DNA synthesized after removal of nalidixic acid. The results will be the subject of a future report.

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